Alcohol intake and cardiovascular function of black South Africans: a 5-year prospective study

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….. kunye nemithandazo yabazalwane baseBhofolo, eNkukwini (African Gospel Church)

'Owemvelo ke umntu akazamkeli izinto zoMoya kaThixo; kuba zibululenge kuye; kananjalo akanakuzazi, ngokuba zipicothwa ngokoMoya; ke ongowoMoya okunene uphicotha zonke izinto, kodwa yena ngokwakhe akaphicothwa mntu'. 1 Kor 2:14-15.

..... Sibuya kude thina, ...singaduka phi? ....asingabo abantu bokuduka, ...Zulu khaya !!!

Rev T Fatyi, African Gospel Church: EBhofolo, mid-late 70's and early 90's.

"Hamba kahle 'Mgangathi wendlela ebheka eKhaya', usikhonzele koBaba uChiliza, Nondaba, Mseleku, Mxunyelwa, Yiba, Khalala, Tyhali, Siyo, Pewa, Nduku, Toyo, Nofilita kunye nabanye".

DEDICATION

I wish to dedicate this achievement to the following people:

- My grandmothers, Norah Shumikazi Zatu and Thenjiwe Dyantyi (*both late*), for their love, upbringing in God, and making sure I never went to school on an empty stomach.
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SUMMARY

Motivation

Alcohol consumption is one of the major risk factors of cardiovascular disease (CVD). Excessive alcohol drinking is the fifth leading cause of death worldwide and the prevalence of alcohol abuse continues to increase especially in low-income areas of sub-Saharan Africa. The alarming rate of urbanisation seems to be the driving force for excessive alcohol intake in the developing world. In addition to its influence on CVD, heavy drinking also results in a number of non-cardiovascular consequences that include injury, risky sexual behaviour, violent crime and family dysfunction among black South Africans, contributing to high mortality. Moreover, the highest number of individuals with human immunodeficiency virus (HIV) infection in South Africa is partly attributable to high intake of alcohol. HIV remains a major concern in South Africa with significant funding diverted to address the pandemic. The continued increases in mortality from preventable outcomes such as stroke, myocardial infarction and renal failure are largely due to urbanisation, poverty and dysfunctional health systems working with limited budgets. These are some of the factors requiring in-depth study of the scientific aspects of alcohol intake in South Africa. Although there is enough evidence that links excessive drinking with hypertension and CVD, the markers of alcohol intake – self reporting of alcohol, gamma-glutamyltransferase (GGT) and carbohydrate deficient transferrin – are still not specific enough to isolate other confounding factors in the association of alcohol intake with CVD. The markers of alcohol that independently predict CVD and mortality need to be explored. Finally, the severe lack of longitudinal investigations on alcohol-related hypertension development and total mortality in black South Africans has compromised the early identification of risk factors associated with these outcomes. This study will therefore attempt to address the limited availability of longitudinal studies and stimulate interest for continued investigation.

Aim

The aim of this study was to investigate whether alcohol intake of black South Africans is related to specific measures of cardiovascular function (change in blood pressure (BP), hypertension development) and mortality over a period of 5 years.

Methodology

This study was based on the international Prospective Urban and Rural Epidemiology (PURE) study which includes 26 countries, investigating the cause and development of cardiovascular risk factors in low, middle and high income countries. This South African leg of the PURE study started in 2005 in which the baseline data was collected from 2021 black South Africans from rural and urban areas in Ikageng, Ganyesa and Tlakgameng in the North West Province. Eleven participants presented with missing data, leaving 2010 participants with complete datasets at baseline. However, data from these 11 participants was useful, especially for Chapter 4. All participants gave informed consent and the Ethics committee of the North-West University (Potchefstroom Campus) approved the study. The follow-up data collection was done in 2010. General health questionnaires, anthropometric measurements, lipid profiles and cardiovascular measurements were taken both at baseline and follow-up using appropriate methods. We also collected blood samples and performed biochemical analyses for lipid markers, liver enzymes, inflammatory markers and percentage carbohydrate deficient transferrin (%CDT). Finally, we obtained data on cardiovascular and non-cardiovascular mortality through verbal autopsy and death certificates.

We made use of analysis of variance (ANOVA) and Chi-square tests to compare means and proportions, respectively. We used dependent t-tests and the McNemar test to compare baseline and follow-up variables. Furthermore, we employed single and partial linear regression analyses to correlate alcohol markers with each other and with the cardiovascular measures. Multiple regression analyses were used to correlate dependent variables in the study with various independent variables as required. Finally, we employed multivariable-adjusted Cox regression analyses to assess the association of the selected alcohol markers with mortality while adjusting for several independent variables.

Results and Conclusions of each manuscript

- With the first research article (Chapter 4), we aimed to compare self-reported alcohol intake estimates with GGT and %CDT, considering their relationship with percentage change in brachial blood pressure (BP) and central systolic blood pressure (cSBP) over 5 years. The

results indicated that only self-reported alcohol intake independently predicted % change in brachial BP and cSBP. This was not found for the biochemical markers GGT and %CDT. Self-reported alcohol intake seems to be an important measure to implement by health systems in low income areas of sub-Saharan Africa, where honest reporting is expected.

- Given the likely presence of high GGT levels in both alcohol consumption and non-alcoholic fatty liver disease (NAFLD), the second manuscript (Chapter 5) aimed to compare the cardiovascular and metabolic characteristics of excessive alcohol users and individuals with suspected NAFLD (confirmed with self-report, GGT and %CDT). We found that different sex and cardiometabolic profiles characterised excessive alcohol users and individuals suspected with NAFLD. Lean body mass and male sex were the dominant characteristics in excessive alcohol use while the NAFLD group had a dysmetabolic profile with obese women making up the higher proportion of this group. In excessive alcohol users systolic blood pressure and pulse pressure were independently associated with high-density lipoprotein cholesterol. Diastolic blood pressure showed a significant correlation with waist circumference. These disparate profiles may guide healthcare practitioners in primary healthcare clinics to identify individuals with elevated GGT levels who may suffer from NAFLD or alcohol overuse. These results emphasise the importance of modifiable risk factors as the main contributors to CVD and that lifestyle change should be the main focus in developing countries such as South Africa.
- The third manuscript (Chapter 6) aimed to determine the measure of alcohol intake (self-reported alcohol intake, GGT and %CDT) that related best with hypertension development, cardiovascular and all-cause mortality over 5 years in the same population of black South Africans. We found that GGT was the only independent predictor of hypertension development, cardiovascular as well as all-cause mortality. Moreover, self-reporting of alcohol intake predicted incident hypertension, confirming our findings from Chapter 4. The third marker, %CDT, a highly specific marker of alcohol intake, was not related with any outcome variable, perhaps due to its low sensitivity. Although self-reported alcohol intake is useful in low-resource primary healthcare settings, measurement of GGT is encouraged due to its

predictive value for hypertension and mortality. GGT represents alcohol intake, non-alcoholic steatohepatitis and obesity - all known to have severe cardiovascular consequences.

Discussion and Conclusions

Excessive alcohol intake remains a major concern in the development of hypertension, CVD and premature death in sub-Saharan Africa. Despite their weaknesses such as bias and non-specificity, self-reporting of alcohol consumption and GGT emerged as reliable alcohol markers that independently predicted 5-year change in BP, hypertension development and total mortality in this population. Serum %CDT did not show any association with the mentioned cardiovascular markers. Finally, we were also able to show that black South Africans with suspected NAFLD (i.e. with high GGT levels who do not consume alcohol) are typically obese women, whereas lean men were more likely to have high alcohol consumption. Further prospective investigations are encouraged regarding (a) these mentioned associations, as well as (b) other self-reporting estimates such as quantity and frequency of drinking and (c) the use of %CDT as a highly specific marker of alcohol intake. The simultaneous presence of HIV infection in alcohol abuse in this population also warrants further investigation.

Keywords: self-reported alcohol consumption, gamma-glutamyltransferase, percentage carbohydrate deficient transferrin, percentage change in blood pressure, hypertension, mortality, high-density lipoprotein cholesterol, non-alcoholic fatty liver disease, HIV infection, low socio-economic status, Africans

AFRIKAANSE OPSOMMING

Motivering

Alkoholinname is een van die belangrikste risikofaktore vir kardiovaskulêre siekte (KVS). Oormatige alkoholinname is die vyfde grootste oorsaak van mortaliteit in die wêreld en daar is steeds ‘n toename in die voorkoms van alkoholmisbruik veral in die lae-inkomste gebiede van sub-Sahara Afrika. Die drastiese toename in verstedeliking blyk om die dryfveer te wees vir oormatige alkoholinname in die ontwikkelende wêreld. Behalwe vir die invloed op KVS, lei oormatige alkoholgebruik ook tot ‘n aantal nie-kardiovaskulêre gevolge wat beserings, hoë risiko seksuele gedrag, geweld, misdaad en gesinsprobleme by swart Suid Afrikaners, wat verder aanleiding gee tot mortaliteit. Verder beskik Suid-Afrika ook oor die grootste aantal persone wat geïnfekteer is met die menslike immuniteitsgebreksvirus (MIV), wat gedeeltelik ook toe te wy is aan hoë alkoholinname. MIV bly ‘n ernstige kommer in Suid-Afrika, met groot hoeveelhede befondsing gefokus om die pandemie aan te spreek. Die voortdurende toenames in mortaliteit vanweë uitkomstes soos beroerte, miokardiale infarksie en nierversaking word grootliks toegeskryf aan verstedeliking, armoede en swak gesondheidsstelsels waar met beperkte fondse gewerk word. Hierdie is sommige van die faktore wat in-diepte studie benodig wanneer die wetenskaplike aspekte van alkoholinname in Suid-Afrika ondersoek word. Hoewel daar voldoende wetenskaplike bewyse bestaan dat oormatige alkoholgebruik met hipertensie en KVS verband hou, is die merkers van alkoholinname – self-gerapporteerde alkoholinname, gamma-glutamieltransferase (GGT) en koolhidraattekort transferrien – steeds nie spesifiek genoeg om die merkers te isoleer van ander faktore wat dit mag beïnvloed nie. Die merkers van alkohol wat KVS en mortaliteit onafhanklik voorspel moet dus verder ondersoek word. Laastens, die ernstige tekort aan longitudinale ondersoeke m.b.t. alkohol-verwante hipertensie ontwikkeling en mortaliteit in swart Suid Afrikaners, het die gevolg dat daar onvoldoende navorsing bestaan rakende die vroeë identifisering van risikofaktore van hierdie uitkomste. Hierdie studie wil daarom poog om die beperkte beskikbaarheid van longitudinale studies aan te spreek, en verdere belangstelling te stimuleer vir voortgesette ondersoeke.

Doel

Die doel van die studie was om te bepaal of alkoholinname in swart Suid Afrikaners verband hou met spesifieke aspekte van kardiovaskulêre funksie (verandering in bloeddruk, hipertensie ontwikkeling) en mortaliteit oor ‘n periode van 5 jaar.

Metodologie

Die studie is gebaseer op die internasionale ‘Prospective Urban Rural Epidemiology (PURE)’ studie wat 26 lande insluit, en wat die oorsake en ontwikkeling van kardiovaskulêre risikofaktore in lae, middel en hoë inkomste lande ondersoek. Die Suid Afrikaanse been van die PURE-studie het in 2005 begin waartydens die basislyndata by 2021 swart Suid Afrikaners versamel is. Dit is gedoen in landelike en verstedelikte gebiede in Ikageng, Ganyesa en Tlakgameng in die Noordwes Provinsie. By 11 proefpersone was daar onvolledige data, en daarom is 2010 proefpersone met volledige basislyndata ingesluit. Nietemin, in sekere gevalle was die data van die 11 proefpersone wel bruikbaar, veral in Hoofstuk 4. Alle proefpersone het ingeligte toestemming verleen en die Etiekkomitee van die Noordwes-Universiteit (Potchefstroomkampus) het die studie goedgekeur. Die opvolgdata is versamel in 2010. Algemene gesondheidsvraelyste, antropometriese metings, lipiedprofiele en kardiovaskulêre metings is geneem tydens basislyn en opvolgopnames volgens aanvaarde metodologie. Ons het bloedmonsters versamel en biochemiese analises vir lipiede, lewerensieme, inflammatoriese merkers en persentasie koolhidraattekort transferrien (%CDT) bepaal. Ons het ook data rakende kardiovaskulêre en nie-kardiovaskulêre mortaliteit verkry d.m.v. verbale outopsies en doodsertifikate.

Ons het van variansie analises (ANOVA) en Chi-kwadraattoetse gebruik gemaak om gemiddelde en proporsies te vergelyk. Ons het afhanklike t-toetse en die McNemartoets gebruik om basislyn en opvolgdata te vergelyk. Verder het ons enkel en parsiële regressie analises gebruik om alkoholmerkers met mekaar te korreleer, asook met kardiovaskulêre metings. Meervoudige regressie analises is gebruik om die afhanklike veranderlikes van die studie met verskeie onafhanklike veranderlikes te korreleer. Laastens is Cox-regressie analises gebruik waartydens aanpassings vir verskeie onafhanklike veranderlikes aangebring is, om die verwantskap te ondersoek tussen die geselekteerde alkoholmerkers en mortaliteit.

Resultate en Gevolgtrekkings van elke manuskrip

- Met die eerste navorsingsartikel (Hoofstuk 4) was ons doelwit om self-gerapporteerde alkoholinname te vergelyk met GGT en %CDT met betrekking tot die verwantskap met persentasie verandering in bragiale bloeddruk en sentrale sistoliese bloeddruk (cSBP) oor 5 jaar. Die resultate het getoon dat slegs self-gerapporteerde alkoholinname die % verandering in bragiale bloeddruk en cSBP onafhanklik voorspel het. Dit is nie gevind vir die biochemiese merkers, GGT en %CDT, nie. Self-gerapporteerde alkoholinname blyk om 'n belangrike meting te wees wat in gesondheidsstelsels geïmplementeer kan word in lae inkomste gebiede van sub-Sahara Afrika, waar eerlike rapportering verwag word.
- Volgens die literatuur kom hoë GGT vlakke voor by beide hoë alkoholinname en nie-alkoholverwante lewervervetting. Met die tweede manuskrip (Hoofstuk 5) is daar gepoog om die kardiovaskulêre en metaboliese eienskappe van oormatige alkoholgebruikers (bevestig met self-rapportering, GGT en %CDT) en persone met nie-alkoholiese vervette lewersiekte te vergelyk (NAFLD). Lae liggaamsmassa en manlike geslag was dominante eienskappe by oormatige alkoholgebruikers terwyl die NAFLD groep 'n dismetaboliese profiel getoon het waar obese vroue die grootste proporsie van die groep gevorm het. By oormatige alkoholgebruikers het sistoliese bloeddruk en polsdruk onafhanklik verband gehou met hoë-digtheid lipoproteïen cholesterol. Diastoliese bloeddruk het 'n betekenisvolle korrelasie met middel-omtrek getoon. Hierdie verskillende profiele kan gesondheidswerkers in primêre gesondheidsorgklinieke moontlik help om individue te identifiseer met verhoogde GGT-vlakke wat aan NAFLD ly of alkohol misbruik. Hierdie resultate beklemtoon die belangrikheid van aanpasbare risikofaktore as belangrike bydraers tot KVS en dat lewenstylveranderinge steeds die hooffokus in ontwikkelende lande soos Suid-Afrika behoort te wees.
- Die derde manuskrip (Hoofstuk 6) het bepaal watter meting van alkoholinname (self-gerapporteerde alkohol inname, GGT en %CDT) die beste met die ontwikkeling van hipertensie verband hou, asook met kardiovaskulêre mortaliteit en totale mortaliteit oor 5 jaar. In dieselfde groep swart Suid Afrikaners het ons bevind dat GGT die enigste onafhanklike voorspeller van hipertensie ontwikkeling, kardiovaskulêre en totale mortaliteit was. Bykomend het self-gerapporteerde alkoholinname hipertensie insidensie voorspel, wat ons bevindinge

van Hoofstuk 4 bevestig. Die derde merker, %CDT, ‘n hoogs spesifieke merker van alkoholinname, het nie met enige uitkomste verband gehou nie, moontlik vanweë die lae sensitiwiteit daarvan. Hoewel self-gerapporteerde alkoholinname bruikbaar is by primêre gesondheidsorg klinieke met min hulpbronne, word die meting van GGT steeds aangemoedig vanweë die voorspellingswaarde vir beide hipertensie en mortaliteit. GGT verteenwoordig alkoholinname, nie-alkoholverwante steatohepatitis en obesiteit – alles toestande wat ernstige kardiovaskulêre gevolge het.

Bespreking en Gevolgtrekkings

Oormatige alkoholgebruik bly ‘n ernstige bydraende faktor tot die ontwikkeling van hipertensie, KVS en vroeë dood in sub-Sahara Afrika. Ten spyte van beperkinge soos vooroordeel en non-spesifisiteit, het ons resultate getoon dat self-gerapporteerde alkoholinname en GGT betroubare alkoholmerkers is wat onafhanklik die 5-jaar veranderinge in bloeddruk voorspel het, asook hipertensie en totale mortaliteit. Serum %CDT het geen assosiasie met die genoemde kardiovaskulêre merkers getoon nie. Laastens, ons het getoon dat swart Suid Afrikaners wat vermoedelik aan NAFLD ly (i.e. hoë GGT vlakke, maar wat nie alkohol gebruik nie) tipies bestaan uit obese vroue, waar skraal mans tipies ‘n hoë alkoholinname toon. Verdere langtermynstudies word aangemoedig om (a) die genoemde verwantskappe verder te ondersoek, asook om (b) ander self-gerapporteerde aanduidings soos hoeveelheid en frekwensie van alkoholgebruik, en (c) %CDT as ‘n hoogs spesifieke merker van alkoholinname te ondersoek. Die simultane teenwoordigheid van beide MIV infeksie en alkoholmisbruik in die bevolkingsgroep moet ook verder ondersoek word.

Sleutelwoorde: self-gerapporteerde alkoholinname, gamma-glutamieltransferase, persentasie koolhidraattekort transferrien, persentasie verandering in bloeddruk, hipertensie, mortaliteit, hoë-digtheid lipoproteïen cholesterol, nie-alkoholiese lewervervetting, MIV infeksie, lae sosio-ekonomiese status, Afrikane

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LIST OF ABBREVIATIONS

%CDT – Percentage carbohydrate deficient transferrin

%DBP – Percentage change in diastolic blood pressure

%SBP – Percentage change in systolic blood pressure

ABPM – Ambulatory blood pressure monitoring

AFLD – Alcoholic fatty liver disease

AIDS – Acquired immunodeficiency syndrome

ALT – Alanine transaminase

ANOVA – Analysis of variance

ASH – Alcoholic steatohepatitis

AST – Aspartate transaminase

AST/ALT – Aspartate transaminase alanine transaminase ratio

AUDIT – Alcohol Use Disorders Identification Test

BMI – Body mass index

BP – Blood pressure

CAD – Coronary artery disease

CDT – Carbohydrate deficient transferrin

CRP – C-reactive protein

cSBP – Central systolic blood pressure

CVD – Cardiovascular disease

DBP – Diastolic blood pressure

EDAC – Early Detection of Alcohol Consumption

EtG – Ethyl glucuronide

EtS – Ethyl sulphate

FAEEs – Fatty acid ethyl esters

GGT – Gamma-glutamyltransferase

GSH – Glutathione

HbA1c – Glycosylated haemoglobin

HDL-C – High-density lipoprotein cholesterol

HIV – Human immunodeficiency virus

hsCRP – High-sensitivity C-reactive protein

IGF-1 – Insulin-like growth factor-1

LDL-C – Low-density lipoprotein cholesterol

MAST – Michigan Alcohol Screening Test

MCV – Mean corpuscular volume

NAFLD – Non-alcoholic fatty liver disease

NASH – Non-alcoholic steatohepatitis

NCDs – Non-communicable diseases

NO – Nitric oxide

PAI-1 – Plasminogen activator inhibitor-1

PEth – Phosphatidyl ethanol

PP – Pulse pressure

PURE – Prospective Urban and Rural Epidemiology

PWV – Pulse wave velocity

QFFQ – Quantitative Food Frequency Questionnaire

RAAS – Renin-angiotensin–aldosterone system

ROS – Reactive oxygen species

SBP – Systolic blood pressure

TC – Total cholesterol

TC:HDL – Total cholesterol and high-density lipoprotein ratio

TG – Triglycerides

VEGF – Vascular endothelial growth factor

WC – Waist circumference

WHO – World Health Organization

PREFACE

- This thesis is presented in article format as approved by the North-West University and indicated as such in guidelines for postgraduate studies.
- Chapter 1 consists of the general introduction, motivation and problem statement for the thesis.
- Chapter 2 includes the literature study which forms the background to discuss the results of the research articles, in addition to the literature included in each research article. It also includes the motivation, aims, objectives and hypotheses for each of the manuscripts.
- Chapter 3 is the discussion of the study protocol together with all the procedures on the materials and methods used to collect and obtain the data.
- Chapter 4 investigated the usefulness of self-reported alcohol intake as the better estimate of 5-year change in blood pressure than biochemical markers in low resource settings. These results were published in the *Journal of Hypertension* in April 2014.
- Chapter 5 compares the cardiometabolic profile of black South Africans with suspected NAFLD and excessive alcohol use. The article from this chapter has been accepted for publication by the *Alcohol* Journal in November 2014.
- Chapter 6 is titled 'Alcohol intake, hypertension development and mortality in black South Africans'. The article from this chapter has been accepted for publication by the *European Journal of Preventive Cardiology* in November 2014.
- Finally, a summary of all the results and general conclusions, including recommendations for future studies are provided in Chapter 7.

The relevant references are provided at the end of each chapter. They are written following the author's instructions of the specific journal in which each research article was submitted for publication. The referencing style for Chapters 1, 2, 3 and 7 is according to the Vancouver style.

DECLARATION BY AUTHORS

The researchers listed below contributed to this study in ways explained below:

Mr. MC Zatu

Responsible for writing the initial proposal and literature study; critical evaluation of the study protocol and methodology; design, planning and writing of the research papers; and statistical analyses, interpretation and writing of the thesis.

Prof. AE Schutte

Promoter. Supervised the design and planning of the thesis and research articles; data collection, intellectual input on statistical analyses and the final writing of the thesis.

Prof. JM van Rooyen

Co-promoter. Co-supervised the design and planning of the thesis and research articles; data collection, intellectual input on statistical analyses and the final writing of the thesis.

Prof. Du-T Loots

Data collection, academic input in the methodology and research articles (Chapters 4 and 5).

Prof. E Wentzel-Viljoen

Data collection, academic input in the methodology and research articles (Chapter 4).

Prof. M Greeff

Data collection, academic input in the methodology and research articles (Chapters 4 and 5).

Prof. A Kruger

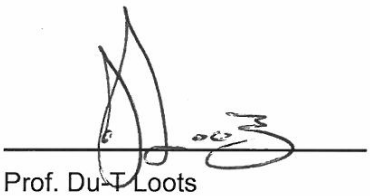
Data collection, academic input in the methodology and research articles (Chapter 6).

The following is a statement from the co-authors confirming their individual roles in the study and giving their permission that the research articles may form part of this dissertation.

I declare that I have approved the above-mentioned manuscripts, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give consent that they may be published as part of the PhD thesis of MC Zatu.

Prof. AE Schutte

Prof. JM van Rooyen



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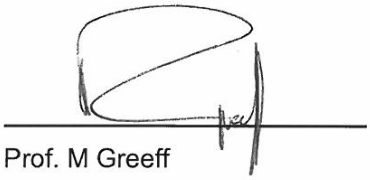
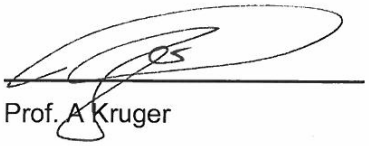
Prof. E Wentzel-Viljoen



Prof. M Greeff



Prof. A Kruger

Chapter 1

General Introduction

1. INTRODUCTION

Cardiovascular disease (CVD) progression has increased dramatically over the last few decades with hypertension as one of the main risk factors in sub-Saharan Africa.[1,2] Obesity, physical inactivity, smoking, and alcohol overuse are also known modifiable risk factors - associated with lifestyle changes accompanying urbanisation.[1,3-5] Furthermore, obesity and low physical activity are also linked to non-alcoholic fatty liver disease (NAFLD), insulin resistance and type 2 diabetes.[6-8]

In South Africa, CVD is the second major leading cause of morbidity and mortality after human immunodeficiency virus (HIV) infection.[1] Among the factors that lead to the rise in mortality from hypertension and CVD is excessive alcohol consumption.[9-11] Alcohol abuse is also an important risk factor for liver injury,[11-13] stroke[14,15] and premature death.[16,17] Heavy drinking may also lead to high levels of HIV infection as alcohol abuse tends to be associated with multiple non-regular sexual partners.[18-21] In turn, HIV infected individuals may abuse alcohol as a coping mechanism in societies in which HIV infection is stigmatised, especially in low- to middle-income countries of sub-Saharan Africa.[22]

Most studies on CVD and alcohol intake in sub-Saharan Africa are cross-sectional. The paucity of longitudinal studies on the association between alcohol intake, hypertension and mortality has hampered the development of interventions that address problematic use of alcohol consumption and its role on cardiovascular outcomes. As a result, and as emphasized by others,[3,23] there is a growing need to perform prospective studies that aim at identifying and preventing the risk factors associated with CVD. With this thesis, I will attempt to increase our understanding of the long-term influence of alcohol abuse on CVD in South Africa. Hypertension and CVD are predicted to increase many fold in sub-Saharan Africa in the near future[13] and to help reduce this health burden, early identification of risk factors is crucial. In addition to high blood pressure (BP), liver damage and mortality risk, alcohol overuse has adverse effects on several organs of the body (Fig. 1.1).

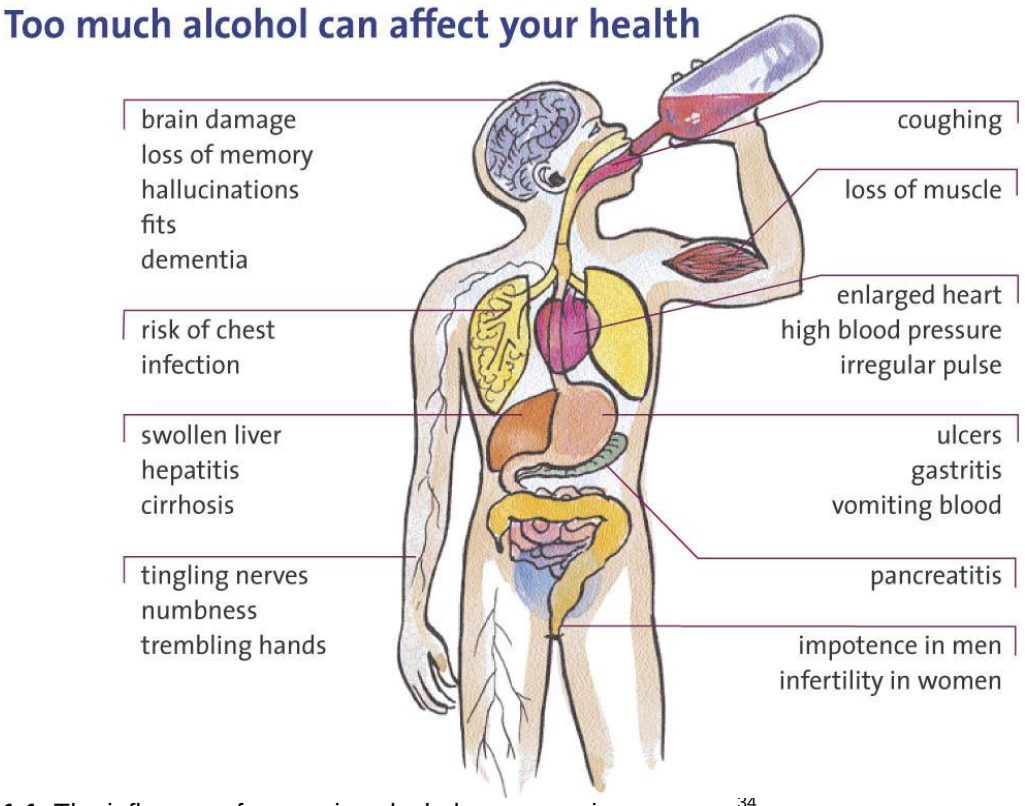


Figure 1.1. The influence of excessive alcohol use on various organs.[34]

2. PROBLEM STATEMENT AND MOTIVATION

Urbanisation and a westernised lifestyle in South Africa have shifted the dietary intakes of Africans from carbohydrate and dietary fibre to a diet consisting predominantly of increased fat and animal protein. A high fat and protein diet is associated with obesity – an important risk factor for CVD.[24] Urbanisation and dietary changes are generally accompanied by excessive smoking and alcohol consumption, which are also independent risk factors for hypertension in black South Africans.[10,25] Alcohol consumption is the fifth leading cause of death worldwide with increasing prevalence of excessive drinking in the developing world, especially in Africa.[26] Sub-Saharan Africa, which once had low rates of hypertension, currently has prevalences higher than those in the developed world; with alcohol consumption as one of the main contributing factors, with an average alcohol intake of 19.5 L per drinker.[10,27] Moreover, South Africa is known to have one of the highest prevalences of hypertension in the world.[28]

Heavy alcohol intake is also associated with cardiovascular outcomes such as stroke and mortality. A significant increase in stroke risk was observed in a large cohort study followed for over two decades on participants consuming alcohol excessively.[29] Stroke is more prevalent in low to middle income countries as poverty leads to poor knowledge that may lead to heavy alcohol intake, poor diet, limited access to health care and thus underdiagnosis of CVD – all serious risk factors for stroke.[5,15,30] Alcohol consumption, an important risk factor for stroke itself, is also closely linked to hypertension and diabetes, which in turn are important determinants of stroke.[15] Hypertension, earlier reported to be very high in South Africa, is the well known predictor of stroke and there is evidence of increasing prevalence of stroke at young age in sub-Saharan Africa.[3] Moreover, poor awareness and control of hypertension means that most people present with advanced stages of disease outcomes at diagnosis with severe complications.[3,28] This is further evidence supporting early identification of modifiabkle risk factors for CVD. The outcome of cardiovascular complications such as alcoholic heart disease and stroke adds to a high mortality rate. As a result, the life expectancy in sub-Saharan Africa is significantly lower when compared with the western world, perhaps also due to infectious diseases.[31,32]

This means that a low level of education, poverty, unemployment and dysfunctional health systems in the low resource settings of South Africa are some of the leading factors in the rise of alcohol abuse. As a result, CVD and health outcomes such as stroke and premature death become the eventualities of excessive alcohol consumption.[33,34] Poverty and unemployment are also linked to advanced disease progression at diagnosis due to lack of awareness and access to screening in low to middle income compared to high-income countries.[3] Interventions through education of the population at risk about the possible risk factors - smoking, high cholesterol, physical inactivity, insufficient fruit and vegetable consumption and excessive alcohol intake - is important in reducing the prevalence of CVD and associated fatal outcomes.[35,36] Whereas the increasing prevalence of hypertension, stroke and mortality in African population is well-reported, the actual contribution of alcohol abuse on these outcomes remains limited in black South Africans – a gap that needs to be filled in our understanding.

Although HIV infection will not form a specific focus of this thesis, it is important to understand the link between HIV and alcohol overuse, including the resultant impact on cardiovascular outcomes. The association of alcohol abuse and HIV infection is a major challenge in poverty-stricken sub-Saharan Africa.[37] HIV infection is associated with an increased inflammatory profile and disease progression in Africans.[22,38] The co-existence of alcohol abuse with HIV infection can hasten the onset of cardiometabolic disease especially in cases where there is compromised adherence to HIV treatment due to excessive alcohol intake.[39,40] Moreover, there is evidence that both alcohol overuse and HIV infection can be attributable to each other with serious consequences such as reinfection and risky sexual behaviour.[37] However, both low to moderate alcohol intake lead to favourable changes in both lipid and hemostatic profiles[41-42] improve endothelial function and plasma oxidant status,[43] and may possibly be associated with reduced cardiovascular risk and HIV infection.

The increasing prevalence of stroke and total mortality – largely associated with rapid urbanisation, poverty and dysfunctional health systems[1,44] – are some of the factors that provide calls for in-depth studies on the scientific aspects of alcohol intake in South Africa. The cardioprotective effects of moderate alcohol intake are based mostly on evidence from the developed world, with limited studies in sub-Saharan Africa.[45] The well-known J-shaped association of alcohol consumption with BP or mortality implies that the risk is low (lower than not taking alcohol) with beneficial effects when alcohol is consumed moderately while heavy use of alcohol increased the risk of both hypertension and mortality.[46] A linear association, on the other hand, indicates that the CVD risk increases steadily with increase in alcohol consumption.[47] Therefore, high alcohol consumption and its role in end stage CVD forms another motivation for this study.

Despite the well-known association of GGT with hypertension and mortality, ethnic-related GGT levels do impact on the influence of alcohol intake and cardiovascular outcomes. Africans are known to have high GGT levels[48,49] with evidence of high GGT not predicting hypertension development or linked to alcohol consumption in this ethnic group.[50] Given these findings, the

association of GGT and hypertension or mortality in this African study population will be explored, and whether GGT levels in this population are indeed alcohol-related.

The available literature on alcohol abuse in South Africa is limited to the effects of alcohol abuse on risky sexual behaviour, violence, crime, injury and dysfunctional family structures, leading to children leaving homes and staying in the streets.[51] To our knowledge there are no studies performed in sub-Saharan Africa on excessive alcohol intake with hypertension and mortality.

We made use of the South African leg of the multi-national Prospective Urban and Rural Epidemiology (PURE) study in which 2021 Africans were recruited at baseline from rural and urban areas of the North West Province. The central focus of this thesis is therefore to investigate alcohol intake in black South Africans and how it relates to cardiovascular health outcomes and mortality over a period of 5 years.

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Chapter 2

Literature Study

TABLE OF CONTENTS

1. INTRODUCTION

One of the main causes of the growing prevalence of cardiovascular disease (CVD) in sub-Saharan Africa is excessive intake of alcohol.[1-3] It is estimated that about 2 billion people in the world consume alcohol.[4] Historically, Africans lived a rural lifestyle with low prevalence of non-communicable diseases (NCDs). But excessive alcohol consumption is now a major risk factor for hypertension, stroke and mortality in South Africa.[1] With South Africa having one of the highest human immunodeficiency virus (HIV) infection in the world,[5] abuse of alcohol by HIV infected individuals promote disease progression and spread to the uninfected population.[6] Alcohol abuse in low resource settings is also associated with injury, violence, malnutrition and psychological distress.[7]

The influence of alcohol intake as a risk factor for CVD is well documented in the literature.[8-12] With regards to South African studies, this prospective study will shed light by investigating the association of alcohol overuse on blood pressure (BP) change, hypertension development, and mortality in black South Africans over a 5 year period. It is envisaged that this study will increase our understanding of the negative effects of excessive alcohol use on the cardiovascular system in the black South African population.

This chapter includes a detailed literature review which, in addition to the literature background in each of the manuscripts, forms the background information for data analyses and discussion of the results. Alcohol intake and CVD in South Africa are explored with the main emphasis on the detrimental effects of excessive alcohol use. This is followed by a discussion of the measures of alcohol use such as liver enzymes and self-reporting of alcohol intake. Finally, the general overview of alcohol-related elevation in BP, hypertension development and mortality are discussed.

2. CARDIOVASCULAR DISEASE AND ALCOHOL INTAKE OF BLACK SOUTH AFRICANS

Urbanisation and a western lifestyle in Africans have resulted in the growing prevalence of CVD.[13,14] Moreover, as a result of urbanisation, there is high prevalence of physical inactivity, obesity and smoking in black South Africans. Finally, the loss of social support and family disorganisation as a result of heavy drinking is evident leading to poverty and increased psychosocial stress among the black urban population.[15]

2.1 Alcohol intake in South Africa

Alcohol overuse is one of the main social, economic and health problems among black South Africans.[16] In addition to CVD, other adverse health consequences are liver damage, dysfunctional families, physical injuries, violent crime and loss of memory due to neuronal degeneration - all contributing to increased prevalence of premature death.[17-19]

Traditionally, Africans use alcohol as a form of entertainment at weddings, traditional ceremonies held for the spirit of an adult who had died, propitiation of ancestral spirits and graduation ceremonies following successful circumcision.[20,21] Extreme care was taken on who should drink and how much, leading to old males as the dominant drinkers over women and young people.[22-24] The rural existence, in which many people were mainly dependent on small scale farming, and living away from the cities, meant that few people could afford buying commercial brands of alcoholic drinks of high ethanol content. As a result, alcohol abuse did not exist and the prevalence of alcohol-related diseases was very low.[13] Generally, one of the main reasons for drinking is believed to bring about a change of mood in order to feel better, such as to relieve stress or cheer oneself up, especially in the African population.[25]

Alcohol abuse among Africans is traced back to the time of Dutch Settlers and French Huguenots as wine farmers in the Cape. Africans worked as labourers on these farms and were given wine as a top-up in addition to their low wages. Urbanisation and industrialisation in South Africa also

contributed to easy access to commercially available alcoholic drinks and invention of ways of brewing home-made brands faster.[26]

Today, the prevalence of alcohol consumption is higher in urban compared to rural areas. There is more alcohol consumption by the working class and male sex, and a growing prevalence of binge drinking in youth in South Africa.[27,28] This is largely due to affordability and availability of high ethanol content alcoholic drinks in urban areas.[29] Moreover, the number of informal liquor outlets, which function outside the boundaries of the South African Liquor Act of 2003, have increased dramatically in urban areas.[30] Additionally, poverty and unemployment have also been mentioned as further causes of alcohol abuse,[16,28] perhaps due to stress and anxiety caused by these factors.[31] While traditionally women did not abuse alcohol, there seems to be a rise in abuse of alcohol by this sex, especially at a young age and in most urban areas of sub-Saharan Africa.[32,33]

There is a strong association of alcohol abuse with violence, crime and risky sexual behaviour.[27,29] Violence is evident when the male partner is an alcoholic and becomes worse when the female partner is also abusing alcohol,[34] resulting to dysfunctional families and child maltreatment.[35] Arrestees of serious crimes such as rape, murder and house breaking have been found with high alcohol content in their blood.[9] Alcohol abuse and the resulting risky sexual behaviour, as discussed later in this review, is also responsible for the high incidence of traffic accidents, unwanted pregnancy and high HIV infection in South Africa.[36-38]

Given the highest rate of HIV prevalence in South Africa, alcohol abuse by HIV infected individuals is a major concern. Alcohol consumption is indeed a serious risk factor for hypertension and mortality in HIV infected people.[39,40] HIV infection is likely to lead to increased alcohol intake as a form of coping with the stress and stigma associated with HIV/AIDS,[41] especially in poverty-stricken populations. Moreover, risky sexual behaviour commonly reported in alcohol abuse may in turn be responsible for the high prevalence of HIV infection in sub-Saharan Africa.[36] Furthermore, since alcohol can adversely affect immune function, nutritional status and adherence to medication,[42-45] it is highly recommended that health care providers must

screen for unhealthy alcohol use in HIV positive patients as alcohol consumption can also interfere with hepatic metabolism and lead to hepatotoxity.[46]

2.2 Alcohol intake and cardiovascular disease

There is either a J-shaped (Fig. 2.1) or linear association between alcohol intake and CVD or mortality.[47-49] The J-shaped association implies a beneficial effect when alcohol consumption is low to moderate, and higher risk of CVD or mortality with excessive drinking. A meta-analysis of many longitudinal studies reported 10 000 deaths from over a million participants and confirmed a J-shaped association between alcohol and total mortality (Fig. 2.1).[47] The linear association, on the other hand, means that the beneficial effects are only observed during abstinence, and that high volume or quantity of alcohol is associated with increased risk of hypertension and mortality.[50] Most published studies[51-53] associate low levels of alcohol intake with cardioprotection and prevention against CVD. Moderate drinking is defined as one drink for women and two drinks per day for men (44 ml whisky, 148 ml wine or 355 ml of beer).[10]

In the South African context, alcohol consumption follows a linear pattern with a dichotomy between either no alcohol intake or heavy episodic drinking mainly on weekends and big traditional occasions.[9,54] The most prevalent age of binge drinking in South Africa is between 18 and 35 years by mostly men in urban areas.[54,55] Generally, a linear association of alcohol intake with BP seems to be more prevalent in men while in women a J-shaped relationship is more commonly observed.[56]

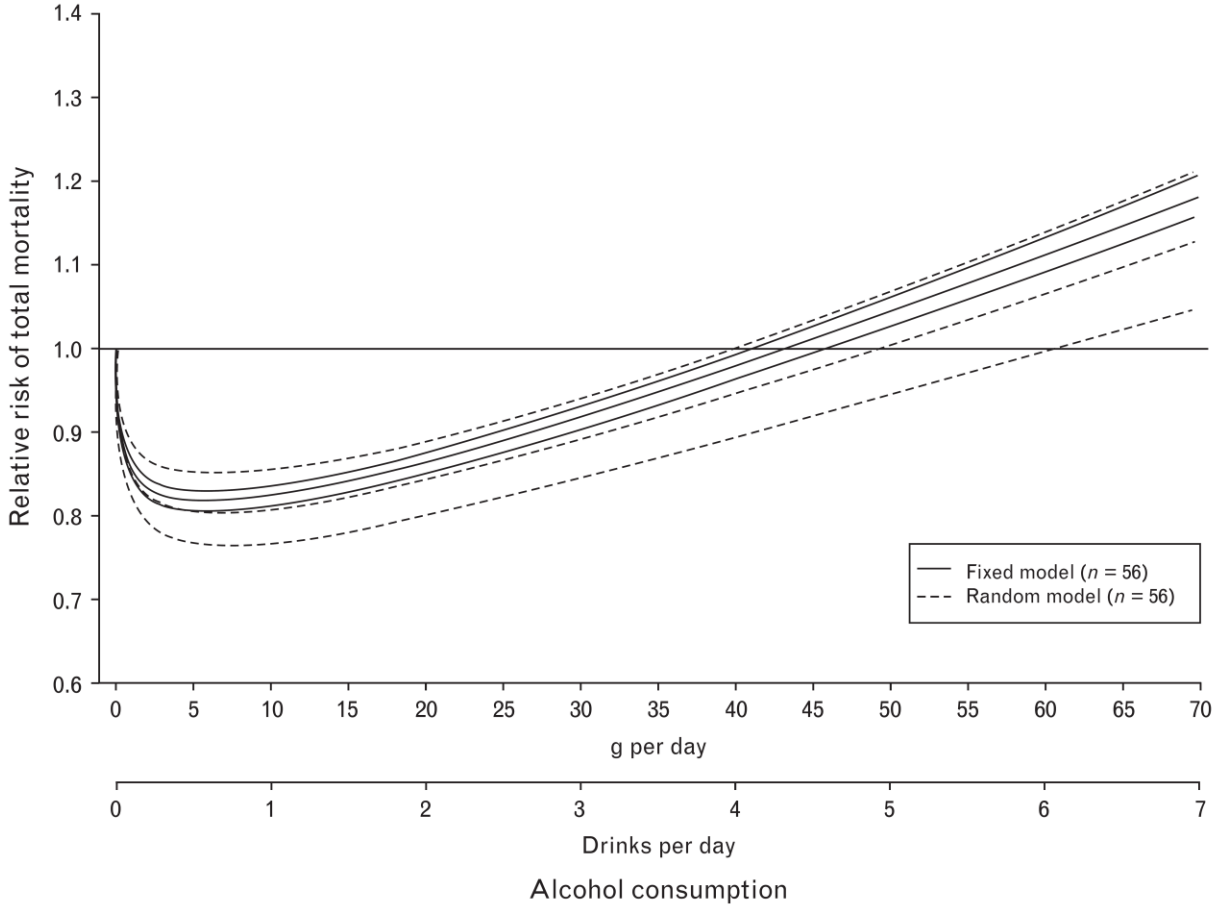

Figure 2.1. Relative risk of mortality with increased quantity of alcohol consumption.[47]

2.2.1 The beneficial effects of moderate alcohol consumption

The ways in which alcohol induces cardioprotection are wide-ranging and related to changes in serum lipids, lipoproteins, blood clotting proteins, platelets, inflammatory cytokines and insulin resistance.[57,58] Increased levels of high-density lipoprotein cholesterol (HDL-C) and decreased low-density lipoprotein cholesterol (LDL-C) are associated with reduced risk of CVD, and HDL-C are elevated with increased alcohol intake.[59,60] The mechanism by which HDL-C reduces the risk of CVD lies in the prevention of atherosclerosis progression. HDL-C protects against atherosclerosis by inhibiting LDL-C oxidation, preventing foam cell formation as well as play a role in reverse cholesterol transport and diminishing the formation of tissue factor.[17,61] Alcohol-related HDL-C increase is also important in its anti-inflammatory action. Alcohol tends to interfere with the activity of pro-inflammatory factors although the actual role of HDL-C in reducing the risk of CVD in alcohol consumption is not fully understood.[62]

Alcoholic beverages such as red wine have a specific advantage by lowering oxidation of LDL-C,[63,64] preventing atherosclerotic plaque formation through their polyphenol content.[65,66] Furthermore, formation of phosphatidylcholine (PEth) following alcohol intake increases HDL-C binding to endothelial cells eventually increasing vascular endothelial growth factor (VEGF), which protects against atherosclerotic plaque formation.[67] Alcohol intake increases apoA-I levels and paraoxonase (PON)-1 activity - antioxidants related to HDL-C. However, these antioxidants seem to increase on moderate alcohol consumption but not with heavy alcohol intake.[68] Moderate alcohol intake further reduces platelet aggregation, fibrinogen levels and increase fibrinolysis.[53,61,69] Moderate drinking also improves insulin sensitivity. The proposed mechanism of improved insulin sensitivity is that ethanol enhances the synthesis of adenosine monophosphate (AMP) which stimulates protein kinase. Protein kinase in turn stimulates the synthesis of long-acting proteins that boost insulin sensitivity.[61,70-73]

In conclusion, although it is generally known that moderate drinking is beneficial, some studies attribute this benefit only to individuals at increased risk for CVD, to older subjects or to those with poor nutritional status.[74,75] The French paradox is the phenomenon of a low CVD risk seen in French population despite a diet high in saturated fats and increased alcohol consumption.[48] It remains to be seen whether this well-known paradox is applicable to Africans.

2.2.2 *Harmful effects of chronic alcohol overuse*

According to the World Health Organisation (WHO),[8] alcohol-related death is more prevalent in developing countries.[76] Excessive alcohol consumption does not only result in loss of cardioprotective effects shown by moderate drinking, but is also linked with myocardial dysfunction, hypertension development, coronary artery disease (CAD), cardiomyopathy, atherosclerosis and stroke.[51,77]

Alcohol intake, endothelial function and oxidative stress

Alcohol abuse results in cardiomyocyte damage through several mechanisms that include oxidative stress, changes in calcium handling and mitochondrial dysfunction. With increased alcohol intake oxidant chemicals are elevated, leading to depletion of antioxidants such as

glutathione (GSH), enhancing myocyte susceptibility to oxidant injury.[78] Alcohol abuse also damages contractile proteins, interfere with calcium signalling, reduces myofiber calcium sensitivity, – promoting calcium overloading, which results in apoptosis, necrosis, and reduced contractile function.[78] Chronic alcohol intake can lead to sudden cardiac death (Fig. 2.2).[79,80] Potential alcohol-related mechanisms include an increased QT interval, leading to ventricular tachyarrhythmia, electrolyte abnormalities, sympathoadrenal stimulation and decreased vagal input.[81,82] Moreover, alcohol-induced reactive oxygen species (ROS) impair myocyte calcium handling and cause hypertrophy, alter gene expression and induce apoptosis.[83,84] Apoptosis may be caused by increased calcium influx in the mitochondrion, which triggers caspase activation and the apoptotic cell death pathway.[10]

The vascular endothelium is also the target of many alcohol-induced harmful substances leading to its dysfunctional state. Alcohol regulates endothelial cell genes involved in inflammation.[85] ROS produced as a result of alcohol abuse react with nitric oxide leading to reduced vasodilation and this may also contribute to high BP seen in chronic alcohol abusers.[10,85] High gamma-glutamyltransferase (GGT) levels associated with alcohol abuse seem to be followed by oxidative stress, similarly to that seen in obesity.[86,87] Excess fat deposition in the liver as a result of excessive alcohol intake increases ROS such as peroxides, leading to overconsumption of GSH with a compensatory increase in GGT synthesis.[11,88]

Alcohol intake and blood pressure

Regular alcohol overuse is associated with higher BP and the development of hypertension,[89] while acute intake seems to lower BP through vasodilation.[90] The mechanisms of alcohol-induced hypertension include the potentiation of the renin-angiotensin –aldosterone system (RAAS). Alcohol abuse leads to high angiotensin II levels and increased cardiac expression of angiotension type I receptors.[91] Alcohol intake leads to weight gain which in turn varies directly with BP.[92] Alcohol abuse is also associated with increased psychological stress and poor coping mechanisms, which are also risk factors for hypertension.[7,93] Results from the large cohort INTERSALT study[94] indicated that after adjusting for body mass index (BMI), smoking and

urinary excretion of sodium and potassium, systolic blood pressure (SBP) continued to increase with the volume of alcohol intake. Moreover, a population-based survey has indicated a positive association between alcohol consumption and BP in current non-hypertensive drinkers.[95]

Alcohol intake, target organ damage and cardiovascular events

Excessive alcohol intake is known to affect several organs directly, including the liver, brain, heart and kidneys – partially via the effects on the cardiovascular system. Although evidence exists that moderate drinking protects against stroke and myocardial infarction, people who use alcohol excessively have an increased risk of stroke.[96,97] It seems that moderate intake of alcohol protects against ischemic but not hemorrhagic stroke, which is more closely associated with heavy drinking.[12] Although there is no clear mechanism linking moderate drinking and reduced stroke risk, several pathways that are likely to play a role include an improved lipid profile, reduced insulin resistance and favourable coagulation activity.[12] The excessive alcohol consumption related to stroke may be mediated by an alcohol-induced increase in BP and may also interfere with normal blood clotting processes resulting in bleeding with the likely onset of hemorrhagic stroke.[98,99] Kolapo and colleagues[100] reported that excessive alcohol intake is a strong predictor for stroke, and that alcohol-induced stroke incidence seems to increase with age. Although the prevalence of stroke seems lower in low-income than in high-income countries, the disability resulting from stroke is as prevalent as in developed countries.[101]

The best method of reducing the risk of stroke is by modification of the risk factors such as reducing BP and the alcohol intake.[102] Alcohol consumption was independently associated with a high-estimated 10-year stroke risk in a large cohort study in Thailand in which most participants affected were from a low-socio economic background.[103] The association of stroke with excessive alcohol intake, high smoking levels and male sex prevalence is confirmed by other studies.[100,103,104]

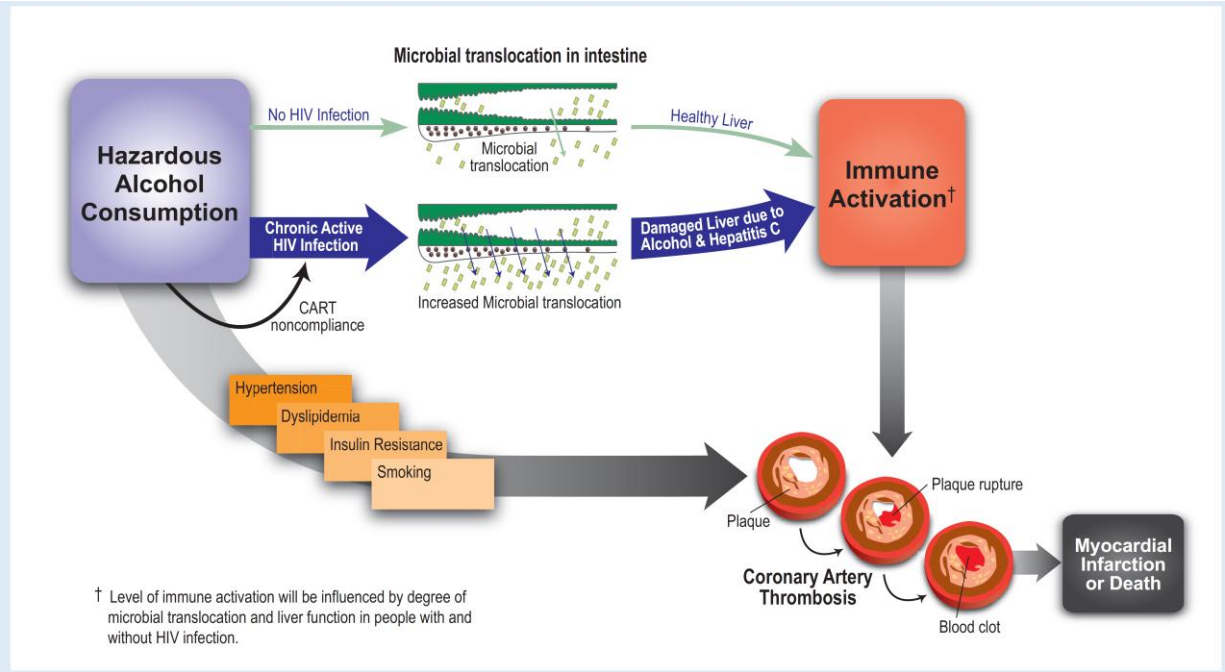

Figure 2.2. The association between excessive alcohol intake, HIV infection and cardiovascular disease.[105] HIV, human immunodeficiency virus; CART, combination antiretroviral therapy

The association of coronary heart disease (CHD) and alcohol consumption is strongly related to the quantity and pattern of drinking – another example of a J-shaped pattern.[77,106] These findings show pathways through which moderate drinking contributes to reduced risk of CVD and also support that excessive alcohol intake is linked to an increased risk of myocardial infarction or death. This seems more evident in HIV infection especially in cases in which treatment is not taken regularly (Fig. 2.2). As indicated earlier, the positive association of HDL-C with alcohol consumption is beneficial, whereas elevation of HDL-C in alcohol abuse is linked to the risk of CVD. Other lipids such as triglycerides (TG) and LDL-C, together with BP, are elevated in heavy drinking, increasing the risk of heart attack.[107] Inflammatory markers, including C-reactive protein (CRP) are elevated in alcohol abusers and are important risk factors for CVD.[105] Alcohol abuse is implicated in cardiomyopathy. Structural and functional changes occur in contractile proteins and intracellular organelle damage of the myocytes occurs, which leads to changes in heart contractility and ventricular dysfunction (Fig. 2.3).[108,109] Alcohol abuse also damages the nerves supplying the heart and blood vessels and may thus alter BP towards higher values.[110] In addition

to pattern and quantity of drinking, the potential damage to the heart related to alcohol abuse seems to vary with population, individual, or genetic and environmental factors.[111]

The damaging effects of alcohol on the kidneys could also raise BP as damaged kidneys exert pressure on the arteries.[112] Excessive alcohol consumption is one of the serious risk factors for kidney disease, contributing to over 1.4 million cases of renal replacement therapy in the world.[113] High GGT levels were found in smokers with chronic kidney diseases and this was closely associated with alcohol consumption.[114]

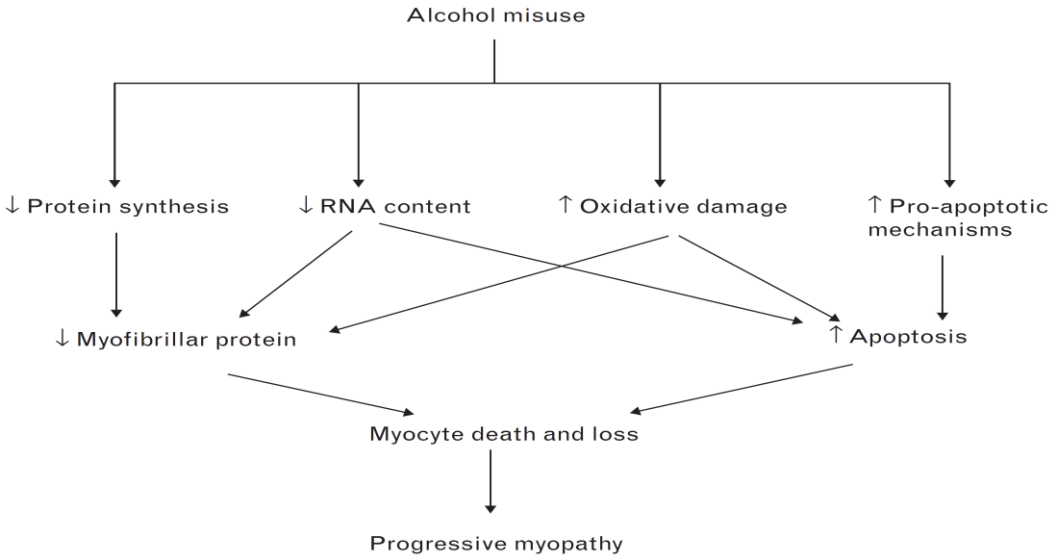

Figure 2.3. Pathophysiology of alcohol abuse in CHD.[115] CHD, coronary heart disease; RNA, ribonucleic acid.

Alcohol intake and mortality

Excessive alcohol intake is an independent risk factor for mortality. The known biomarkers of alcohol consumption have been found to have positive associations with cardiovascular[40,116,117] and all-cause mortality.[40,118,119] A large cohort study reported interesting results in which high alcohol consumption and low physical activity were independently associated with both cardiovascular and all-cause mortality. They further indicated that moderate drinking and high physical activity correlated positively with reduced risk of total mortality.[120] This confirms the well-known J-shaped association of alcohol consumption with both cardiovascular and all-cause mortality. Similarly, heavy drinking and smoking have increased risk of mortality, while the

opposite is associated with reduced mortality risk,[121] with moderate drinking associated with reduced mortality in comparison with non-drinking.[122]

2.2.3 HDL-C and excessive alcohol intake

Despite the well-known beneficial association of alcohol intake with elevated HDL-C and reduced risk of CVD in moderate drinking, a further rise of HDL-C increases CVD risk. Increases in BP and inflammation that normally follow high HDL-C appear to predominate during excessive alcohol intake.[62] While moderate alcohol intake raises the levels of smaller particle-sized HDL-C, associated with reduced risk of CVD, heavy drinking is associated with high levels of larger HDL-C particle size.[123,124] Contrary to this, some recent findings support the association of small-sized HDL-C particles with heavy alcohol consumption,[125,126] suggesting that both small and large-sized HDL-C particles are potential risk factors for CVD. Therefore, elevated HDL-C which is known to be beneficial in moderate alcohol consumption may also have detrimental cardiovascular effects based on the HDL-C particle size. These negative outcomes may also be influenced by genetic and metabolic factors influencing the HDL-C particle size.[126]

The mechanisms of the beneficial effects of HDL-C on moderate drinking have been discussed already in this review (p.18). Alcohol abuse may lead to the rise in certain components of HDL-C including polyunsaturated fatty acid content of HDL-C. This increases HDL-C particle fluidity that may significantly alter various HDL-C functions,[127] possibly resulting in reduced benefit of HDL-C. Moreover, production of fatty acid ethyl esters (FAEEs) as a result of heavy drinking promotes fibrosis or hepatic damage.[62,127] Although contradicting evidence exists regarding the genetic modulation of HDL-C response to alcohol intake, black and white people seem to have similar alcohol dose responses for increases in HDL-C and apo A-I levels.[128]

Finally, in addition to HDL-C, LDL-C is another well-known lipid marker generally reduced in moderate alcohol intake.[129,130] However, elevated LDL-C levels are seen in individuals with high alcohol consumption.[50,131] Excessive alcohol intake brings about several unfavourable changes in hepatic cholesterol metabolism that finally lead to decreased extracellular signal-related kinase

activation in the liver, leading to elevation of LDL-C.[62] Genetic factors, explained in detail elsewhere,[131] seems to be the main influence in these discrepancies.

2.2.4 Body composition and alcohol consumption

Excessive alcohol use is generally linked to lean body mass while increased weight gain is associated with moderate to low intake of alcohol.[78] Body composition also depends on the type, quantity and volume of alcohol consumed. Furthermore, alcohol increases the levels of orexin in the brain, which in turn stimulates excess fat intake, increasing body weight and finally elevating TG levels.[132] There is generally a U-shaped relation between alcohol intake and TG levels, and alcohol consumption in Africans and in men seems to lead to elevated TG levels.[128] There is also evidence of increased food intake during episodes of alcohol consumption.[133,134] Alcohol intake can also cause dyslipidemia, increased fat mass, and disrupt adipokine profiles such as leptin and adiponectin.[135]

Underweight as a result of alcohol abuse may be caused by decreased food consumption or malabsorption resulting in decreased micronutrient availability and changes in tissue growth factors.[136] Moreover, decreased muscle protein synthesis, accelerated muscle proteolysis, increased amino acid oxidation, decreased circulating levels of androgens and insulin-like growth factor-1 (IGF-1) and upregulation of myostatin are factors that lead to decreased body mass seen as a result of excessive alcohol consumption.[78] Alcohol abuse is also associated with altered bone metabolism, decreased bone mineral density and an increased risk of fractures,[137] contributing further to lean body mass. Decreased body mass in chronic alcohol intake is also due to altered neuroendocrine function resulting in increased cortisol release.[138] Lean alcohol users are at serious increased risk of stroke. The elevated BP as a result of alcohol abuse damages and constricts the microvasculature in the brain, depriving the brain cells of oxygen and nutrients increasing the risk of stroke.[139] Finally, it is important to note that genetic and environmental influences are the main factors that determine body composition in alcohol consumption.[78]

2.3 Measures of alcoholic intake

In order to assess alcohol consumption, numerous biomarkers of alcohol intake are described in the literature and include ethanol, GGT, aminotransferases, mean corpuscular volume (MCV) and percentage carbohydrate-deficient transferrin (%CDT).[140] Recently new biomarkers such as ethyl glucuronide (EtG), ethyl sulphate (EtS) and phosphatidylethanol (PEth) have also been identified.[141] These alcohol biomarkers differ from each other in sensitivity, specificity, reliability, and half-life in the blood.[142] Although screening alcohol intake by means of a questionnaire proves beneficial because of low cost and increased sensitivity, biomarkers can be useful for detecting unhealthy use of alcohol among those who are not honest in their reporting of alcohol use.[46]

2.3.1 Self-reported alcohol intake

Biochemical markers of alcohol intake are generally known to be better estimates than self-reporting of alcohol. However, self-reported alcohol intake is an easier and cheaper method to assess alcohol intake. The potential inaccuracy associated with self-reporting of alcohol consumption can lead to either under- or overestimation of alcohol reporting.[143-145] This is especially true when comparing populations from different ethnical backgrounds with differing social perceptions of alcohol consumption. Self-reporting of alcohol use has, in many instances, been used with laboratory biomarkers to detect alcohol usage. In addition to prevailing attitudes towards drinking among the reporting subjects, questionnaire surveys require good skills in pursuing clinical interviews, and are time consuming.[146,147]

Self-reporting investigation tools to screen alcohol abuse include general health questionnaires with yes/no responses and questions on volume, type of alcohol, frequency and duration of drinking. The Alcohol Use Disorders Identification Test (AUDIT)[148] has demonstrated good returns of both sensitivity (86%) and median specificity (89%) in various studies.[147] It also displayed a good pattern of drinking in South Africa, in which men, participants with low level of education and staying in urban residences were more likely to report current alcohol consumption.[54] Moreover, CAGE questions and the Michigan Alcohol Screening Test (MAST) are

often used as tools for assessing problem drinking.[149] A validated quantitative food frequency questionnaire (QFFQ) is also widely used and has a low rate of false positive responses[150] although a low level of accuracy has often been detected.[143] The validity of the QFFQ was reported in a Japanese study in which nutrient intake of each individual was assessed[151]– has also been validated in South Africa.[150-153]

A simple self-administered questionnaire can provide useful estimates of alcohol intake over an extended period of time.[154] Self-reporting of alcohol intake showed a significant association between alcohol intake and CRP[155] or BP.[156] Lee et al[157] also showed that self-reporting showed a positive association with biochemical markers when they compared the validity of an 8-item semiquantitative food frequency questionnaire (SFFQ) with four spot urine samples collected over a 12-month period in a dietary isoflavone assessment validation study. The strong association between self-reporting of alcohol intake with biomarkers indicating alcohol use such as GGT and %CDT by some large cohort studies[156,158] confirmed the honesty and reliability of reporting alcohol use. Finally, the use of self-administered questionnaires only without validation by alcohol intake biomarkers by Sasaki and colleagues,[159] successfully showed the significant association between alcohol consumption and arterial stiffness.

Some authors have however reported that questionnaire data on alcohol intake does not always correspond to alcohol biomarkers as underreporting is common among alcohol abusers, especially in high socio-economic populations.[146,160,162] GGT prevailed as the only independent predictor of CVD[163] and stroke[164] when compared with self-reported alcohol intake in Chinese and Finnish populations, respectively. This casts doubt on the reliability of self-reported alcohol intake especially in adults in the high income bracket, suggesting that self-reported alcohol intake may be influenced by socio-economic status and level of education.[165]

Other self-reported estimates of alcohol such as volume and frequency of alcohol intake may perhaps be more difficult to report on in populations from low resource settings, mostly with a low level of education.[165] Increasing the number of items (more food items or alcohol types) in a questionnaire might result in an overestimation of the intake, whereas combining several similar items into a single item could lead to underestimation.[165]

2.3.2 Ethanol

Ethanol is measured in blood, breath or urine samples and is more suitable for use in subjects where alcohol intoxication is suspected.[142] It is rarely used to investigate long-term alcohol abuse because of its short half-life, although increased tolerance of ethanol in some people may give an indication of alcohol dependence.[166] Ethanol measurements are specific, simple to perform and therefore suitable for screening. They are also useful in follow-up studies to assess non-compliance for abstinence.[140] Moreover, FAEEs, minor ethanol metabolites that can accumulate in hair, are useful in the assessment of chronic alcohol consumption.

In a recent study[167] FAEEs reached specificity levels of 87% in assessing excessive drinking when the identified false positives were excluded from data elaboration. FAEEs are also found in the liver and adipose tissue, and have been useful as postmortem markers of premortem ethanol intake. Ethanol is also a suitable marker for detection of alcohol overuse in pregnancy because of high specificity and a long detection window in the matrix hair.[168] Ethanol and other biomarkers such as serotonin metabolites and sialic acids have limited use despite their good diagnostic characteristics. Their association with hypertension or mortality in clinical practice is sparse due to the costly equipment necessary for their measurement.[169] Some studies have investigated the association between ethanol and traumatic brain injury where alcohol intoxication contributed significantly to high prevalence of traumatic brain injury.[170,171] However, the presence of ethanol in the blood of patients with traumatic brain injury associated significantly with higher survival of these patients from their injuries.[171] Further research is recommended to validate potential therapeutic implications of this neuroprotective role of ethanol.[172]

2.3.3 Mean corpuscular volume (MCV)

Mean corpuscular volume (MCV), the determination of the red blood cell size, is a useful screening procedure for the detection of chronic alcohol abuse.[173] MCV is more sensitive in women,[140] has stronger correlation with self-reported drinking behaviour and has more specificity than GGT in most populations.[174] MCV responds slowly to abstinence, and its normalisation may

require 2 to 4 months.[142] The specificity of MCV is, however, decreased by vitamin B_{12} deficiency, liver disease, hematological disease, hypothyroidism, or reticulocytosis.[140,142]

Alcohol increases the MCV by interfering with cell structure and metabolism. Moreover, ethanol has a direct bone marrow toxic effect, causing reduced bone marrow cellularity and vacuolisation of red cell precursors.[175] Acetaldehyde, an important metabolite of ethanol, has been found inside red blood cells of many alcoholics, in which it forms adducts with proteins and other cell constituents, thereby damaging and reducing the half-life of these cells.[176,177] Occult bleeding is associated with alcohol dependence. As a result, bleeding or hemolysis introduces young red blood cells, reticulocytes, with large cell volume.[140] In addition to high MCV, excessive ethanol consumption may be determined through blood cell counts,[178] mean corpuscular haemoglobin (MCH) and low platelet count,[178] and the occurrence of sideroblastic anemia.[179] However, MCV was found to be the least reliable marker of alcohol consumption in one study in which all the alcohol biomarkers were compared.[180]

2.3.4 Aminotransferases (ALT and AST)

Alanine transaminase (ALT) and aspartate transaminase (AST) are two well-known liver enzymes that are sensitive indicators of liver injury.[140] ALT catalyses the transamination between L-alanine and *α*-ketoglutarate to form pyruvate and L-glutamate. AST, catalyses the following reaction: L-aspartate + 2-oxoglutarate ⇋ oxaloacetate + L-glutamate.[181] However, the correlation between the degree of liver damage and these enzymes is generally poor.[182] These aminotransferases are less sensitive and show poor specificity, particularly AST, which can be found in various organs outside the liver.[140,183]

Although these liver enzymes are suitable for the assessment of chronic intake of alcohol, their correlation with alcohol consumption is generally weak, and rather non-specific.[140] However, an association between AST and chronic alcohol intake does exist in adults.[158] ALT and AST are more sensitive to hazardous drinking patterns than volume of alcohol intake.[184] As a result, some authors suggest AST:ALT ratio as a better assessment of alcohol consumption.[140] A ratio of >2 is considered to be highly suggestive that alcohol is the cause of the patient's liver injury. In their

study, Nyblom and colleagues[185] concluded that an AST:ALT ratio below 2 may be indicative of high alcohol consumption but without severe liver disease - confirmed by Kazemi-Shirazi et al.[186] An AST:ALT ratio of <2 is also associated with non-alcoholic steatohepatis (NASH).[187-189] Africans have high levels of liver enzymes, and it is unclear whether this is due to genetic or environmental influences.[183]

Both AST and ALT, despite being known markers of liver injury, are also linked with CVD risk. For example, association of ALT with endothelial dysfunction, and its positive association with CRP has been reported.[190] ALT showed a significant association with CHD after adjustment for the other components of the metabolic syndrome and traditional risk factors.[181] There is evidence that ALT is associated with GGT, markers of inflammation and oxidative stress - all risk factors for CVD.[191,192] Finally, elevated ALT was shown to be independently associated with increased CVD in a study by Yun and colleagues[193] and they suggested that ALT may serve as a surrogate predictor of CVD among the Korean population.

2.3.5 Gamma-glutamyltransferase (GGT)

GGT, a liver enzyme widely used as a marker of excessive alcohol intake, is a glycoprotein enzyme that catalyzes the hydrolysis of gamma-glutamyl bonds in glutathione.[194] GGT is found mainly in the liver but is also produced by the kidneys, heart and the spleen. Glutathione, the substrate for GGT, is the most abundant antioxidant molecule in cells, stores and transports nitric oxide, protects cells against oxidative stress, and is also involved in the detoxification of harmful compounds in the liver.[195] The sequence of human GGT contains seven N-glycosylation sites with four cysteines likely to be involved in the formation of disulphide bonds. The enzymatic reaction of GGT on GSH involves the cleavage of gamma-glutamyl bonds and the transfer of gamma-glutamyl moieties to either water to form glutamate or to amino acids, forming new gamma-glutamyl compounds (Fig. 2.4).[195]

The positive correlation between alcohol consumption and serum GGT is well established.[140,156,158,196] But GGT levels are also generally higher in men,[197] in obesity and old age,[198,199] and elevated in non-alcoholic fatty liver disease (NAFLD).[200] Evidence exists that

alcohol intake has an independent association with elevated GGT levels.[58,196] As with other liver enzymes, Africans show higher GGT levels than other ethnic groups.[201] Black South Africans consistently showed higher levels of GGT than whites in various studies by our group.[202,203]

Shankar et al.[204] found a positive association between GGT and peripheral arterial disease in Caucasian men but not in women or Afro-American men. This suggests that association of GGT and cardiovascular markers may also be sex and ethnic specific. Factors such as CRP, markers of oxidative stress and glycosylated haemoglobin (HbA1c) are reportedly higher in Africans and possibly cause elevation of GGT levels independent of alcohol intake.[204,205] The clinical value of GGT in the assessment of excessive alcohol intake could thus be markedly improved if these confounders are more carefully controlled. GGT correlates positively with BP,[163] obesity, diabetes mellitus, dyslipidemia and fibrinogen levels while a negative correlation exists with antioxidants (Fig. 2.5).[157] GGT is also considered to be proatherogenic in atherosclerotic CVD[116] and found with lipoproteins in atherosclerotic plaques.[142]

Gamma-glutamyltransferase and non-alcoholic fatty liver disease (NAFLD)

GGT has low specificity for alcohol intake and is influenced by several other factors as mentioned earlier.[199] NAFLD is among the well-known factors that associates with high GGT levels, independent of other confounding factors. NAFLD refers to a spectrum of diseases of the liver ranging from fat infiltration (steatosis) to NASH, characterised by inflammation and necrosis.[206] NASH is therefore the advanced stage of NAFLD as alcoholic steatohepatitis (ASH) is the advanced form of alcoholic fatty liver disease (AFLD).[207] These conditions are increasingly becoming apparent because of their association with obesity and diabetes mellitus,[208] and while both sexes are affected,[209] African women seem to be at more risk in sub-Saharan Africa because of the highest prevalence of obesity, type 2 diabetes and metabolic syndrome in this population group.[210-212] The highest rates of obesity were found among the least educated and poverty-stricken populations.[213]

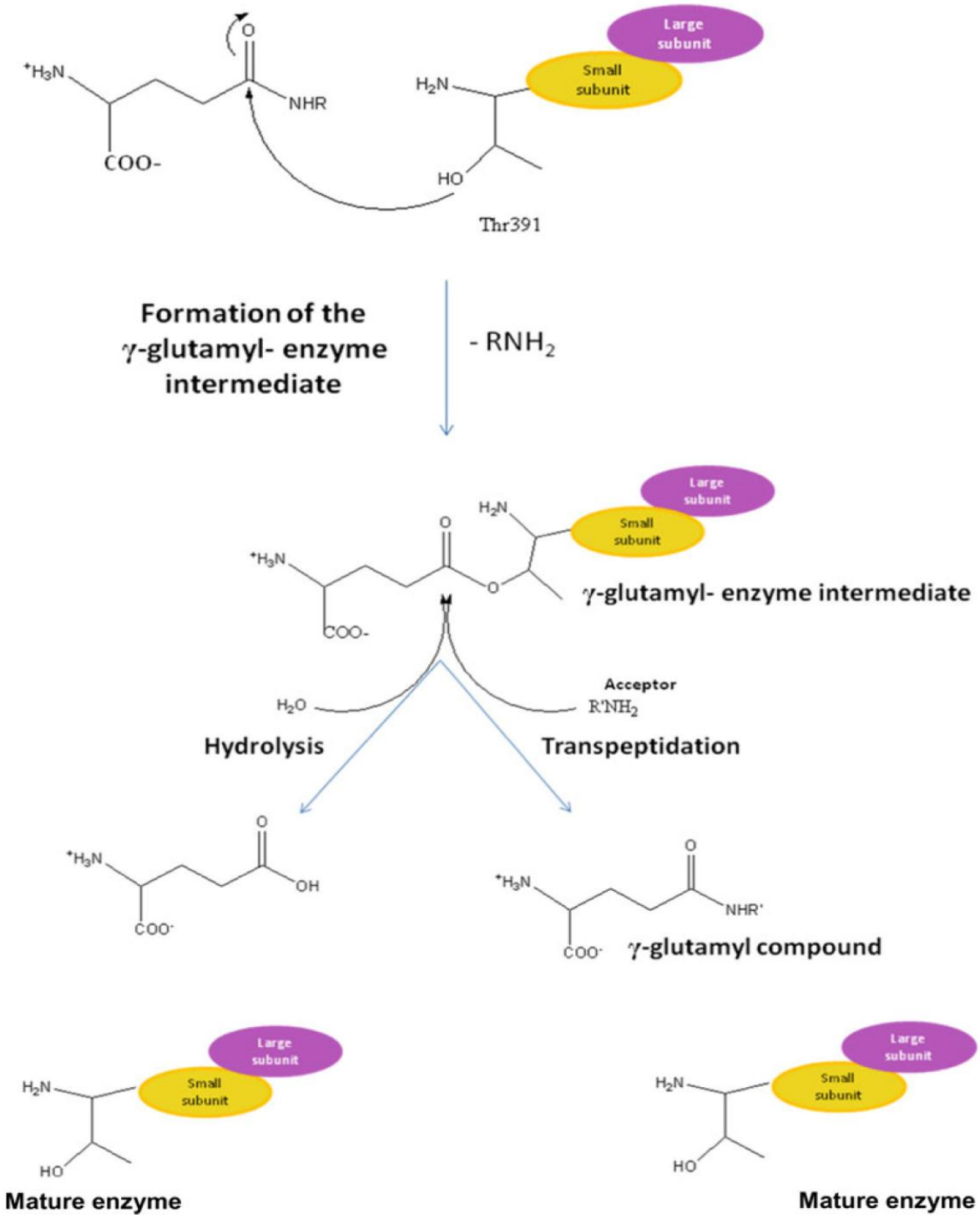

Figure 2.4. Proposed reaction mechanism of GGT on glutathione.[195] GGT, gamma-glutamyltransferase

Ethnically, Africans are the least affected in American-based studies[214-216] perhaps due to their genotype of the gene that predisposes NAFLD,[217,218] or as a result of underdiagnosis.[219] Similar findings have been confirmed in a recent South African study.[220] Another likely reason for the low

prevalence of NAFLD may be the more subcutaneous to visceral fat profile and also a different lipoprotein metabolism in Africans.[221] Subcutaneous adiposity is associated with a 'healthy' adipose tissue while visceral obesity is linked to a dysfunctional adipose tissue.[222]

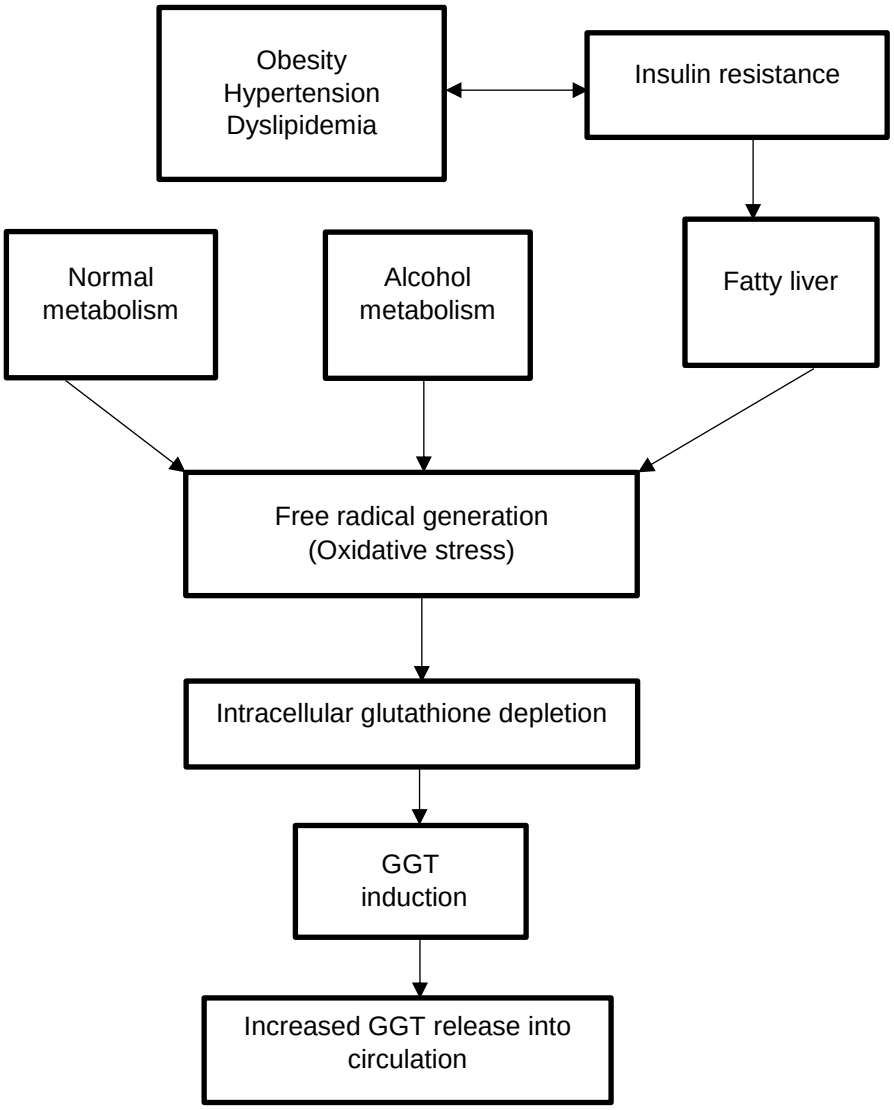

Figure 2.5. The relationship of obesity, fatty liver and alcohol intake with GGT and oxidative stress.[88] GGT, gamma-glutamyltransferase

The close association of NAFLD with obesity, other components of the metabolic syndrome and other risk factors is well documented (Fig. 2.6).[208,216,223-225] The 'two hit' hypothesis in the progression of NAFLD involves accumulation of TG in hepatocytes (simple steatosis) followed by uncontrolled production of cytokines leading to oxidative stress and necrosis, characteristic of NASH.[226] This observation is relevant in South Africa in which more than 40% of the adult population is overweight or obese with a very high unemployment rate.[210,227] This suggests that

NAFLD and obesity may be common and yet underdiagnosed in low resource settings in sub-Saharan Africa. Obesity is closely associated with insulin resistance, which in turn leads to NAFLD or NASH, characterised by high GGT levels in the absence of alcohol consumption.[228]

Liver enzymes are elevated in NAFLD. The AST:ALT ratio, one of the better indicators of NAFLD diagnosis, is usually less than 2.[207,224] Amongst the components of the metabolic syndrome, insulin resistance seems to be the main contributor to NAFLD. Insulin resistance, like obesity, leads to increased deposition of fatty acids in liver cells.[215] The inflammatory response that results from fat deposition may lead to progression of cirrhosis and CVD.[229-231]

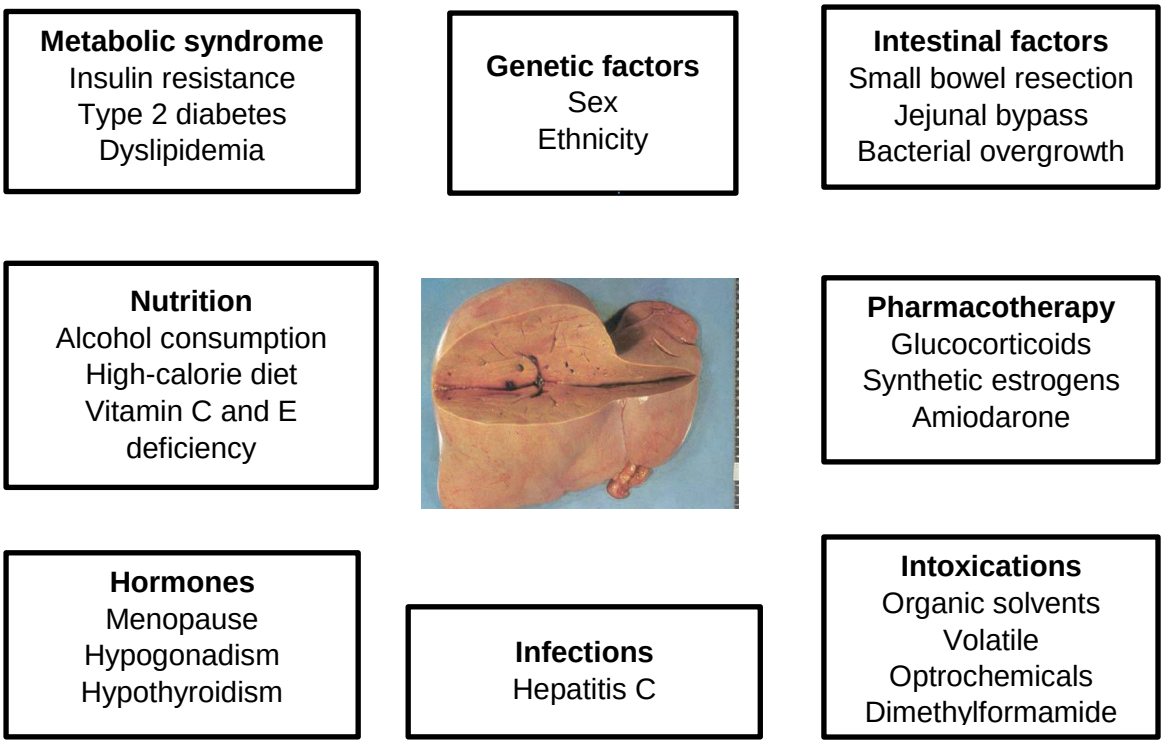

Figure 2.6. Some of the risk factors for fatty liver disease.[216]

The close association of NAFLD with insulin resistance, the metabolic syndrome and lipid profiles is a clear indication that NAFLD is a risk factor for CVD. Moreover, fatty liver, one of the main symptoms of NAFLD, is associated with increased levels of fibrinogen, CRP, plasminogen activator inhibitor 1 (PAI-1) - all established risk factors for CVD (Fig. 2.7).[232,233] NAFLD or NASH-related mortality from coronary events is well documented[234-237] leading to the assumption that

the leading cause of death from NAFLD or NASH is CVD rather than liver damage.[235] This association suggests strongly that NAFLD is an important marker of CVD.[200]

Another concept worth mentioning is the association of serum iron and uric acid with liver injury. Fasting serum ferritin and transferrin-iron saturation are elevated in NAFLD and linked to fat accumulation in the liver.[238] Iron overload is also known to be associated with diabtetes, obesity and metabolic syndrome, which have an important link with NAFLD.[239,240] While studies have reported on positive association between adiposity and iron deficiency,[241] high serum ferritin concentrations have also shown an inverse association with body weight.[242] Iron overload is implicated in AFLD and seems to reduce the activation of adenosine monophosphate protein kinase associated with ethanol.[196] The consumption of traditional beers brewed in iron pots seem to contribute to iron overlaod in sub-Saharan Africa.[243] High serum iron levels are also linked to oxidative stress[244] and severity of atherosclerosis.[245] Uric acid on the other hand, is elevated in obesity and is associated with hypertension,[246,247] with evidence of high uric acid levels in Africans.[240]

Finally, NAFLD is known to improve dramatically following early detection accompanied by appropriate lifestyle changes such as weight loss and exercise.[224] Given that the most reliable form of diagnosis is an invasive and expensive liver biopsy,[206] education of the population about risk factors and change of lifestyle will prove beneficial in sub-Saharan Africa. However, in order to achieve this, prospective studies that assess the long-term natural history of NASH with validated biomarkers are urgently needed. This will also help to determine whether or not NAFLD carries independent risk beyond the traditionally-considered cardiovascular risk factors.

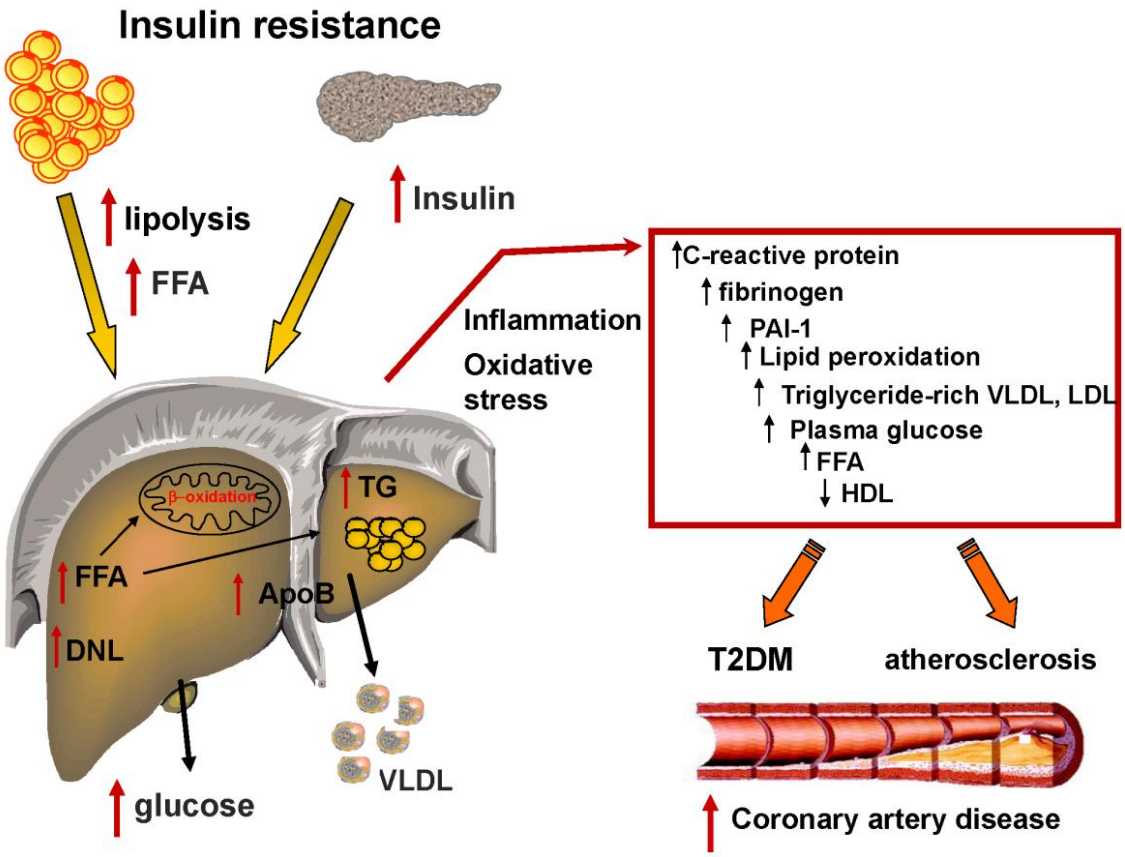

Figure 2.7. Mechanisms that link metabolic syndrome, NAFLD and markers of CVD.[233] FFA, free fatty acids; PAI-1, plasminogen activator inhibitor-1; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; T2DM, type 2 diabetes mellitus; TG, triglycerides; DNL, de novo lipogenesis

2.3.6 Carbohydrate deficient transferrin (CDT)

Carbohydrate deficient transferrin (CDT) is a relatively new, highly specific and possibly the most reliable biomarker of chronic alcohol intake.[184,248,249] Human transferrin is a serum iron transporting protein which usually binds carbohydrate chains of variable composition.[142] The structure of CDT is only partially known. However, available studies[250,251] emphasise the loss of the entire glycan side chains and not of individual sialic acid moieties of a transferrin protein.

Alcohol disturbs the synthesis of the transferrin carbohydrate chain by interfering with the transferase enzymes.[252-255] Furthermore, ethanol or acetaldehyde may enhance the sialidase enzyme that removes the carbohydrate groups from transferrin. Disturbed function of various liver cell receptors could possibly also influence the serum concentrations of the desialylated fractions.[142,256] As a result of these mechanisms, a defective transferrin carbohydrate structure,

especially a sialic acid deficiency, in serum, commonly from alcoholics, is formed. The well-known method of CDT measurement is expressed as %CDT, which measures the relative amount of CDT isoforms in proportion to total transferrin.[257] This method is better than absolute CDT values and accommodates the natural variability in transferrin.[257]

The serum %CDT varies directly with increased alcohol intake.[58,255,258] High %CDT was seen in excessive alcohol intake together with high CRP in one Chinese study.[259] This is an indication that %CDT is a possible risk marker for CVD. Generally a serum %CDT of more than 2% is indicative of chronic alcohol intake and %CDT is more sensitive in men.[258] Other factors such as old age and pregnancy are also known to influence %CDT values to higher levels.[260] Liver diseases, chronic iron deficiency, inborn errors of glycoprotein metabolism and rare genetic D-variants of transferrin are among other factors that elevate serum %CDT.[254,261,262]

There is a positive correlation between %CDT and BP or CVD.[263,264] The study by Baros and colleagues[263] showed that reduction in alcohol consumption in heavy drinkers was associated with reductions in both BP and biomarkers of alcohol consumption, including %CDT. Moreover, while there is a positive correlation of GGT with CHD reported in obe study, an inverse association was also observed with %CDT.[265] Because of its better sensitivity than other biomarkers of alcohol intake, %CDT was investigated in unhealthy drinking of HIV infected people. However, it was not sufficiently sensitive for use in screening for excessive drinking in individuals with HIV infection.[46]

2.3.7 Other biochemical markers of alcohol intake: Ethyl glucuronide (EtG), ethyl sulphate (EtS) and phosphatidylethanol (PEth)

Since none of the biomarkers to date are completely accurate in assessing alcohol consumption, novel biomarkers have since been investigated. These biomarkers are ethyl glucuronide (EtG), ethyl sulphate (EtS) and phosphatidylethanol (PEth).[266] EtG and EtS are two specific metabolites of ethanol synthesized via conjugation with activated UDP-glucuronic acid and 3′-phosphoadenosine 5′-phosphosulphate, respectively.[267] They are detectable in urine after consumption of about 5 g of ethanol with its assessment possible up to five days after

consumption. Furthermore, EtG may undergo sulphate conjugation to produce EtS. As a result, individuals positive in urinary EtG are also positive for EtS.[268] EtG is also a useful marker for assessing fitness to drive in suspected drinking while driving compared to CDT.[269]

PEth is a direct ethanol metabolite with a half-life of 4 days and can be detectable in blood for more than two weeks after sustained ethanol intake. PEth is synthesized by phospholipase from phosphatidylcholine in the presence of ethanol (Fig. 2.8). PEth synthesis is specifically dependent on ethanol, thus the diagnostic specificity of PEth as an alcohol biomarker is theoretically very high.[270] One study found a significant correlation between PEth and other established markers of alcohol intake such as GGT, CDT and MCV.[271] Moreover, PEth can be a more sensitive indicator of alcohol consumption than %CDT and GGT or a combination of these.[272]

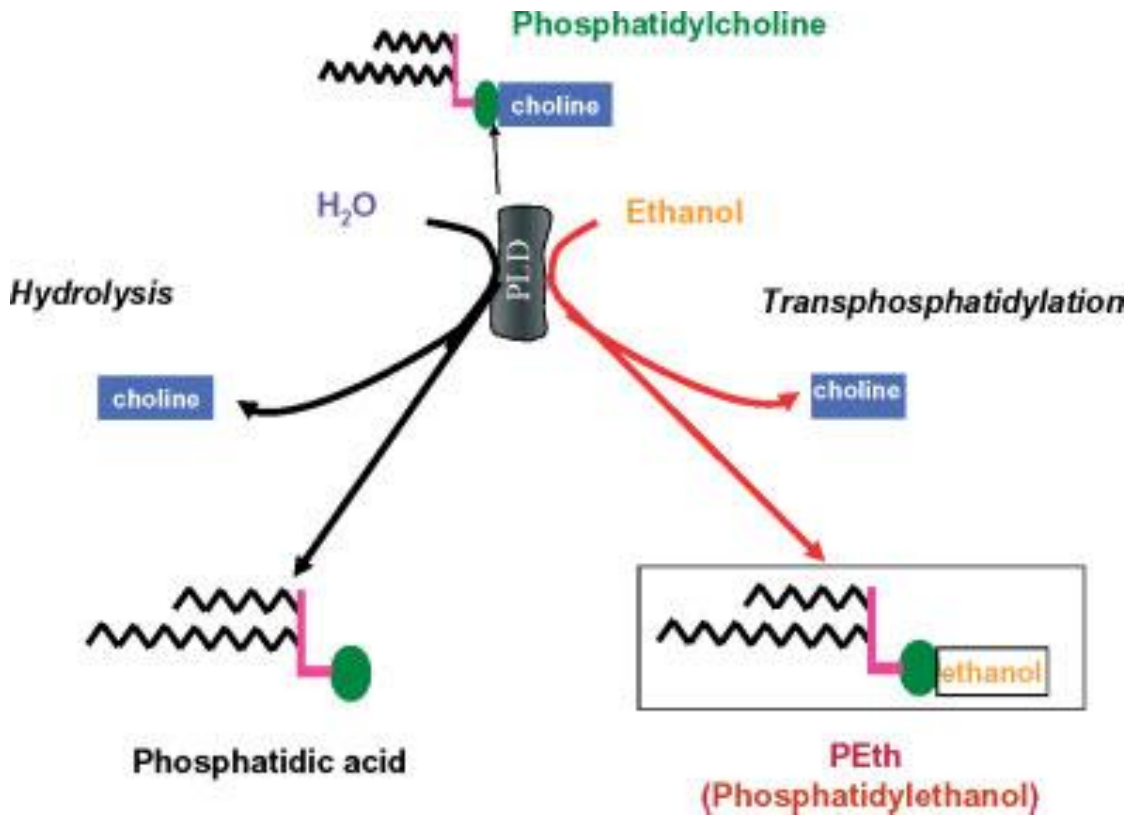

Figure 2.8. The synthesis of PEth from ethanol.[270] PEth, phosphatidylethanol

The validity of these novel biomarkers of alcohol was shown where their association with mortality was stronger than liver enzymes.[273] The highest sensitivity of PEth than frequently used biomarkers[274] and positive correlation with self-reported alcohol intake suggests these novel

markers are promising in their accurate detection of alcohol consumption.[275,276] Although these markers may be the most reliable, they are expensive and not a realistic option for population studies or clinical practice in low-resource settings.

3. BLOOD PRESSURE AND CARDIOVASCULAR DISEASE

3.1 Brachial and central blood pressure (BP)

Scientists make use of various cardiovascular measures in order to detect the association of alcohol intake with CVD. High BP is one of the main measures of cardiovascular dysfunction. BP measurements are used to draw conclusions about the prevalence and risk factors for CVD.[277,278] Brachial BP, the force exerted by the blood on the blood vessel walls has, for over a century, been a reliable predictor of CVD and mortality.[279,280] Despite the long history of its use and reliability, the use of brachial BP has recently been associated with challenges, especially when used in isolation. Challenges of brachial BP measurement have been brought forward by researchers,[279,281,282] who expressed concern of inaccuracy as a result of the 'white-coat effect' and the unevenness of the SBP in different sites of the arterial tree. This means that brachial SBP value may not be a true reflection of the pressure experienced by the central organs such as the kidneys, brain – organs more adversely affected by high BP.[283,284]

The more effective and reliable method to record BP is ambulatory blood pressure monitoring (ABPM). ABPM is measured continuously during the course of the day over a 24-hr period and has become important for the diagnosis and management of hypertension.[285] Readings in ABPM can be set into time windows such as mean daytime or night time values. Daytime clinic BP is associated with bias that includes white coat effect and so ABPM seems suitable as it can be taken from a non-medical setting. Moreover, daytime BP monitoring is better associated with cardiovascular mortality than night time BP monitoring.[286] During sleep, BP typically decreases, or dips, such that mean sleep BP is lower than mean awake BP.[287] Moreover, there is evidence of the important association of ABPM with target organ damage, superiority to casual BP in prediction of mortality. The association of alcohol consumption and ABPM can provide useful information on the risks linked to hypertension or benefits of moderate alcohol consumption.[288]

Although ABPM is very reliable and useful in hypertension clinics, it is not feasible to perform in large population-based epidemiological studies.

Finally, central BP, the pressure in the aorta in contrast to brachial BP (bBP) indicative of the pressure in the brachial arteries, seems promising as a more accurate predictor of cardiovascular events.[279] Central BP normally reflects lower values than bBP, especially in younger subjects[289,290] due to more stiffer muscular arteries.[279] Central BP is generated by a forward wave from the left ventricle and a backward wave from the arterial tree.[279,291] Elasticity of the large arteries causes the reflected wave to arrive back to the heart during diastole, thereby contributing less to central BP. Stiffer arteries, on the other hand, drive the reflected wave early during systole, adding to the central BP.[291,292] Age is an important determinant of arterial wave reflection pattern, with forward and backward waves faster in old age, causing the reflected wave to occur in systole with greater amplitude (Fig. 2.9).[291]

Aortic stiffness and central pulse pressure (PP) are predictors of cardiovascular events relating well with target organ damage.[293] Early wave reflections in a damaged elastic artery elevate central SBP with subsequent increase in central PP.[292] While there is evidence that brachial BP associates significantly with CVD,[294] some findings are in favour of central BP as a better indicator in this regard.[284,295-297] Peripheral BP seems to regain its importance over the age of 55 when the degree of stiffness in elastic arteries is close to the muscular arteries.[292] In addition to younger subjects, central BP is also an important cardiovascular predictor during antihypertensive treatment.[292,297,298]

There is a general trend of increased alcohol intake and central BP, possibly caused by the vascular damage or atherosclerosis induced by alcohol usage.[10,17] Despite the support of central BP over brachial BP, there are studies that still provide enough evidence of peripheral BP as the relevant marker to predict cardiovascular risk.[300,301] Studies on the association of chronic alcohol consumption and central BP are scant in sub-Saharan Africa. In South African studies, peripheral BP correlated well with other cardiovascular variables and proved to be a reliable predictor of cardiovascular risk.[1,302,303] Moreover, lifestyle risk factors such as smoking and alcohol

consumption showed a positive correlation with peripheral BP in studies performed in black South Africans.[153,304]

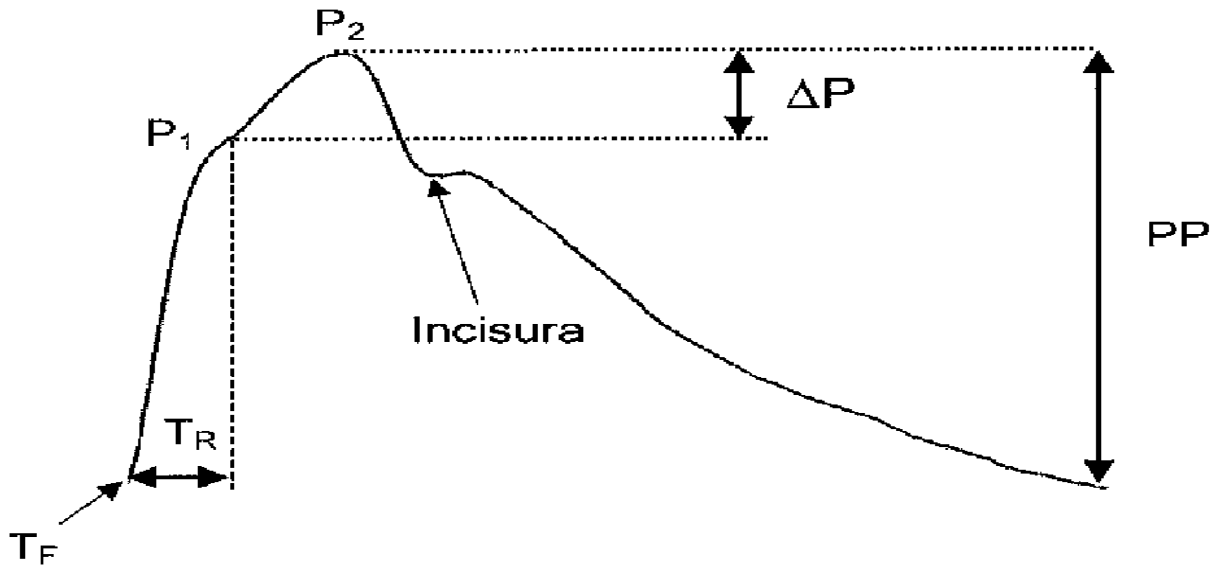

Figure 2.9. Central aortic pressure wave forms.[299] P_1, ejected wave; P_2, reflected wave; ΔP, augmentation index; PP, pulse pressure; T_F, time between the foot of the wave and incisura; T_R, time between T_F and the inflection point

3.2 Hypertension and mortality in sub-Saharan Africa

Hypertension continues to be one of the main challenges in sub-Saharan Africa and is the greatest health challenge after HIV and AIDS.[305] As indicated earlier, excessive drinking is a major risk factor for hypertension and CVD.[1,28] Age is an important non-modifiable risk factor for hypertension and age-related hypertension prevalence is high in low resource settings due to poverty and financial constraints in treating hypertension.[1,306] The prevalence of hypertension increases steadily with age in both rural and urban environments of sub-Saharan Africa with higher cases in urban settings.[2] Age-related hypertension is characterised by reduced elasticity of the conduit arteries leading to increased arterial stiffness. The result is the faster forward ventricular ejection wave and early reflected wave which increases central BP in old age.[291,292]

Ethnic differences in the prevalence of hypertension exist. Several studies indicate that hypertension is more common among black Africans when compared with other ethnic groups.[307-309] This trend however tends to level up by old age (about 70 years) possibly because more severe

hypertension in younger black Africans leads to a high mortality rate leaving less black hypertensives to old age.[1] One of the reasons for the high mortality rate in Africans is that hypertension in Africa is more often underdiagnosed and frequently undertreated, as patients cannot afford treatment or treatment is not always readily available in clinics.[2]

As a result, adverse health outcomes in the form of stroke, myocardial infarction, and renal failure are more common leading to high mortality.[310,311] Other factors such as sodium sensitivity, low plasma renin levels and increased peripheral vascular resistance contribute more to the high incidence of hypertension-related cerebrovascular and renal complications among Africans.[1,312] Moreover, Africans are known to have very high obesity levels[210,227] and obesity-related hypertension in black South Africans support this association.[156] Obesity is associated with urbanisation in Africans with consequent high rate of hypertension in black South African women in urban settings.[278]

The shift to a westernised lifestyle as a result of urbanisation has also caused changes in behavioural patterns among African people. Black Africans in urban areas suffer from psychosocial distress,[7,313] show high levels of smoking[304,314] and demonstrate excessive intake of alcohol.[28,161] The prevalence of hypertension in the younger population continues to grow.[1,2] Schutte and colleagues[302] observed elevated arterial stiffness at a young age in Africans - another important risk factor for hypertension. Given these early signs, prospective early detection of predictors of hypertension in sub-Saharan Africa cannot be overemphasized.

In conclusion, the levels of detection, treatment and control of hypertension in sub-Saharan Africa are still very poor, meaning that adverse effects such as stroke, heart failure, renal dysfunction and mortality will become even more apparent in years to come.[1,315-318] The estimated number of hypertensives in 2008 was nearly four times than in 2005, and there is a projected increase of 68% in 2025.[2] It is as a result of these findings and projections that longitudinal studies of hypertension and CVD are urgently needed in Africa.[319,320] Healthy lifestyle and public awareness through education programs on the dangers of alcohol abuse must be encouraged by health policy makers in order to reduce the risk of hypertension and CVD.

4. MOTIVATION, AIMS, OBJECTIVES AND HYPOTHESES FOR EACH MANUSCRIPT

This thesis includes three manuscripts submitted for publication. Since the relevant literature background for each manuscript is discussed in the papers and in the literature review above, only a brief motivation together with aims, objectives and hypotheses for each article will be provided below. The central aim of this study was to investigate whether alcohol intake of black South Africans is related to specific measures of cardiovascular function (change in BP, hypertension development) and mortality over a period of 5 years.

4.1 Self-reported alcohol intake is a better estimate of 5-year change in blood pressure than biochemical markers in low resource settings: the PURE study (Chapter 4)

Motivation

The importance and reliability of biomarkers of alcohol use such as GGT and %CDT are well documented.[158,199,259] Moreover, both GGT and %CDT have shown positive associations with BP and other cardiovascular variables.[156,259,263] However, these biomarkers of alcohol use are expensive and not readily available in low resource settings of sub-Saharan Africa. Moreover, they are influenced by obesity, liver damage, old age and ethnicity.[161,198,199] Self-reported alcohol use, on the other hand, is easier and cheaper to use but less reliable with under- and overestimation often associated with it.[164,321] With the burden of CVD and excessive alcohol use highly prevalent, and due to limited resources to assess alcohol intake using expensive biomarkers in sub-Saharan Africa, the usefulness of self-reported alcohol use regarding its association with BP should be evaluated as an affordable measure of alcohol use in populations with low socio-economic status.

Aim

To compare self-reported alcohol estimates with biochemical measures (GGT and %CDT) regarding their relationship with percentage change in brachial BP (%bSBP, %bDBP) and central SBP over a 5-year period, in a black South African population.

Objectives

To establish which marker of alcohol intake is better associated with percentage change in BP and central SBP over 5 years in black South Africans.

Hypotheses

Based on the literature, the following hypothesis was formulated:

- GGT and %CDT have a better positive association with %BP and central SBP than self-reported alcohol intake.

4.2 The comparison of the cardiometabolic profile of black South Africans with suspected non-alcoholic fatty liver disease (NAFLD) and excessive alcohol use (Chapter 5)

Motivation

In sub-Saharan Africa CVD remains one of the leading contributors to morbidity and mortality.[2,308] With urbanisation, lifestyle change has taken the centre stage in the African population leading to high prevalence of obesity and chronic diseases of lifestyle such as hypertension, diabetes and the metabolic syndrome. The risk factors for CVD as a result of lifestyle change include an unhealthy diet high in fat intake, smoking, physical inactivity and excessive alcohol use.[1,308] These factors elevate BP and may also lead to liver damage that may begin as AFLD in the case of excessive alcohol intake, and NAFLD when alcohol intake is low or moderate.[207,322,323] It is a major challenge to treat CVD in sub-Saharan Africa.[324] Therefore, it is important to identify the possible risk factors of early cardiovascular dysfunction. Moreover, there is scant literature on NAFLD and alcohol consumption in Africa regarding prevalence and risk factors. The early identification and prevention of these risk factors will help reduce the burden of CVD in poverty-stricken sub-Saharan Africa.

Aim

To compare the cardiovascular and metabolic characteristics of Africans with suspected NAFLD and excessive alcohol use; and their associations with BP in black South Africans.

Objectives

- To compare the cardiometabolic profiles of three groups of Africans: excessive alcohol users, those with suspected NAFLD and non-alcohol users;
- To establish if there are associations between the cardiometabolic profiles of each group with BP.

Hypotheses

Based on literature, the following hypotheses were formulated:

- Metabolic and cardiovascular markers are elevated in excessive alcohol users, while lipid markers stay favourable with HDL-C elevated and having a significant positive association with BP;
- NAFLD is closely associated with obesity, the components of metabolic syndrome and high BP.

4.3 Alcohol intake, hypertension development and mortality in black South Africans (Chapter 6)

Motivation

The high prevalence of urbanisation in sub-Saharan Africa has, among other behavioural factors, led to a high rate of alcohol consumption, which is a modifiable risk factor for hypertension and mortality.[1,156,325] Although light to moderate intake of alcohol is beneficial and associated with reduced risk of CVD,[288] alcohol abuse is associated with stroke, myocardial infarction, renal damage and mortality.[100,326] Furthermore, the well-known J-shaped association between alcohol consumption and BP or mortality seems not relevant in black South Africans. Instead, a linear pattern with either none or excessive drinking dichotomy has been reported.[9,54] Since high mortality can be attributed to excessive alcohol intake in sub-Saharan Africa,[41] including high rates of HIV infection,[327] there is a need to determine if alcohol intake predicts total mortality in a low resource African population. By also identifying the alcohol marker that predicts cardiovascular outcomes best, this may aid in reducing the burden of alcohol-related CVD and mortality that is highly prevalent in sub-Saharan Africa.

Aim

To determine which biomarker of alcohol use relates best with cardiovascular and all-cause mortality, and predict the development of hypertension over 5 years in a population of black South Africans.

Objectives

- To investigate which alcohol biomarker (self-reported alcohol intake, GGT or %CDT) predicts mortality; and
- incident hypertension in black South Africans over a 5 year period.

Hypotheses

Based on the literature, the following hypotheses were formulated:

- GGT predicts alcohol-related mortality better than %CDT and self-report in black South Africans;
- GGT also indicates the strongest association with incident hypertension over a 5 year period.

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Chapter 3

Methodology

1. STUDY DESIGN AND SUBJECT SELECTION

This study is a sub-study of the international Prospective Urban and Rural Epidemiology (PURE) study which investigates the health transition in urban and rural subjects. The PURE study is an on-going investigation that involves the follow-up of 180 000 individuals across 26 countries worldwide. Communities that participated in the study had to meet certain inclusion criteria. The main criterion was that there should be migration stability within the chosen rural and urban communities. The selected communities in this South African leg of PURE were part of the North West Province. Moreover, the chosen communities had to be large enough to allow random selection of subjects. The rural communities included Ganyesa, 450 km west of Potchefstroom on the way to Botswana; and Tlakgameng, 35 km east of Ganyesa, only accessible by gravel road (Fig. 3.1). Both communities were under tribal law. The urban communities were chosen in Potchefstroom and were the established urban township of Ikageng and informal settlements surrounding Ikageng (Fig. 3.1).

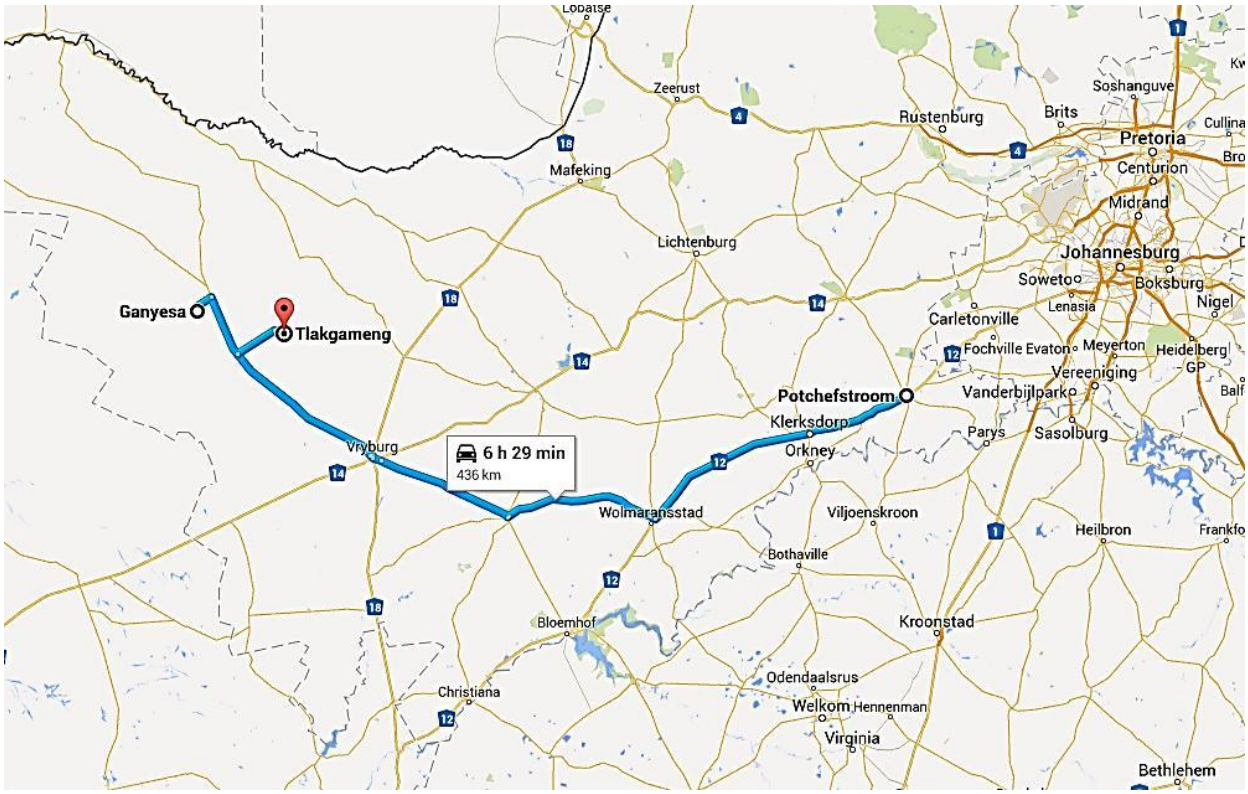

Figure 3.1. Geographical locations of Potchefstroom, Tlakgameng and Ganyesa in the North West Province, South Africa.

The two sets of data collection for this study took place in 2005 (baseline), with the follow-up data collected in 2010. Trained African field workers who spoke the participant's native language were employed and responsible to keep track of participants over the 5-year follow-up period. The participants were contacted by the field workers every three months. Initially, a total of 2021 Africans, 30 years and older from 6000 households were originally recruited from selected urban and rural areas of the North West Province. The recruited subjects were visited at their homes and gave voluntary informed consent.

2. MATERIALS AND METHODS

Ethical considerations

The study was approved by the Ethics committee of the North-West University, South Africa (ethics number: 04M10) and complied with the Helsinki Declaration. Permission to conduct the study was given by the provincial Department of Health of the North West Province, community leaders, tribal chiefs and mayors in charge of the selected communities. All subjects were informed about the objectives and procedures. The field workers were available to do the translation in the participant's native language. Confidentiality and anonymity of all the results were assured. An agreement with local clinics and hospitals serving the selected communities was reached, so that subjects identified with chronic illnesses such as hypertension and Human Immunodeficiency Virus (HIV) were referred with a standardised referral letter. Participants received meals and remuneration for travelling expenses during the study, in addition to the necessary counselling as appropriate.

Questionnaires

The participants were interviewed using structured demographic, socio-economic, lifestyle and physical activity questionnaires developed and standardised for the international PURE study,[1] validated and adapted for South Africa. The questionnaires included questions on alcohol consumption behaviour including a yes/no question on alcohol use (yes, current or former use; no, never used), the quantity, frequency of intake and the type of alcoholic beverage. Different beverages of alcohol were assessed separately in these questions (Appendix A).

Additionally, a quantified food frequency questionnaire (QFFQ)[2,3] which was developed and validated for this population and tested for validity and reproducibility in the PURE study, was completed by each participant with the help of trained fieldworkers (Appendix B). Participants estimated portion sizes by using a food-portion photograph book and other suitable tools.[4,5] Portion sizes were then converted to weights by using standard tables.[6] The food and alcohol intake was coded and analysed using the South African Food Composition Tables.[3,7-8] The dietary questionnaire data (QFFQ and 24-hour recall) were coded by two dieticians and sent to the Medical Research Council of South Africa for computerisation, cleaning and nutrient analyses. The alcohol intake was estimated by the amount of alcohol consumed per day and expressed as intake of pure alcohol (ethanol) in grams (g) per day. Beer, home-made brews, spirits and wine were considered to contain 3.6 g, 3 g, 36 g and 9.4 g of pure alcohol per 100 g of beverage, respectively.

Anthropometric measurements

Weight, height and waist circumference were measured using calibrated instruments by anthropometrists (Precision Health Scale, A & D company, Tokyo, Japan; Invicta Stadiometer, IP 1465, London, UK; Holtain unstretchable metal tape).[9,10] Weight was determined with the use of a digital scale with the participant standing still without support with the weight evenly distributed over the centre of the scale, looking straight ahead while the weight is recorded, to the nearest 0.1 kg. The height was taken as the maximum vertical distance from the floor to the vertex of the head. The participant stood erect with heels together and touching the base of the stadiometer and arms hanging by the sides. The anthropometrist brought the headpiece firmly down the hair and having made contact with the vertex, made a pencil mark on the paper tape level with the underside of the headpiece. Measurements were made before the participant exhaled. After the participant stepped aside, the headpiece was removed and the vertical distance from the floor to pencil mark measured with the retractable metal tape stadiometer and read to the nearest 0.1 cm. The waist circumference was measured in triplicate at the midpoint between the lower margin of the last rib and the top of the iliac crest[11] while the participant was standing erect looking straight ahead with a metallic measuring tape (Holtain unstretchable metal tape).

Cardiovascular measurements

After a 10-min rest period, duplicate measurements, 5 minutes apart, of blood pressure (systolic, diastolic) and heart rate were taken with the validated Omron HEM-757 apparatus (Omron Healthcare, Kyoto, Japan) both in 2005 and at follow-up in 2010 while the participants were seated upright with the right arm at heart level. Central systolic blood pressure and pulse pressure were also taken at follow-up using the Omron HEM-9000AI (Omron HealthCare, Kyoto, Japan) whilst the participant was in a sitting position.

Blood, serum and plasma samples

Before blood samples were taken, all participants were requested to fast for approximately 8-10 hours. A registered nurse took a blood sample from the ante-brachial vein branches. Blood was centrifuged within two hours of collection and stored at -80 °C until analysis. Serum samples were prepared by collecting whole blood into tubes that did not contain any anticoagulant. This blood was then allowed to clot at room temperature for 30 minutes and centrifuged at 2 000 g for 15 minutes at 10 °C. Collected serum was subsequently transferred to cryo tubes and then stored at -80 °C until analysis. As for the preparation of plasma samples, blood collection tubes containing ethylenediamine tetra acetic acid (EDTA) were filled (vacutainers) to capacity. This ensured optimal blood to anticoagulant ratios. These tubes were centrifuged at 2 000 g for 15 minutes at 4 °C. Plasma was transferred to cryo tubes and stored at -80°C until analysis. In cases where blood samples could not be processed immediately, they were immediately frozen and stored at -18°C for no longer than five days, transported to a laboratory facility and stored at -80°C until analysis.

Biochemical analyses

From the blood samples collected from the participants, we measured a series of biochemical markers. Serum glucose, uric acid, liver enzymes [GGT, alanine aminotransferase (ALT), aspartate aminotransferase (AST)], high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides and high-sensitivity C-reactive protein (hsCRP) were determined using the Konelab20i auto analyser (Thermo Scientific, Vantaa, Finland). Furthermore, serum iron was

determined using immunological, colorimetric and high-performance liquid chromatography methods. Glycosylated haemoglobin (HbA1c) was measured on-site in EDTA-treated whole blood using the D-10 Haemoglobin testing system (Bio-Rad Laboratories, Hercules, CA). Serum percentage carbohydrate deficient transferrin (%CDT) analyses were performed by using an in vitro heterogeneous immunoassay with column separation followed by a turbidimetric measurement (Axis-Shield %CDT kit, Oslo, Norway). The coefficient of variance (CV) for all assays was < 10%.

HIV testing

The HIV status was determined in 2005 by using the First Response (PMC Medical, India) rapid HIV card test with whole blood. The test was repeated by using the Pareeshak (BHAT Bio-tech, India) card test. Appropriate guidelines of the National Department of Health, South Africa were followed in determining the HIV status of all the participants. Everyone received pre-test counselling before the test was done and then post-test counselling for those who opted to know their results.

Mortality

In order to assess both cardiovascular and non-cardiovascular mortality, we obtained the cause of death through verbal autopsy coded by a physician according to the International Classification of Disease codes for the immediate and underlying causes, and from the family's death certificate. Cardiovascular mortality was defined as death from congestive cardiac failure, ischemic heart disease, diabetes mellitus, stroke and myocardial infarction. All-cause mortality included cardiovascular and non-cardiovascular mortality.

Statistical analyses

Statistical analyses of all the results were performed using Statistica software (StatSoft, Inc., Tulsa, OK). Variables with a non-Gaussian distribution were logarithmically transformed and the central tendency and spread were represented by the geometric mean and the 5^{th} and 95^{th} percentile intervals. Means and proportions were compared by analyses of variance (ANOVA)

and Chi-square tests, respectively. Dependent t-tests and the McNemar test (for proportions) were used to compare baseline and follow-up variables. Single and partial linear regression analyses were employed to correlate alcohol markers with each other and with the cardiovascular variables. Multiple regression analyses were used to correlate dependent variables in the study with various independent variables as required. Multivariable-adjusted Cox regression analyses were used to assess the association of the selected alcohol markers with cardiovascular and all-cause mortality, as well as incident hypertension while adjusting for several independent variables.

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Chapter 4

Self-reported alcohol intake and 5-year change in blood pressure

INSTRUCTIONS FOR AUTHORS (J Hypertens)

The Journal of Hypertension considers original, clinical and experimental research papers of high standard which contribute to the advancement of knowledge in the field of hypertension. The Journal publishes full papers and reviews or editorials.

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Authors must state all possible conflicts of interest in the manuscript, including financial, consultant, institutional and other relationships that might lead to bias. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript.

Patients have a right to privacy that should not be infringed without informed consent. When informed consent has been obtained it should be indicated in the published article. All authors must sign a declaration that the research was conducted within the guidelines of Journal of Hypertension and under the terms of all relevant local legislation.

All authors must sign the letter accompanying their submission to confirm that they have read and approved the paper, that they have met the criteria for authorship as established by the International Committee of Medical Journal Editors, that they believe that the paper represents honest work, and that they are able to verify the validity of the results reported In addition to those from the ICJME the International Society for Medical Publication Professionals, ISMPP (www.ismpp.org) have produced some useful guidelines on authorship of studies sponsored by companies: Good Publication Practice (GPP2) (www.ismpp.org/initiatives/gpp2.html).

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The title page should have the full title of the paper, consisting of no more than 20 words; a brief short running title, all authors' full names, the affiliations of all the authors; when authors are

affiliated to more than one institution, their names should be connected using a,b,c, etc. The sources of any support, for all authors, for the work in the form of grants should be stated in the title page. The name and address of the author responsible for correspondence concerning the manuscript, and the name and address of the author to whom requests for reprints should appear in the title page.

2. Abstracts

The abstract should be no more than 250 words and should state the objective(s) of the study or investigation, basic methods, main results (giving specific data and their statistical significance, if possible), and the principal conclusions. It should emphasise new and important aspects of the study or observations.

3. Article text

The full orginal papers should be divided into Introduction, Methods, including ethical and statistical information, Results and Discussion including conclusion. Acknowledgements should be made only to those who have made substantial contribution to the study.

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References should be numbered consecutively in the order in which they first appear in the text. They should be assigned Arabic numericals in brackets, e.g. [18] or [13,14]. Six authors in an article should all be listed and et al in cases of more than six authors. However, if an article has seven authors, all seven authors must be listed.

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More than seven authors: Grassi G, Vailati S, Bertinieri G, Seravalle G, Stella ML, Dell'Oro R, et al. Heart rate as a marker of sympathetic activity. *J Hypertens* 1998; 16:1635-1639.

Books: Katz AM, Konstam MA. Heart Failure. Pathophysiology, molecular biology, and clinical management. Philadelphia: Lippincott Williams & Wilkins; 2008, pp. 137-153.

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Each table should be typed on a separate page and should be assigned an Arabic numerical, e.g. Table 1, and a brief title. Explanatory matter, including full explanations of the abbreviations used, should be placed in footnotes, not in the heading. Standard statistical measures of variation such as standard deviation and standard error of the mean should also be explained in the footnote. The tables and figures must be must be cited in the text. The figures must be numbered in order in which they are discussed.

Self-reported alcohol intake is a better estimate of 5-year change in blood pressure than biochemical markers in low resource settings: the PURE study

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Short title: Self-reported alcohol intake and 5-year change in BP.

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ABSTRACT

Background: Despite criticism of self-reported alcohol intake, it is a valuable tool to screen for alcohol abuse as a risk factor for cardiovascular disease. We aimed to compare various self-reported estimates of alcohol use with gamma-glutamyltransferase (GGT) and percentage carbohydrate deficient transferrin (%CDT) considering their relationship with blood pressure changes (%BP) over a 5-year period in black South Africans.

Method: We recruited 2021 participants and collected 5-year followed up data (N=1246). Participants completed questionnaires on alcohol intake indicating their former and current alcohol use ('yes' response and 'no' if alcohol was never used). We assessed alcohol intake (in grams) using a quantified food frequency questionnaire. We collected blood samples and measured GGT and %CDT. Brachial blood pressure (bBP) was measured at baseline and follow-up and central BP (cBP) at follow-up only.

Results: Self-reported alcohol intake associated significantly with the 5-year change in bBP before and after adjusting for confounders (%bSBP: R^2=0.263, β=0.06, p=0.023; %bDBP: R^2=0.326, β=0.08 p=0.005), as well as cSBP (R^2=0.286, β=0.09, p=0.010) and central pulse pressure (R^2=0.254, β=0.06, p=0.020). GGT and %CDT correlated well with self-reported alcohol intake (r=0.44; p=0.001; r=0.34 p=0.001), but did not associate significantly with %bBP or cBP at follow-up.

Conclusions: Self-reported alcohol use associated strongly with a 5-year increase in BP in Africans with a low socio-economic status. This was not found for biochemical measures, GGT and %CDT. Self-reported alcohol intake could be an important measure to implement in primary healthcare settings in middle to low income countries, where honest reporting is expected.

Key words: Self-reported alcohol intake, GGT, %CDT, blood pressure, low socio-economic status, hypertension, cardiovascular disease.

INTRODUCTION

Cardiovascular disease (CVD) is a major challenge in developing countries and is one of the main causes of morbidity and mortality [1,2]. Rapid urbanisation in sub-Saharan Africa (SSA) drives many lifestyle changes, including unhealthy high fat diets, stressful lifestyle and chronic excessive alcohol abuse [3,4], the latter of which is regarded as a critical risk factor for hypertension, stroke and coronary heart disease [5,6]. Although the detrimental consequences of alcohol abuse are constantly emphasised [7,8], excessive alcohol consumption remains a significant concern in SSA [9]. Apart from the devastating cardiovascular consequences of alcohol abuse in urbanising SSA, it poses additional threats in the form of premature deaths through malnutrition, stress, violence and crime [10].

Biochemical markers for alcohol use are accepted as superior to self-reported estimates. Although self-reporting is the easier and cheaper method for assessing alcohol intake, its potential inaccuracy may lead to an underestimation or overestimation of alcohol intake, especially when comparing participants from different ethnical backgrounds with differing social perception to alcohol consumption [11]. The biochemical markers that are commonly used in screening for alcohol abuse are gamma-glutamyltransferase (GGT) and percentage carbohydrate deficient transferrin (%CDT). GGT is the most widely used marker of excessive drinking [12], but high GGT levels could also be associated with aging, obesity and liver damage [13,14]. Percentage CDT, on the other hand, is another well-known marker of alcohol intake, and is characterised by a higher specificity and lower sensitivity to alcohol consumption, when compared to GGT [12].

Due to limited resources in SSA to test for biochemical measures of alcohol abuse, we aimed to compare self-reported estimates with these biochemical measures (GGT and %CDT), considering their relationship with percentage change in brachial blood pressure (%bSBP, %bDBP) and central systolic blood pressure (SBP) over a 5-year period, in a black South African population.

METHODOLOGY

Study design and subject selection

This study is based on the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study which investigated the health transition in urban and rural subjects in the North West Province. This is an on-going investigation that involves a series of follow-up studies over a 12-year period. The baseline data used in the present study was collected in 2005, with the first follow-up data obtained in 2010. To keep track of participants over the 5-year follow-up period, trained field workers contacted the participants every three months. A total of 2021 African volunteers (30 years and older) from 6000 households were originally recruited from urban and rural areas. The recruited participants were visited at their homes and gave voluntary informed consent.

The study was approved by the Ethics committee of the North-West University, South Africa. The provincial Department of Health of the North West Province, community leaders, tribal chiefs and mayors also gave permission to conduct the study. All subjects were informed about the objectives and procedures of the study and were asked to fast for 10 hours prior to sample collection. Field workers were available to do the translation in the participant's native language. Confidentiality and anonymity of all the results were assured, and individuals identified with chronic illnesses such as hypertension and Human Immunodeficiency Virus (HIV) were referred to their local clinics and hospitals. Participants received remuneration for travelling expenses during the study, in addition to the necessary counselling.

Questionnaires

The participants were also interviewed using structured demographic, socio-economic, lifestyle and physical activity questionnaires developed and standardised for the international PURE study [15]. The questionnaires had questions on alcohol consumption behaviour including a yes/no question on alcohol use (yes, current or former use; no, never used), the quantity, frequency of intake and the type of alcoholic beverage. In these questions, intakes of different beverages were assessed separately.

Additionally, a validated [16,17] food frequency questionnaire was completed for each participant by trained fieldworkers. The food intake was coded and analysed using the South African Food Composition database [17,18]. Alcohol consumed was expressed as pure alcohol in grams per day. Beer, home-made brews, spirits and wine were considered to contain 3.6 g, 3 g, 36 g and 9.4 g of pure alcohol per 100 g of beverage, respectively.

Cardiovascular measurements

An appointment was secured with each participant to measure the cardiovascular variables. After a 10-min rest period, blood pressure (systolic, diastolic) and heart rate were measured in duplicate, 5 min apart, with the validated Omron HEM-757 apparatus (Omron Healthcare, Kyoto, Japan) while the participants were seated upright with the right arm at heart level. Additional cardiovascular measurements, namely central SBP and pulse pressure (cPP) were taken at follow-up in 2010 using the Omron HEM-9000AI (Omron HealthCare, Kyoto, Japan) whilst the participant was in a sitting position.

Anthropometric measurements

Height, weight, waist and hip circumferences were measured (Precision Health Scale, A & D company, Japan; Invicta Stadiometer, IP 1465, UK; Holtain unstretchable metal tape) using standardised methods [19].

Blood, serum and plasma samples

After fasting for approximately 10 hours, a registered nurse took a blood sample from the ante-brachial vein branches. Samples were prepared according to appropriate methods and stored at -80°C until analyses. Blood samples were immediately frozen and stored at -18°C for no longer than 5 days, transported to a laboratory facility and stored at -80°C until analysis.

Biochemical analyses

From the blood samples, we measured GGT, alanine aminotransferase (ALT), aspartate aminotransferase (AST), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC),

triglycerides, C-reactive protein (CRP) and serum glucose (Konelab20i auto analyser; Thermo Scientific, Vantaa, Finland). Furthermore, glycosylated haemoglobin (HbA1c) was determined in EDTA-treated whole blood using the D-10 Haemoglobin testing system (Bio-Rad Laboratories, Hercules, CA) and serum %CDT analyses were performed by using an in vitro heterogeneous immunoassay with column separation followed by a turbidimetric measurement (Axis-Shield %CDT kit, Oslo, Norway). The coefficient of variance (CV) for all assays was less than 10%.

The protocol of the National Department of Health, South Africa, was followed in determining the HIV status of all the participants. HIV status was determined in 2005 by using the First Response (PMC Medical, India) rapid HIV card test with whole blood. The test was repeated by using the Pareeshak (BHAT Bio-tech, India) card test. Everyone received pre-test counselling before the test was done and then post-test counselling for those who tested positive and opted to know their results.

Statistical analyses

Statistica version 11 (StatSoft, Inc., Tulsa, OK) was used to compare the means and proportions in this study. Variables with a non-Gaussian distribution were logarithmically transformed. Analysis of variance (ANOVA) was used to compare the variables when the 2005 characteristics of the participants were divided into three groups: (1) those that were successfully followed from 2005 to 2010; (2) those lost during the follow-up period; and (3) those who passed away. Chi-square tests were used to compare proportions for categorical variables. Dependent t-tests and the McNemar test were used to compare baseline and follow-up variables. Single and partial linear regression analyses were employed to correlate alcohol markers with each other and with the cardiovascular variables. The multiple regression models employed percentage change in brachial SBP (%bSBP), DBP (%bDBP), and cSBP and cPP (taken at follow-up) as dependent variables with a single alcohol marker, and the following variables included as independent variables: age, rural/urban, sex, body mass index (BMI), HbA1c, total cholesterol to HDL ratio (TC:HDL), CRP, smoking, physical activity, hypertension medication at baseline and HIV status.

RESULTS

Table 1 presents baseline characteristics of those followed, lost to follow-up and those who passed away. Over this 5-year follow-up study, we were able to collect data from 1473 (73%) of the original 1994 recruited participants, of whom 227 (11.3%) passed away. At baseline, participants who passed away had a significantly lower BMI and higher proportion than the other groups reported that they use alcohol, confirmed by all the biochemical markers of alcohol abuse at baseline ($p<0.001$). Those participants lost to follow-up were generally younger and showed the shortest duration of alcohol consumption. The three groups were comparable in terms of blood pressure (BP).

Table 2 indicates the changes in characteristics of the follow-up group (n=1246) over a period of 5 years. Obesity markers (weight, BMI, waist circumference), metabolic markers (glucose, HbA1c, TC:HDL, CRP) and BP increased despite a lowering in reported alcohol intake and serum liver enzymes.

To determine whether self-reported alcohol measures are related to the biochemical measures, we compared the correlations of self-reported and biochemical alcohol markers in Table 3. Self-reported alcohol intake (no/yes) showed highly significant associations with all the biochemical markers ($p\leq0.001$), and showed a stronger correlation with GGT ($r=0.44$; $p=0.001$) and %CDT ($r=0.34$; $p=0.001$) than the intercorrelation between %CDT and GGT ($r=0.23$; $p<0.001$).

Table 4 shows the independent associations of alcohol markers with the 5-year percentage change in BP (%SBP or %DBP), and cSBP or cPP. Self-reported alcohol intake was the only alcohol measure that was associated with %bSBP, %bDBP, cSBP and cPP at follow up, independent of the various confounders. Furthermore, %bDBP associated positively with the volume of alcohol intake whereas the duration of drinking was negatively associated with cPP.

Table 1. Descriptive characteristics at baseline of the study population who were followed up, lost or passed away by 2010.

	2005			
	Follow-up (n=1246)	**Lost to follow-up (n=548)**	**Passed away (n=227)**	**p-value**
Socio-demographic profile				
Age, years	50.0 ± 10.16[ab]	46.4 ± 9.39[ac]	52.6 ± 11.82[bc]	**<0.001**
Body mass index, kg/m^2	25.1 ± 7.10[a]	24.98 ± 7.12[b]	22.2 ± 6.14[ab]	**<0.001**
sex, women n (%)	824/1246 (66.1)[a]	324/537 (60.3)[ab]	116/227 (51.1)[ab]	**<0.001**
Location, rural, n (%)	669/1246 (53.7)[a]	238/547 (43.5)[a]	108/227 (47.6)	**<0.001**
HIV infected, n (%)	148/1239 (12.0)[ac]	97/548 (18.0)[ab]	75/224 (33.5)[bc]	**<0.001**
Smoking, n (%)	674/1241 (54.3)[a]	303/533 (56.9)	142/226 (62.8)[a]	**0.053**
Biochemical measurements				
Serum glucose, mmol/L	4.85 (3.50-6.90)	4.80 (3.50-6.20)	4.69 (3.40-6.10)	0.098
Glycosylated haemoglobin, %	5.63 (4.90-6.60)[a]	5.51 (4.80-6.30)[a]	5.54 (4.70-6.60)	**0.001**
Total cholesterol, mmol/L	5.10 ± 1.34[a]	4.97 ± 1.45[b]	4.61 ± 1.33[ab]	**<0.001**
TC:HDL	3.81 ± 2.43	3.90 ± 2.55	4.26 ± 4.42	**<0.001**
C-reactive protein, mg/L	3.10(0.25-39.4)[a]	2.60 (0.27-31.8)[b]	5.18 (0.32-53.6)[ab]	**<0.001**
Markers of alcohol intake				
Self-reported alcohol use, n (%)	517/1239 (41.7)[a]	241/533 (45.2)	124/225 (55.1)[a]	**0.001**
Alcohol intake, g/day*	11.4 ± 23.2[a]	11.2 ± 24.1	15.7 ± 25.3[a]	**<0.001**
Daily frequency of alcohol use, n (%)	167/473 (35.3)[a]	66/228 (29.0)[a]	38/118 (32.2)	**0.050**
>5 drinks/d at least once/month, n (%)	90/366 (25.0)	30/171 (18.0)	23/82 (28.0)	0.102
Started drinking age, years	26.1 ± 8.90	26.0 ± 8.58	25.5 ± 9.18	0.779
Duration of drinking, years	23.0 ± 10.9[ab]	20.5 ± 9.81[ac]	26.2 ± 13.2[bc]	**<0.001**
Volume of alcohol intake, L	3.28 ± 8.00	4.58 ± 17.3	3.24 ± 6.91	0.352
Alanine aminotransferase, IU/L	17.5 (7.71-49.7)[a]	18.7 (8.00-53.0)	19.6 (7.00-69.0)[a]	**<0.001**
Aspartate aminotransferase, U/L	28.0 (13.0-96.1)[a]	29.1 (12.0-102.0)[b]	34.0 (12.0-146.1)[ab]	**<0.001**
Gamma-glutamyltransferase, U/L	53.9 (19.0-350)[a]	53.7 (17.8-347)[b]	74.0 (21.8-488)[ab]	**<0.001**
Carbohydrate deficient transferrin, %	2.92 ± 1.37[a]	2.94 ± 1.49[b]	3.26 ± 1.53[ab]	**0.006**
Cardiovascular variables				
Systolic blood pressure, mmHg	133 ± 23.7	132 ± 24.15	136 ± 29.1	0.163
Diastolic blood pressure, mmHg	87.8 ± 14.1	87.1 ± 14.9	88.4 ± 16.3	0.515
Pulse pressure, mmHg	45.7 ± 15.0	45.3 ± 14.1	47.8 ± 18.0	0.108
Heart rate, bpm	73.4 ± 15.4[a]	72.4 ± 15.6[b]	80.5 ± 19.2[ab]	**<0.001**

Values are interpreted as arithmetic mean ± standard deviation; geometric mean (5th to 95th percentile interval); number of participants (%). Values with the same superscript letter differed significantly (p≤0.05), and bold p-values denote statistical significance where p≤0.05. HIV, human immunodeficiency virus.*Alcohol intake as analysed using the quantified food frequency questionnaire (QFFQ).

Table 2. Comparison of the study population over 5 years (N=1246).

	Baseline (2005)	Follow-up (2010)	p-value
Anthropometric measurements			
Weight, kg	64.0 ± 17.1	65.2 ± 17.9	**<0.001**
Height, m	1.60 ± 0.08	1.59 ± 0.08	**<0.001**
Body mass index, kg/m^2	24.1 (16.5-38.5)	25.0 (16.4-39.8)	**<0.001**
Waist circumference, cm	80.0 ± 13.0	82.0 ± 13.2	**<0.001**
Biochemical measurements			
Serum glucose, mmol/L	4.86 (3.50-6.90)	5.10 (3.90-7.30)	**<0.001**
Glycosylated haemoglobin, %	5.63 (4.90-6.60)	6.02 (5.10-7.30)	**<0.001**
TC:HDL	3.47 (1.85-6.61)	3.65 (1.90-6.95)	**<0.001**
C-reactive protein, mg/L	3.10 (0.25-39.4)	3.51 (0.33-34.0)	**0.013**
Markers of alcohol intake			
Self-reported alcohol use, n (%)	517/1239 (41.7)	430/1169 (36.8)	**<0.001**
Alcohol intake, g/day*	13.5 (1.07-85.1)	11.0 (1.00-107)	**<0.001**
>5 drinks/d at least once/month, n (%)	143/619 (23.1)	-	**-**
Alanine aminotransferase, U/L	17.5 (7.71-49.7)	16.8 (7.70-48.9)	**0.013**
Aspartate aminotransferase, U/L	27.9 (12.6-96.1)	25.7 (13.5-79.7)	**<0.001**
Gamma-glutamyltransferase, U/L	53.9 (19.0-350)	45.1 (12.1-310)	**<0.001**
Carbohydrate deficient transferrin, %	2.63 (1.32-5.62)	-	-
Cardiovascular measurements			
Systolic blood pressure, mmHg	133 ± 23.8	135 ± 24.1	**0.017**
Diastolic blood pressure, mmHg	87.8 ± 14.1	88.4 ± 13.6	0.159
Central systolic BP, mmHg	-	147 ± 24.4	-
Central pulse pressure, mmHg	-	58.3 ± 18.4	-

Values are arithmetic mean ± standard deviation; geometric mean (5th to 95th percentile interval); number of participants (%). Bold p-values denote statistical significance where p≤0.05.
*Alcohol intake as analysed using the quantified food frequency questionnaire (QFFQ).

Table 3. Single linear regression analysis of markers of alcohol intake at baseline (N=1246).

	Daily frequency of alcohol use , n (%)	Volume of alcohol, L	>5 drinks/day at least once/month, n (%)	Duration of drinking, years	Alcohol intake, g/day	ALT, U/L	AST, U/L	GGT, U/L	CDT, %
Self-reported alcohol, n (%)					r=0.42 p=**0.001**	r=0.20 p**<0.001**	r=0.32 p=**0.001**	r=0.44 p=**0.001**	r=0.34 p=**0.001**
Start drinking age, years	r=0.15 **p=0.001**	r=-0.09 p=0.06	r=0.04 p=0.44	r=-0.57 p=**0.001**	r=-0.01 p=0.87	r=-0.10 p=**0.03**	r=-0.18 **p<0.001**	r=-0.06 p=0.17	r=-0.06 p=0.24
Daily frequency of alcohol use, n (%)		r=0.15 p=**0.001**	r=0.02 p=0.72	r=-0.14 p=**0.003**	r=-0.23 **p<0.001**	r=-0.11 p=**0.02**	r=-0.15 p=**0.002**	r=-0.13 p=**0.006**	r=-0.05 p=0.30
Volume of alcohol, L			r=0.23 p=**0.001**	r=0.10 p=**0.04**	r=-0.02 p=0.69	r=-0.07 p=0.14	r=-0.04 p=0.44	r=0.03 p=0.55	r=-0.07 p=0.15
>5 drinks/day at least once per month, n (%)				r=-0.09 p=0.10	r= 0.04 p=0.50	r=0.08 p=0.12	r=0.11 p=**0.05**	r=0.15 p=**0.006**	r=-0.01 p=0.82
Duration of drinking, years					r=-0.03 p=0.55	r=-0.02 p=0.71	r=0.02 p=0.62	r=-0.02 p=0.74	r=-0.04 p=0.41
Alcohol intake, g/day*						r = 0.12 p =**0.03**	r = 0.21 p =**0.001**	r = 0.25 p **=0.001**	r = 0.19 p **<0.001**
ALT, U/L							r=0.62 **p=0.001**	r = 0.60 p **=0.001**	r = 0.12 p **<0.001**
AST, U/L								r = 0.60 p **=0.001**	r = 0.21 p **<0.001**
GGT, U/L									r = 0.23 p **<0.001**

Bold p-values denote statistical significance where p≤0.05. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; CDT, carbohydrate deficient transferrin.*Alcohol intake as analysed using the quantified food frequency questionnaire (QFFQ).

Sensitivity analyses

Due to weak associations between %BP and alcohol markers, we wanted to determine whether baseline BP would yield similar results. When substituting %BP with baseline BP (Supplemental Tables 1 & 2) we found that SBP and DBP correlated significantly with baseline self-reported alcohol intake (both $r=0.17$; $p<0.001$), liver enzymes, and %CDT, also after adjusting for age, sex and BMI ($p\leq0.001$).

We additionally excluded all individuals who changed reporting from “No” to “Yes” or from “Yes” to “No” in 2005 vs. 2010. After repeating the original multiple regression analyses (Supplemental Table 3), we found similar results to those reported in Table 4.

As there is a possibility that changes in body composition over 5 years may associate with the %BP, we performed an additional sensitivity analysis by including change in BMI (%BMI) in our multiple regression models instead of baseline BMI (Supplemental Table 4). However, this did not change our main findings.

Table 4. Multiple regression analyses with BP as dependent variables, and one marker of alcohol intake as the main independent variable.

Alcohol marker	% Brachial Systolic BP[a]				% Brachial Diastolic BP[b]		
	N	R^2	β value (SE)	p-value	R^2	β value (SE)	p-value
Self-reported alcohol, n (%)	1239	0.263	0.06 (0.03)	**0.023**	0.326	0.08 (0.03)	**0.005**
Alcohol intake, g/day*	1221	0.252	0.03 (0.03)	0.36	0.318	0.03 (0.03)	0.30
Daily frequency of alcohol intake, n (%)	473	0.239	0.03 (0.04)	0.57	0.307	-0.03 (0.04)	0.50
>5 drinks/day at least once/month, n (%)	366	0.232	0.01 (0.05)	0.81	0.301	-0.03 (0.05)	0.56
Started drinking age, years	472	0.238	0.01 (0.05)	0.83	0.306	0.003 (0.044)	0.94
Duration of drinking, years	472	0.239	-0.04 (0.06)	0.43	0.306	0.02 (0.05)	0.76
Volume of alcohol intake, L	457	0.242	0.07 (0.04)	0.13	0.314	0.08 (0.04)	**0.040**
ALT, U/L	1163	0.251	-0.02 (0.03)	0.41	0.320	-0.04 (0.03)	0.17
AST, U/L	1164	0.252	-0.04 (0.03)	0.21	0.320	-0.04 (0.03)	0.14
GGT, U/L	1164	0.251	0.01 (0.03)	0.76	0.317	0.01 (0.03)	0.75
CDT, %	1156	0.251	0.01 (0.03)	0.83	0.318	0.04 (0.03)	0.17
	Central Systolic BP[c]				**Central Pulse Pressure[d]**		
Self-reported alcohol, n (%)	1239	0.286	0.09 (0.03)	**0.010**	0.254	0.06 (0.03)	**0.020**
Alcohol intake, g/day*	1221	0.283	0.03 (0.03)	0.30	0.247	0.03 (0.03)	0.27
Daily frequency of alcohol intake, n (%)	473	0.270	0.005 (0.04)	0.89	0.235	0.04 (0.04)	0.35
>5 drinks/day at least once/month, n (%)	366	0.264	0.004 (0.05)	0.93	0.228	0.03 (0.05)	0.52
Started drinking age, years	472	0.272	0.05 (0.05)	0.32	0.236	0.06 (0.05)	0.22
Duration of drinking, years	472	0.273	-0.08 (0.06)	0.16	0.254	-0.12 (0.06)	**0.033**
Volume of alcohol intake, L	457	0.275	0.07 (0.04)	0.083	0.234	0.04 (0.04)	0.41
ALT, U/L	1163	0.282	-0.01 (0.03)	0.65	0.250	0.02 (0.03)	0.48
AST, U/L	1164	0.282	-0.03 (0.03)	0.28	0.246	-0.004 (0.03)	0.86
GGT, U/L	1164	0.282	0.023 (0.03)	0.40	0.247	0.03 (0.03)	0.23
CDT, %	1156	0.282	-0.008 (0.03)	0.77	0.248	-0.05 (0.03)	0.12

N, number of participants; [a]adjusted for baseline systolic blood pressure; [b] adjusted for baseline diastolic blood pressure. [c]adjusted for baseline systolic blood pressure; [d]adjusted for baseline pulse pressure. Bold p-values denote statistical significance where p≤0.05. Independent variables included in each model: a measure of alcohol intake, age, rural/urban, sex, BMI, glycosylated haemoglobin, total cholesterol to HDL ratio (TC:HDL), C-reactive protein (CRP), smoking, physical activity, hypertension medication at baseline and HIV status. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; CDT, carbohydrate deficient transferrin.*Alcohol intake as analysed using the quantified food frequency questionnaire (QFFQ).

DISCUSSION

This study evaluated self-reported estimates of alcohol intake, comparative to the accepted laboratory based biochemical assays, considering their association with BP elevations over 5 years in a black South African population. We found that self-reported alcohol intake significantly predicted the change in BP, which was not seen for the biochemical alcohol markers, GGT and %CDT. Self-reported alcohol intake also associated significantly with central SBP and pulse pressure at follow-up, also not found for the biochemical markers. Given the honesty of the participants, self-reported alcohol intake may perhaps be the most accurate indicator of alcohol use, when considering that biochemical variables are known to be influenced by age, obesity, liver damage and sex [13,14,20,21]. This may especially be of benefit in low resource settings in which biochemical markers are an expensive option in the public health sector, and not readily available in poor resource settings.

Our results confirm findings from previous studies that self-reported alcohol intake correlates well with other measured biochemical markers (such as GGT and %CDT) and BP. Although self-reported intake has for long been regarded as reliable [22,23], effective screening methods may be complicated by cultural differences in response to questions such as frequency of drinking [24]. The reliability of self-reporting is undermined by underreporting of alcohol abuse, which is common in high socio-economic populations, especially among young adults having to report excessive drinking [25,26]. Black South African school teachers with a high socio-economic status (SES) recently reported low use of alcohol but demonstrated high levels of GGT [27]. This places doubt on the honesty of self-reported alcohol intake especially in adults in the high income bracket [24], who are also likely to be involved in excessive drinking [28].

In 1991 Glovannucci et al.[29] indicated that a simple self-administered questionnaire can provide useful estimates of alcohol intake over an extended period of time. Furthermore, large studies have successfully employed self-reported alcohol intake to assess, for example, the association between alcohol consumption and CRP concentration [30], or BP. Tsai et al. [8] recently found

that self-reported excessive drinkers also reflected high GGT concentrations in both men and women after adjustment for confounders, confirming the honesty and reliability of self-reported alcohol use. Furthermore, the projected increase of GGT (about 314%) in excessive drinking men without history of liver damage confirmed the risk of developing cardiovascular disease in individuals abusing alcohol [31].

These findings from Tsai et al. [8] and a study in a Chinese population in Hong Kong [32] support the importance of biochemical markers such as GGT as a risk marker for hypertension and CVD. Furthermore, GGT prevailed as the only marker associated with stroke when used with self-reported alcohol use [33], further exposing the underestimation of reporting alcohol intake in high socio-economic populations. However, our study failed to show an association between elevation in BP and GGT presumably because of other factors such as liver damage, obesity and old age [13,14] that elevated GGT levels irrespective of alcohol intake. Moreover, Africans are known to have elevated GGT levels, independent of alcohol intake [27]. This could also have impacted on the lack of association between GGT and change in BP in this study population.

The strengths of our study are that the participants were from a low socio-economic environment with an average age of 50 ± 10.16 years, which is a group likely to report honesty regarding alcohol intake in an attempt to improve their health. Moreover, the presence of African field workers who would translate the questions into the participants' home language ensured that clear responses were obtained. Honest alcohol reporting is arguably the most accurate measure of alcohol intake, as GGT levels may be influenced by other conditions [13,14]. Although our study population may have reported honesty on alcohol intake, their low level of SES may have limited their ability to accurately report on other alcohol intake behaviour, e.g. the volume of alcohol intake. This study also adds to the limited availability of longitudinal studies on lifestyle and CVD in SSA. This study included participants from the North West Province (South Africa) and although these results may have wider application in other populations, this needs to be investigated. It is therefore recommended that consistency of self-reported use of alcohol be

explored in other populations and income groups. Finally, residual confounding cannot be excluded, although our results remained consistent after multiple adjustments.

We conclude that self-reported alcohol intake is independently associated with the 5-year change in BP of black South Africans with low SES. This association was not found for GGT and %CDT, which are biochemical alcohol markers known to relate to CVD [8,29]. We suggest that self-reported alcohol intake may be a highly useful and affordable measure of alcohol to use in populations wherein honest reporting is expected, such as in those with low SES. The early identification of excessive drinking through self-reporting may contribute in reducing the burden on the national health system regarding high incidence of non-communicable diseases.

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Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF do not accept any liability in regard thereto.

Conflicts of interest

There are no conflicts of interest.

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Supplemental Table 1. Single linear regression analysis of baseline blood pressure with alcohol markers.

Marker of alcohol intake	SBP (mm Hg)	DBP (mm Hg)
Self-reported alcohol use, n (%)	**r=0.17**	**r=0.17**
	p<0.001	**p<0.001**
Alcohol intake, g/day	r=-0.009	r-0.02
	p=0.84	p=0.62
Daily frequency of alcohol intake, n (%)	r=0.05	r=0.05
	p=0.25	p=0.33
>5 drinks/day once/month, n (%)	r=0.01	r=0.11
	p=0.79	p=0.032
Started drinking age, years	r=0.05	r=0.01
	p=0.29	p=0.81
Duration of drinking, years	**r=0.23**	**r=0.10**
	p<0.001	**p=0.039**
Volume of alcohol intake, L	r=0.07	r=0.07
	p=0.11	p=0.12
Alanine aminotransferase, IU/L	**r=0.11**	**r=0.15**
	p<0.001	**p<0.001**
Aspartate aminotransferase, U/L	**r=0.07**	**r=0.12**
	p=0.012	**p<0.001**
Gamma-glutamyltransferase, U/L	**r=0.15**	**r=0.20**
	p<0.001	**p<0.001**
Carbohydrate deficient transferrin, %	r=0.04	r=0.04
	p=0.15	p=0.17

Data in bold denote statistical significance where p≤0.05. SBP, systolic blood pressure; DBP, diastolic blood pressure

Supplemental Table 2. Partial regression analysis of baseline blood pressure with alcohol markers, adjusted for age, sex and body mass index.

Marker of alcohol intake	SBP (mm Hg)	DBP (mm Hg)
Self-reported alcohol use, n (%)	**r=0.21**	**r=0.23**
	p<0.001	**p<0.001**
Alcohol intake, g/day	r=-0.01	-0.003
	p=0.78	p=0.95
Daily frequency of alcohol intake, n (%)	r=0.06	r=0.04
	p=0.23	p=0.46
>5 drinks/day once/month, n (%)	r=0.04	r=0.12
	p=0.50	p=0.023
Started drinking age, years	r=-0.06	r=-0.07
	p=0.18	p=0.17
Duration of drinking, years	r=0.001	r=0.02
	p =0.97	p=0.74
Volume of alcohol intake, L	r=0.07	r=0.08
	p=0.16	p=0.12
Alanine aminotransferase, IU/L	**r=0.12**	**r=0.17**
	p<0.001	**p<0.001**
Aspartate aminotransferase, U/L	**r=0.12**	**r=0.16**
	p<0.001	**p<0.001**
Gamma-glutamyltransferase, U/L	**p=0.17**	**p=0.24**
	p<0.001	**p<0.001**
Carbohydrate deficient transferrin, %	**r=0.10**	**r=0.10**
	p=0.001	**p=0.001**

Data in bold denote statistical significance where p≤0.05. SBP, systolic blood pressure; DBP, diastolic blood pressure

Supplemental Table 3. Multiple regression analyses with percentage change in blood pressure as dependent variables, and one marker of alcohol intake as the main independent variable, after exclusion of participants who changed their reporting on alcohol intake over 5 years ("No" to "Yes" or "Yes" to "No").

Alcohol marker	% Brachial Systolic BP[a]				% Brachial Diastolic BP[b]		
	N	R^2	β value (SE)	p-value	R^2	β value (SE)	p-value
Self-reported alcohol, n (%)	899	0.261	0.17 (0.04)	**<0.001**	0.323	0.15 (0.04)	**<0.001**
Alcohol intake, g/day*	355	0.231	0.06 (0.05)	0.26	0.297	0.04 (0.05)	0.40
Daily frequency of alcohol intake, n (%)	311	0.225	-0.01 (0.05)	0.81	0.299	-0.08 (0.05)	0.11
>5 drinks/day at least once/month, n (%)	256	0.221	0.06 (0.06)	0.31	0.286	-0.007 (0.06)	0.89
Started drinking age, years	308	0.224	-0.002 (0.06)	0.97	0.293	-0.03 (0.06)	0.59
Duration of drinking, years	308	0.224	0.02 (0.07)	0.81	0.297	0.10 (0.07)	0.15
Volume of alcohol intake, L	305	0.227	0.06 (0.05)	0.26	0.300	0.09 (0.05)	0.07
ALT, U/L	837	0.245	-0.02 (0.03)	0.49	0.313	-0.04 (0.03)	0.16
AST, U/L	837	0.247	-0.04 (0.03)	0.17	0.313	-0.05 (0.03)	0.10
GGT, U/L	838	0.245	0.007 (0.03)	0.82	0.311	0.001 (0.03)	0.97
CDT, %	833	0.245	-0.02 (0.03)	0.56	0.311	0.01 (0.03)	0.74
		Central Systolic BP[c]			Central Pulse Pressure[d]		
Self-reported alcohol, n (%)	899	0.291	0.17 (0.04)	**<0.001**	0.260	0.12 (0.04)	**<0.001**
Alcohol intake, g/day*	355	0.264	0.06 (0.05)	0.23	0.239	0.06 (0.05)	0.27
Daily frequency of alcohol intake, n (%)	311	0.257	-0.006 (0.05)	0.91	0.236	0.07 (0.05)	0.21
>5 drinks/day at least once/month, n (%)	256	0.251	0.03 (0.06)	0.56	0.229	0.06 (0.06)	0.33
Started drinking age, years	308	0.257	-0.004 (0.06)	0.95	0.257	-0.004 (0.06)	0.95
Duration of drinking, years	308	0.258	0.05 (0.07)	0.42	0.232	-0.01 (0.07)	0.87
Volume of alcohol intake, L	305	0.262	0.07 (0.05)	0.15	0.233	0.04 (0.05)	0.45
ALT, U/L	837	0.277	-0.02 (0.03)	0.55	0.252	0.01 (0.03)	0.67
AST, U/L	837	0.277	-0.03 (0.03)	0.30	0.252	-0.001 (0.03)	0.98
GGT, U/L	838	0.277	0.02 (0.03)	0.44	0.254	0.04 (0.03)	0.22
CDT, %	833	0.277	-0.03 (0.03)	0.44	0.254	-0.05 (0.03)	0.14

N, number of participants; [a]adjusted for baseline systolic blood pressure; [b]adjusted for baseline diastolic blood pressure. [C]adjusted for baseline systolic blood pressure; [d]adjusted for baseline pulse pressure. Bold p-values denote statistical significance where p≤0.05. Independent variables included in each model: a measure of alcohol intake, age, rural/urban, sex, BMI, glycosylated haemoglobin, total cholesterol to HDL ratio (TC:HDL), C-reactive protein (CRP), smoking, physical activity, hypertension medication at baseline and HIV status. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; CDT, carbohydrate deficient transferrin.*Alcohol intake as analysed using the quantified food frequency questionnaire (QFFQ).

Supplemental Table 4. Multiple regression analyses with percentage change in BP as dependent variables, and one marker of alcohol intake as the main independent variable, after replacing baseline BMI with %BMI as one of the independent variables.

Alcohol marker	% Brachial Systolic BP[a]				% Brachial Diastolic BP[b]		
	N	R^2	β value (SE)	p-value	R^2	β value (SE)	p-value
Self-reported alcohol, n (%)	1239	0.263	0.09 (0.03)	**<0.001**	0.320	0.07 (0.03)	**0.032**
Alcohol intake, g/day*	535	0.248	0.02 (0.04)	0.59	0.309	0.02 (0.04)	0.55
Daily frequency of alcohol intake, n (%)	473	0.246	0.02 (0.04)	0.66	0.307	-0.03 (0.04)	0.47
>5 drinks/day at least once/month, n (%)	366	0.292	0.03 (0.05)	0.58	0.301	-0.02 (0.05)	0.65
Started drinking age, years	472	0.246	0.009 (0.05)	0.85	0.703	0.02 (0.04)	0.70
Duration of drinking, years	472	0.247	-0.05 (0.06)	0.35	0.306	-0.007 (0.05)	0.90
Volume of alcohol intake, L	457	0.249	0.06 (0.04)	0.13	0.314	0.09 (0.04)	**0.03**
ALT, U/L	1163	0.258	-0.01 (0.03)	0.67	0.318	-0.02 (0.03)	0.36
AST, U/L	1164	0.259	-0.03 (0.03)	0.31	0.319	-0.04 (0.03)	0.14
GGT, U/L	1164	0.258	0.02 (0.03)	0.48	0.318	0.019 (0.03)	0.49
CDT, %	1156	0.292	0.002 (0.03)	0.95	0.318	0.03 (0.03)	0.35
	Central Systolic BP[c]				**Central Pulse Pressure[d]**		
Self-reported alcohol, n (%)	1239	0.296	0.09 (0.03)	**0.005**	0.250	0.09 (0.03)	**0.008**
Alcohol intake, g/day*	535	0.287	0.07 (0.04)	0.06	0.239	0.09 (0.04)	**0.02**
Daily frequency of alcohol intake, n (%)	473	0.279	-0.001 (0.04)	0.97	0.243	0.03 (0.04)	0.43
>5 drinks/day at least once/month, n (%)	366	0.273	0.006 (0.05)	0.90	0.227	0.03 (0.05)	0.56
Started drinking age, years	472	0.280	0.04 (0.04)	0.40	0.234	0.04 (0.05)	0.36
Duration of drinking, years	472	0.283	-0.08 (0.05)	0.14	0.238	-0.10 (0.06)	0.07
Volume of alcohol intake, L	457	0.284	0.07 (0.04)	0.08	0.233	0.04 (0.04)	0.40
ALT, U/L	1163	0.291	-0.001 (0.03)	0.96	0.245	0.02 (0.03)	0.43
AST, U/L	1164	0.291	-0.02 (0.03)	0.46	0.245	0.003 (0.03)	0.92
GGT, U/L	1164	0.292	0.04 (0.03)	0.18	0.246	0.04 (0.03)	0.16
CDT, %	1156	0.291	-0.01 (0.03)	0.71	0.246	-0.04 (0.03)	0.17

N, number of participants; [a]adjusted for baseline systolic blood pressure; [b]adjusted for baseline diastolic blood pressure. [C]adjusted for baseline systolic blood pressure; [d]adjusted for baseline pulse pressure. Bold p-values denote statistical significance where p≤0.05. Independent variables included in each model: a measure of alcohol intake, age, rural/urban, sex, percentage change in BMI, glycosylated haemoglobin, total cholesterol to HDL ratio (TC:HDL), C-reactive protein (CRP), smoking, physical activity, hypertension medication at baseline and HIV status. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; CDT, carbohydrate deficient transferrin. *Alcohol intake as analysed using the quantified food frequency questionnaire (QFFQ).

Chapter 5

A comparison of the cardiometabolic profile of black South Africans with suspected NAFLD and excessive alcohol use

INSTRUCTIONS FOR AUTHORS (*Alcohol* Journal)

The *Alcohol* Journal is devoted to publishing multi-disciplinary biomedical research on all aspects of the actions or effects of alcohol on the nervous system or on other organ systems. Emphasis is given to studies into the causes and consequences of alcohol abuse and alcoholism, and biomedical aspects of diagnosis, etiology, treatment or prevention of alcohol-related health effects.

Papers submitted must not have been published in their current form or a substantially similar form elsewhere, and they are not under consideration by another publication.

Authors must state all possible conflicts of interest in the manuscript, including financial, consultant, institutional and other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript. Participants must sign an informed consent and it should be indicated in the article.

All authors should have made substantial contributions to all of the following: 1. the conception and design of the study, or acquisition of data, or analysis and interpretation of data, 2. drafting the article or revising it critically for important intellectual content, 3. final approval of the version to be submitted.

Manuscript preparation and submission

The structure of the article must be divided into sections: Introduction, Material and methods, Results and Discussion. The introduction should state the objectives of the study and provide adequate background, avoiding a detailed literature survey or a summary of the results. Materials and methods should provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described. Results should be clear and concise. The discussion should explore the significance of the results of the work. Extensive citations and discussion of published literature should be avoided.

Title page information: The title must be concise and informative with no abbreviations or formulae. It must contain authors' full affiliation addresses, including the country name. The corresponding author must be clearly indicated with his/her phone numbers and email address.

Abstracts: The abstract must briefly summarise the work limited to a short continuous paragraph of about 300 words, without sections or sub-headings. The purpose of the study must be clearly stated followed by the following: basic methods and procedures, the most important findings, and principal conclusions. Immediately after the abstract, a maximum of 6 keywords must be provided. Abbreviations in the abstract may be used only if necessary. Terms appearing frequently within the text of the manuscript may be abbreviated.

Acknowledgements: They should be at the end before the references. List here those individuals who provided help during the research.

Tables and Figures: Each table or figure should have a caption and must be cited consecutively in the text. Each table should include the appropriate column heads, and explanatory footnotes. Superscript lowercase letters in descending alphabetic order order as footnote symbols may be used. Statistical measures of variation, standard deviation, standard error of the mean must be identified in the footnotes.

References: All references cited in the text must be present in the reference list. The style for citations and references to be used is that used by the American Psychological Association-5th edition. The following are examples of common types of citations:

1. Original article: Lieber, C.S (1999). Carbohydrate deficient transferrin in alcoholic liver disease: mechanisms and clinical implications. Alcohol *19,* 249-254.
2. An edited book: Liu, Y., and Hunt, W.A., eds (1999). The 'Drunken' Synapse: Studies of alcohol related disorders (New York: Klewer Academic/Plenum Publishers).
3. An authored book: Cohen, J. (1988). Statistical Power Analysis for the Behavioural Sciences, 2nd edn (Hillsdale, NJ: Lawrence Erlbaum Associates).

A comparison of the cardiometabolic profile of black South Africans with suspected non-alcoholic fatty liver disease (NAFLD) and excessive alcohol use

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ABSTRACT

Excessive alcohol use and non-alcoholic fatty liver disease (NAFLD) are putative cardiovascular disease risk factors. In order to ease the identification of these conditions on primary healthcare level, we aimed to determine and compare the demographic and cardiometabolic characteristics of excessive alcohol users and those with suspected NAFLD in black South Africans. In the Prospective Urban and Rural Epidemiology study (North West Province, South Africa, N = 2021, collected in 2005) we selected 338 participants, namely: 1) alcohol users (N = 143) reporting 'yes' to alcohol intake, with high gamma-glutamyltransferase (GGT) ≥ 80 U/L and a percentage of carbohydrate-deficient transferrin (%CDT) ≥2%; 2) non-alcohol users (N = 127) self-reporting 'no' to alcohol intake with GGT ≤ 30 U/L and %CDT ≤ 2%; and 3) NAFLD group (N = 68) who were non-drinkers with GGT levels ≥ 60 U/L and %CDT ≤ 2%. The demographics indicated that the alcohol users were mostly men (73%) with a body mass index (BMI) of 19.8 (15.2–27.3) kg/m^2, 90% of whom were smokers. Systolic blood pressure (SBP) of alcohol users significantly correlated with high-density lipoprotein cholesterol (HDL-C) (β = 0.24; p = 0.003) and waist circumference (WC) (β = 0.22; p = 0.006). Non-alcohol users were mostly women (84%) with a BMI of 26.0 (18.0–39.2) kg/m^2, and blood pressure in this group related positively with triglycerides. The NAFLD group were also mostly women (72%) with a comparatively larger WC (p < 0.001) and an adverse metabolic profile (total cholesterol: 5.55 ± 1.69 mmol/L; glycosylated hemoglobin: 6.03 [4.70–9.40]%). Diastolic blood pressure in the NAFLD group associated positively with WC (β = 0.27; p = 0.018). We therefore found disparate gender and cardiometabolic profiles of black South Africans with suspected NAFLD and excessive alcohol use. The described profiles may aid healthcare practitioners in low resource settings when using these crude screening measures of gender, obesity indices, and self-reported alcohol use to identify individuals at risk.

Keywords: non-alcoholic steatohepatitis; blood pressure; low socioeconomic status; hypertension; cardiovascular disease; high-density lipoprotein cholesterol

INTRODUCTION

Cardiovascular disease (CVD) remains one of the leading causes of morbidity and mortality in sub-Saharan Africa (Opie & Seedat, 2005; Sliwa et al., 2008; Twagirumukiza et al., 2011). South Africa is experiencing a rapid rate of urbanization, which leads to lifestyle changes that contribute to the high prevalence of hypertension and type 2 diabetes (Stewart et al., 2011; Van Rooyen et al., 2000). The levels of alcohol intake and abuse have increased in Africans over the last decade and is considered one of the main contributors to hypertension (Schutte et al., 2012) and liver injury (Nguyen & Thuluvath, 2008) in this ethnic group.

Gamma-glutamyltransferase (GGT) is a well-known marker of alcohol intake and GGT levels are elevated in individuals who use alcohol excessively (Tsai, Ford, Li, & Zhao, 2012). GGT is also known to relate strongly with the development of CVD and may predict cardiovascular outcomes (Mason, Starke, & Van Kirk, 2010). Apart from the association of GGT with excessive alcohol intake, it is also influenced by other factors including age, obesity, and liver injury (Mason et al., 2010; Puukka et al., 2006; Torrente, Freeman, & Vrana, 2012). GGT is also elevated in patients with non-alcoholic fatty liver disease (NAFLD) even in the absence (or during very low intake) of alcohol (Bhatia, Curzen, Calder, & Byrne, 2012; Hall & Cash, 2012).

The prevalence of NAFLD has been shown to be positively associated with body weight (Bayard, Holt, & Boroughs, 2006) and is recently receiving significant attention as a putative CVD risk factor (Bhatia et al., 2012; Targher, Day, & Bonora, 2010; Tsuzaki, Kotani, Fujiwara, Sano, & Sakane, 2014). Obesity is common among black South Africans, contributing to the onset of hypertension (Puoane et al., 2002; Schutte et al., 2008). Although obesity is associated with NAFLD, a study carried out in South Africa did not find any association between the degree of obesity and severity of NAFLD in Africans (Kruger et al., 2010), possibly due to the low number of Africans (N = 12) used in their study. Additionally, patients with NAFLD are mostly asymptomatic, making it difficult to accurately characterize these individuals with NAFLD (Scaglioni, Ciccia, Marino, Bedogni, & Bellentani, 2011). Despite the availability of various tests used for indicating

excessive alcohol use (percentage carbohydrate-deficient transferrin [%CDT], ethyl glucuronide [EtG], ethyl sulfate [EtS], and phosphatidylethanol [PEth]), they are rather costly and not necessarily viable options for use in developing countries (Junghanns et al., 2009; Viel et al., 2012).

In a large African population cohort of 2021 participants, we characterized 3 subgroups for the purpose of this investigation: (1) self-reported excessive alcohol users, (2) abstainers (both the latter confirmed with %CDT and GGT), and (3) individuals with characteristics of NAFLD, who reported no or low alcohol intake, with low %CDT and elevated GGT. We aimed to compare the cardiovascular and metabolic characteristics of these 3 groups in order to obtain a better understanding regarding the risk factors contributing toward elevated blood pressure (BP) in each group.

MATERIALS AND METHODS

Study population

This is a sub-study of the international PURE (Prospective Urban and Rural Epidemiology) study and the detailed methodology has been described elsewhere (Schutte et al., 2012; Teo et al., 2009). This South African leg of the PURE study is based in the North West Province, where data was collected in 2005 from 2021 participants. We identified 338 individuals who complied with the inclusion criteria of the predetermined groups, namely, (1) those who reported 'yes' to alcohol intake, confirmed with a GGT ≥ 80 U/L and %CDT ≥ 2% (named "alcohol users") (N = 143); (2) those that self-reported 'no' to alcohol intake, confirmed by low GGT ≤ 30 U/L and %CDT ≤ 2% (named "non-alcohol users") (N = 127); and 3) those who presented with the characteristics of NAFLD, i.e., reported 'no' to alcohol intake but accompanied with GGT ≥ 60 U/L and %CDT levels ≤ 2% (named "NAFLD group") (N = 68). The cut offs for GGT and %CDT were selected based on the normal ranges of these biomarkers (Kratz, Ferraro, Sluss & Lewandrowski, 2004; Cash & Hall, 2012; Tsai et al 2012).

The study was approved by the Ethics Committee of the North-West University, South Africa and complied with the Helsinki Declaration of 1975, as revised in 2004. Permission was obtained from the Department of Health of the North West Province, community leaders, tribal chiefs, and mayors in this area to conduct the study. All subjects were informed about the objectives and procedures of the study and gave written informed consent prior to voluntary participation. Participants were asked to fast for 10 h prior to blood sample collection. Field workers were available to do the translation in the participant's native language. Confidentiality and anonymity of all the results were assured. Participants identified with chronic illnesses such as hypertension and Human Immunodeficiency Virus (HIV) were referred to their local clinics and hospitals with which prior arrangements were made. Afterward participants received a light meal. If any health risk was identified, they received counseling and a referral letter. Moreover, remuneration was offered to ensure they could go to clinics if referred.

Questionnaires

The participants were requested to complete structured demographic, socioeconomic, lifestyle, and physical activity questionnaires, conducted by trained African fieldworkers, developed and standardized for the international PURE study (Teo et al., 2009). The questions on alcohol consumption included a yes/no question on alcohol use (yes, current or former use; no, never used).

Anthropometric measurements

Each participant had height, body weight, waist, and hip circumferences measured (Precision Health Scale, A & D company, Tokyo, Japan; Invicta Stadiometer, IP 1465, Leicester, UK; Holtain unstretchable metal tape) using standardized methods (Marfel-Jones, Olds, Stewart, & Cartel, 2006).

Cardiovascular measurements

Cardiovascular measurements were executed after a 10-min rest period once BP had stabilized. Systolic and diastolic blood pressure (SBP, DBP) and heart rate were measured in duplicate,

5 min apart, with the validated Omron HEM-757 apparatus (Omron Healthcare, Kyoto, Japan), while the participants were seated upright with the right arm at heart level. Appropriately sized cuffs were used for obese participants. Pulse pressure (PP) was then calculated by subtracting DBP from SBP.

Blood, serum and plasma samples

As indicated earlier, each participant was requested to fast for approximately 10 h before blood sampling commenced. A registered nurse took a blood sample from the ante-brachial vein branches. Samples were prepared according to appropriate methods. In the rural area, samples were immediately frozen and stored at −18 °C for no longer than five days, transported to a laboratory facility and stored at −80 °C until analysis.

Biochemical analyses

From the collected blood samples, liver enzymes (GGT, alanine aminotransferase (ALT), aspartate aminotransferase (AST)), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), uric acid, C-reactive protein (CRP), and serum glucose (Konelab20i auto analyzer; Thermo Scientific, Vantaa, Finland) were determined. Serum iron was determined using immunological, colorimetric and high-performance liquid chromatography methods and glycosylated hemoglobin (HbA1c), in the EDTA-treated whole blood, using the D-10 Hemoglobin testing system (Bio-Rad Laboratories, Hercules, CA). We performed serum percentage carbohydrate deficient transferrin (%CDT) analyses using an *in vitro* heterogeneous immunoassay with column separation followed by a turbidimetric measurement (Axis-Shield %CDT kit, Oslo, Norway). The coefficient of variance (CV) for all assays was < 10%.

The HIV status of each participant was determined using whole blood samples and following the protocol of the National Department of Health, South Africa, using the First Response (PMC Medical, India) rapid HIV card test. The test result was confirmed using the Pareeshak (BHAT Bio-tech, India) card test also. Each participant received pre-test counseling before the test was

performed and post-test counseling for those who tested positive and had opted to know their results.

Statistical analyses

Statistica version 11 (StatSoft, Inc., Tulsa, OK) was used to compare the 3 groups as defined in the aim of the study. Variables with a non-Gaussian distribution, including body mass index (BMI) and all biochemical measurements (except glucose), were logarithmically transformed. Analysis of variance (ANOVA) was used to compare the characteristics of the 3 groups, and chi-square tests to compare proportions of the categorical variables. Using Pearson's correlations, we determined unadjusted associations between BP or PP and cardiometabolic variables. Partial correlations were performed after adjustment for gender and age. We employed forward stepwise multiple regression analyses to determine the independent association of BP or PP (dependent variable) with the following variables included as independent variables: age, gender, smoking, WC, HbA1c, HDL-C, CRP, TG, AST/ALT ratio, iron, uric acid, GGT and %CDT within each of the 3 groups.

RESULTS

The descriptive statistics for the 'alcohol users'; the 'non-alcohol users'; and the group reflecting characteristics of NAFLD are given in Table 1. The alcohol users were mostly men (73%), with a below average mean BMI of 19.8 (15.2–27.3) kg/m^2, comparatively the highest HDL-C levels and AST:ALT ratios ($p < 0.001$), 90% of which were smokers ($p < 0.001$). The non-alcohol users consisted largely of women (84%), with comparatively the lowest SBP and DBP of the three groups (both $p < 0.001$). The NAFLD group also comprised mostly of women (72%), with comparatively less smokers than the alcohol-users group, and characterized by the highest comparative abdominal adiposity of the three groups based on waist circumference ($p < 0.001$).

Moreover, the NAFLD group showed an unfavorable metabolic profile represented by elevated glucose, HbA1c, TG, and CRP (all $p < 0.001$). Serum iron and uric acid levels were comparable between alcohol users and the NAFLD group, but significantly lower in non-alcohol users

Table 1. Descriptive characteristics of the African study population.

	Alcohol users (n=143)	Non-alcohol users (n=127)	NAFLD group (n=68)	p-value
Socio-demographic profile				
Age, years	49.0 ± 8.71	49.3 ± 12.3	51.4 ± 11.9	0.26
Sex, women, n (%)	38 (27.0)[ac]	107 (84.3)[ab]	49 (72.1)[bc]	**<0.001**
Body mass index, kg/m^2	19.8 (15.2-27.3)[ab]	26.0 (18.0-39.2)[a]	28.0 (16.4-43.3)[b]	**<0.001**
Waist circumference, cm	74.3 ± 8.19[ab]	81.0 ± 13.0[ac]	88.0 ± 14.4[bc]	**<0.001**
Location, rural, n (%)	67 (46.9)	67 (52.8)	30 (44.1)	0.45
HIV infected, n (%)	14 (9.80)	18 (14.2)	12 (17.7)	0.25
Smoking, n (%)	128 (90.1)[ab]	44 (34.7)[a]	17 (25.0)[b]	**<0.001**
Unemployed, n (%)	127 (89.0)	113 (89.0)	65 (96.0)	0.250
Education, n (%)	78 (55.0)	78 (61.4)	44 (65.0)	0.302
Biochemical measurements				
Serum glucose, mmol/L	4.60 (3.30-6.25)[a]	4.83 (3.30-6.20)[b]	5.50 (3.60-8.10)[ab]	**<0.001**
Glycosylated haemoglobin, %	5.41 (4.80-6.00)[ab]	5.63 (4.90-6.40)[ac]	6.03 (4.70-9.40)[bc]	**<0.001**
Total cholesterol, mmol/L	5.08 ± 1.45	4.82 ± 1.13[a]	5.55 ± 1.69[a]	**0.003**
HDL-C, mmol/L	1.90 (0.81-3.01)[ab]	1.18 (0.65-2.16)[a]	1.32 (0.76-2.51)[b]	**<0.001**
TC:HDL	2.57 (1.56-5.71)	3.99 (2.25-7.39)[a]	3.99 (2.30-7.70)[a]	**<0.001**
Triglycerides, mmol/L	1.08 (0.56-2.69)[a]	1.06 (0.55-2.13)[b]	1.55 (0.68-4.00)[ab]	**<0.001**
C-reactive protein, mg/L	2.93 (0.27-41.8)	2.60 (0.27-22.2)[a]	4.97 (0.48-25.2)[a]	**0.010**
Iron, U/L	27.0 (9.40-63.2)[a]	16.3 (6.60-31.4)[ab]	24.0 (8.42-54.0)[b]	**<0.001**
Uric acid, mmol/L	0.46 (0.21-1.10)[a]	0.33 (0.17-0.99)[ab]	0.46 (0.21-1.32)[b]	**<0.001**
Markers of alcohol intake				
Self-reported alcohol use, n (%)	143 (100)[ab]	0 (0)[a]	0 (0)[b]	**<0.001**
>5 drinks/day, once/month, n (%)	24/93 (25.8)	0/1 (0)	0/3 (0)	0.504
Alanine aminotransferase, IU/L	30.1 (10.0-90.0)[a]	13.6 (7.00-25.0)[ab]	25.0 (12.0-47.0)[b]	**<0.001**
Aspartate aminotransferase, U/L	59.2 (20.0-180)[ac]	20.2 (9.54-37.0)[ab]	32.0 (14.0-90.0)[bc]	**<0.001**
AST:ALT	1.95 (0.77-4.33)[ab]	1.48 (0.65-3.00)[a]	1.26 (0.65-2.85)[b]	**<0.001**
ɣ-glutamyl transferase, U/L	186 (84.7-743)[ab]	23.1 (17.0-30.0)[ac]	102 (61.2-323)[bc]	**<0.001**
Carbohydrate deficient transferrin, %	5.10 (3.72-7.98)[ab]	1.48 (0.96-1.90)[a]	1.40 (0.96-1.98)[b]	**<0.001**
Cardiovascular variables at baseline				
Systolic blood pressure, mmHg	137 ± 23.7[a]	130 ± 23.5[ab]	139 ± 25.3[b]	**0.001**
Diastolic blood pressure, mmHg	90.4 ± 16.0[a]	85.2 ± 14.4[a]	90.0 ± 13.1	**0.001**
Pulse pressure, mmHg	46.6 ± 13.6	44.5 ± 15.2	49.5 ± 17.5	0.098
Heart rate, bpm	78.3 ± 18.0[a]	71.0 ± 13.4[a]	72.0 ± 13.8	**<0.001**
Anti-hypertensive medication, n (%)	5 (3.50)[ab]	15 (11.8)[a]	13 (19.1)[b]	**0.001**

Values are interpreted as arithmetic mean ± standard deviation; geometric mean (5th to 95th percentile interval); number of participants (%). Values with the same superscript letter differ significantly, and bold p-values denote statistical significance where p≤0.05. NAFLD, non-alcoholic fatty liver disease; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus.

($p < 0.05$). When directly comparing men and women (Supplementary Table 1), we found women to have significantly higher BMI, WC, and lower self-reported alcohol intake, GGT and %CDT (all $p < 0.001$).

We performed single linear regression (Fig. 1; Supplementary Table 2) and partial regression (Table 2) analyses between BP and several independent variables (BMI, WC, HbA1c, iron, uric acid, CRP, HDL-C, TG, smoking, AST/ALT, GGT, and %CDT) in each of the 3 groups. In alcohol users, SBP was positively associated with HDL-C and WC (Fig. 1); and PP showed positive associations with age and HDL-C (Supplementary Table 2). In non-alcohol users, SBP was associated significantly with age and TG; DBP with TG; and PP with age and TG (Fig. 1 and Supplementary Table 2). In the NAFLD group both SBP and DBP correlated positively with age, and DBP further showed a positive association with WC (Supplementary Table 2).

After adjusting for confounders (gender and age), in alcohol users BP remained significantly associated with HDL-C and WC, and PP showed positive associations with iron ($r = 0.17$, $p = 0.050$) (Table 2). While in non-alcohol users both SBP and DBP remained significantly associated with TG, positive associations with iron and uric acid emerged. In NAFLD group DBP was strongly associated with WC.

Forward stepwise regression analyses with BP or PP as dependent variables are shown in Table 3. In all the groups, SBP and PP were independently associated with age. In alcohol users, SBP was independently associated with WC and HDL-C ($\beta = 0.22$ and $\beta = 0.24$, respectively); DBP related positively with gender, WC, and AST:ALT, and PP showed significant positive associations with HDL-C. In non-alcohol users, SBP, DBP and PP related positively to TG ($\beta = 0.25$ to 0.28; $p \leq 0.004$), while SBP and DBP showed positive associations with iron and uric acid, respectively. Finally, in the NAFLD group, DBP was significantly associated with WC ($\beta = 0.27$; $p = 0.018$).

Sensitivity analyses

We excluded all individuals who tested positive for HIV infection and repeated the analyses. The results remained the same irrespective of the HIV status of the participants.

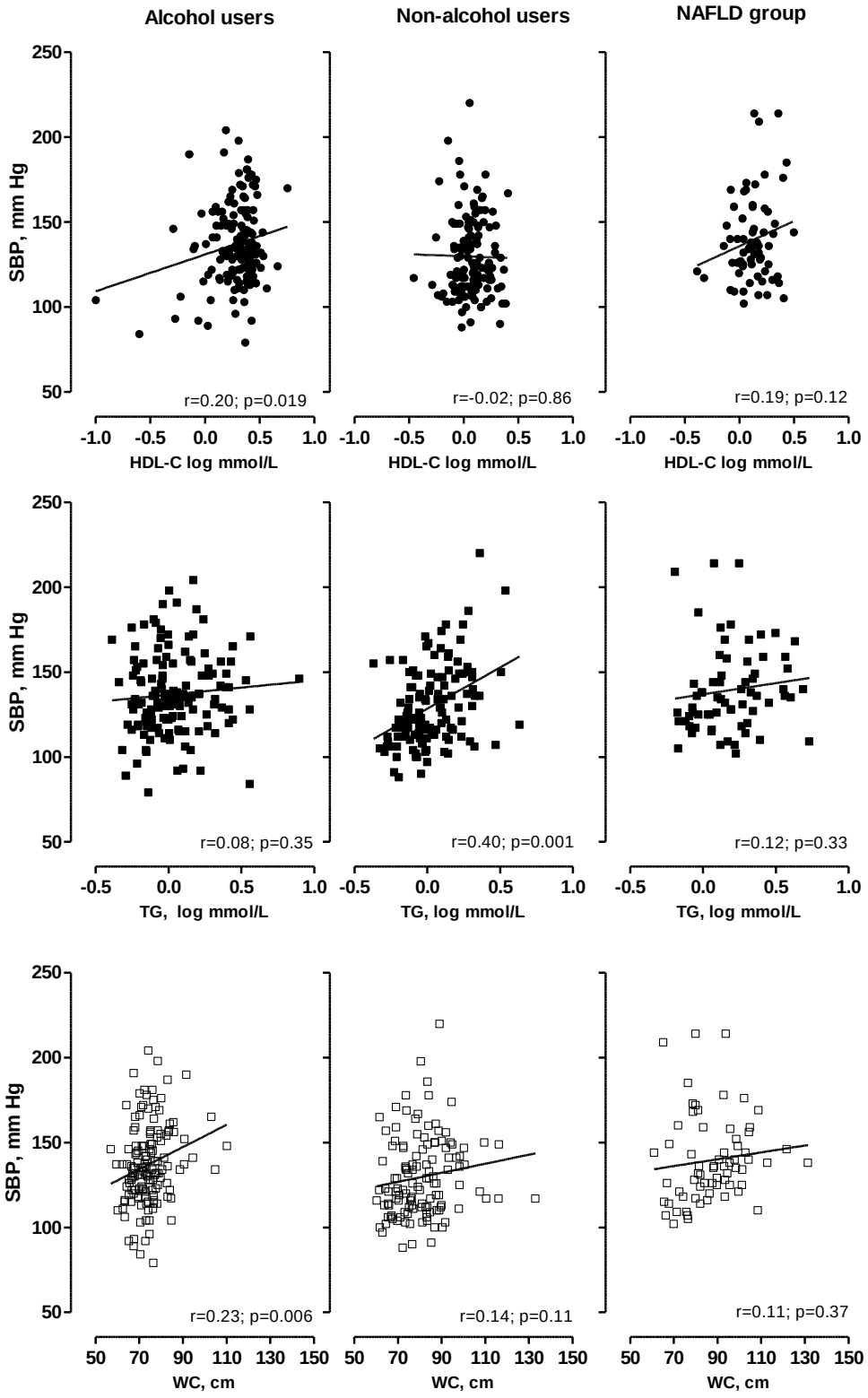

Figure 1. Association of SBP with HDL-C, TG and WC in alcohol users, non-alcohol users and NAFLD group. SBP, systolic blood pressure; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; WC, waist circumference.

Table 2. Partial correlations of blood pressure with cardiometabolic variables, adjusted for sex and age.

	Alcohol users (N=143)			Non-alcohol users (N=127)			NAFLD group (N=68)		
Independent Variable	**SBP, mm Hg**	**DBP, mm Hg**	**PP, mm Hg**	**SBP, mm Hg**	**DBP, mm Hg**	**PP, mm Hg**	**SBP, mm Hg**	**DBP, mm Hg**	**PP, mm Hg**
BMI, kg/m^2	**r=0.20;p=0.016**	**r=0.21;p=0.013**	r=0.11;p=0.17	r=0.13;p=0.16	r=0.16;p=0.08	r=0.04;p=0.68	r=0.09;p=0.47	**r=0.30; p=0.016**	r=-0.12;p=0.35
WC, cm	**r=0.20;p=0.020**	**r=0.20;p=0.021**	r=0.13;p=0.12	r=0.11;p=0.23	r=0.11;p=0.23	r=0.06;p=0.52	r=0.08;p=0.51	**r=0.28; p=0.025**	r=-0.11;p=0.37
HbA1c, %	r=-0.08;p=0.36	r=-0.14;p=0.11	r=0.02;p=0.77	r=0.04;p=0.64	r=-0.02;p=0.85	r=0.09; p=0.35	r=-0.07;p=0.59	r=-0.08; p=0.50	r=-0.17;p=0.18
Iron, U/L	r=0.08;p=0.38	r=-0.03;p=0.74	**r=0.17;p=0.050**	**r=0.22;p=0.013**	r=0.17;p=0.07	**r=0.19;p=0.042**	r=-0.13;p=0.30	r=-0.08; p=0.54	r=-0.12; p=0.34
Uric acid, mmol/L	r=0.03;p=0.70	r=0.05;p=0.57	r=0.0002;p=0.99	r=0.10;p=0.26	**r=0.20;p=0.030**	r=-0.04;p=0.66	r=-0.02;p=0.89	r=0.09; p=0.43	r=-0.11;p=0.39
CRP, mg/L	r=-0.07;p=0.44	r=-0.04;p=0.60	r=-0.06;p=0.45	r=-0.02;p=0.84	r=-0.01;p=0.90	r=-0.02;p=0.84	r=0.09;p=0.49	r=0.05; p=0.68	r=0.08;p=0.52
HDL-C, mmol/L	**r=0.21;p=0.011**	r=0.14;p=0.11	**r=0.22;p=0.010**	r=-0.03;p=0.71	r=-0.01;p=0.90	r=-0.04;p=0.64	r=0.15;p=0.24	r=0.16; p=0.19	r=0.07;p=0.56
TG, mmol/L	r=0.03;p=0.69	r=0.005;p=0.96	r=0.05;p=0.52	**r=0.30;p=0.001**	**r=0.22;p=0.014**	**r=0.24;p=0.007**	r=0.02;p=0.88	r=0.03; p=0.83	r=0.005;p=0.97
Smoking, n (%)	r=-0.007;p=0.93	r=-0.04;p=0.61	r=0.04;p=0.65	r=-0.10;p=0.26	r=-0.02;p=0.81	r=-0.14;p=0.13	r=-0.07;p=0.59	r=-0.11;p=0.37	r=-0.002;p=0.99
AST/ALT	r=0.02;p=0.83	r=0.12;p=0.18	r=-0.10;p=0.23	r=-0.08;p=0.40	r=0.002;p=0.98	r=-0.13;p=0.17	r=-0.03;p=0.84	r=-0.08; p=0.55	r=0.03;p=0.83
GGT, U/L	r=0.10;p=0.24	r=0.14;p=0.09	r=0.009;p=0.92	r=0.05;p=0.58	r=0.06;p=0.54	r=0.02;p=0.81	r=-0.06;p=0.65	r=-0.05; p=0.67	r=-0.04;p=0.77
CDT, %	r=0.12;p=0.17	r=0.009;p=0.92	r=0.10;p=0.25	r=0.05;p=0.60	r=-0.03;p=0.76	r=0.11;p=0.25	r=0.18;p=0.15	r=0.22; p=0.080	r=0.07;p=0.57

Bold data denote statistical significance where p≤0.05. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; HbA1c, glycosylated haemoglobin; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; CDT, carbohydrate deficient transferrin.

Table 3. Forward stepwise regression analysis with blood pressure (SBP, DBP) and pulse pressure (PP) as dependent variables.

Alcohol users (n=143)						
	SBP, mm Hg R^2=0.146		**DBP, mm Hg** R^2=0.136		**PP, mm Hg** R^2=0.144	
Independent variable	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value
Age, years	0.25 (0.08)	**0.002**			0.35 (0.08)	**<0.001**
Sex (0, men; 1, women)	0.16 (0.09)	0.06	0.30 (0.08)	**<0.001**		
WC, cm	0.22 (0.08)	**0.006**	0.25 (0.08)	**0.003**		
HDL-C, mmol/L	0.24 (0.08)	**0.003**	0.15 (0.09)	0.09	0.19 (0.08)	**0.020**
AST/ALT			0.19 (0.08)	**0.030**		
Non-alcohol users (N=127)						
	SBP, mm Hg R^2=0.245		**DBP, mm Hg** R^2=0.090		**PP, mm Hg** R^2=0.251	
Independent variable	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value
Age, years	0.31 (0.09)	**<0.001**			0.33 (0.08)	**<0.001**
Triglycerides, mmol/L	0.28 (0.08)	**0.001**	0.25 (0.09)	**0.004**	0.26 (0.08)	**0.003**
Iron, U/L	0.19 (0.08)	**0.020**				
Uric acid, mmol/L			0.19 (0.09)	**0.030**		
Smoking, n (%)					-0.16 (0.08)	**0.040**
NAFLD group (N=68)						
	SBP, mm Hg R^2=0.326		**DBP, mm Hg** R^2=0.167		**PP, mm Hg** R^2=0.329	
Independent variable	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value
Age, years	0.58 (0.10)	**<0.001**	0.33 (0.11)	**0.005**	0.58 (0.10)	**<0.001**
Waist circumference, cm			0.27 (0.11)	**0.018**		

Bold p-values denote statistical significance where p≤0.05. N, number of participants; NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol. Independent variables included in the model: age, sex, waist circumference, smoking, glycosylated haemoglobin, high-density lipoprotein cholesterol, triglycerides, C-reactive protein (CRP), iron, uric acid, AST/ALT, gamma-glutamyltransferase (GGT), percentage carbohydrate deficient transferrin (%CDT).

DISCUSSION

We compared the cardiometabolic profiles of black South Africans according to three categories related to liver function, namely alcohol users, non-alcohol users, and those with suspected NAFLD. Although no age differences were encountered, we found gender, metabolic, and cardiovascular differences characterizing the 3 groups.

We found that alcohol users consisted mostly of lean men (73%), with a high percentage of smokers (90%). The most recent South African National Health and Nutrition Examination Survey (Shisana et al., 2013) reported that 31% of adult men were alcohol consumers, in contrast to only 9.3% of adult women. Traditionally, alcohol in Africans was only used by adult men socially and as a sign of masculinity, while drinking in women was associated with derision, condemnation, and even divorce (Marques-Vidal et al., 2010; Setlalentoa, Pisa, Thekisho, Ryke, & Loots, 2010). In our study, we confirmed that men are the main alcohol users in many of the households of the North West Province impoverished areas.

Black South Africans are known to have a favorable lipid profile (Opie & Seedat, 2005), with or without alcohol intake (Wakabayashi, 2008). However, we found that during states of alcohol overuse HDL-C levels are elevated, and resultant increase in BP ensues. This association is likely to predict the later development of hypertension in excessive alcohol users (Ariesen, Claus, Rinkel, & Algra, 2003; Schutte et al., 2012).

Excessive alcohol consumption is a risk factor for stroke (Ariesen et al., 2003), and despite the general inverse association between HDL-C and stroke (Wang et al., 2011), Bots et al. (2002) reported a positive association between these. Specific types of polymorphisms of HDL-C are known to lead to very high serum HDL-C levels of larger particle size, which has been associated with an increased risk for CVD (Van der Steeg et al., 2008). To the contrary, excessive alcohol intake was very recently positively associated with small-sized HDL-C particles and increased risk for coronary heart disease (Akinkuolie, Paynter, Padmanabhan, & Mora, 2014; Tsuzaki et al., 2014). This suggests that the elevated HDL-C previously reported to be associated with alcohol

overuse may have detrimental cardiovascular effects based on the HDL-C particle size and may also be influenced by genetic and metabolic factors influencing HDL-C particle size. Future studies in the black South African population should take into account analyses of HDL-C particle size to shed more light on understanding the positive association with BP.

Body composition was distinctly different in our groups with alcohol users being underweight to lean compared to overweight to obese in non-alcohol users and the NAFLD group. This may be explained by the interaction of smoking and alcohol overuse to influence weight, since only 10% of our group did not smoke. According to Koppes, Twisk, Van Mechelen, Snel, & Kemper, 2005 smoking is a major confounder in the association between alcohol intake and body weight. Smoking increases energy expenditure and reduces appetite, leading to weight loss (Chiolero, Faeh, Paccaud, & Cornuz, 2008). However, alcohol intake alone has also been associated with weight loss (Breslow & Smothers, 2005; Lukasiewicz et al., 2005). Contradictory to this, Yeomans (2010) reported a positive relationship between alcohol consumption and energy intake, suggesting that alcohol increases subsequent food intake, confirmed by Schröder et al. (2007) indicating a positive correlation between measures of obesity and alcohol. Alcohol consumption in small amounts is generally considered an appetite enhancer, however, these associations need to be interpreted with care, as there are many physiological, genetic, psychological, societal, and economic factors that are at play when looking at the association of alcohol consumption and weight.

In our study, the excessive alcohol use and the associated lean body mass reported in the alcohol-users group, suggest they are at risk for stroke (Andersen & Olsen, 2013; Kolapo & Vento, 2011). Apart from our previous argument linking elevated HDL-C to stroke, the elevated BP caused by alcohol overuse can additionally damage and narrow the microvasculature in the brain, depriving the brain cells of oxygen and nutrients, increasing the risk of stroke (Tikhonoff, Zhang, Richart, & Staessen, 2009). On the other hand, it is well known that moderate alcohol consumption is associated with a reduced risk for stroke (Elkind et al., 2006). Considering this, it is currently highly debatable as to whether health education in low resource settings should

promote any alcohol use whatsoever, due to lack of education and potential misinterpretation of this information.

The NAFLD group of this study consisted mostly of overweight or obese women, with DBP significantly correlating with abdominal adiposity. Furthermore, this group had the highest blood pressures, dysmetabolic profiles and inflammatory markers comparatively. As previously reported, obesity and hypertension are highly prevalent in black South Africans and has subsequently characterized this group as high risk for developing both CVD (Puoane et al., 2002; Schutte et al., 2008) and NAFLD (Vernon, Baranova, & Younossi, 2011). NAFLD has become a putative CVD risk factor, possibly because of its close association with insulin resistance, obesity, and a dysmetabolic profile (Bhatia et al., 2012). Obesity and type 2 diabetes are the two risk factors for NAFLD that are more common in black women (Hilawe, Yatsuya, Kawaguchi, & Aoyama, 2013; Tuei, Maiyoh, & Ha, 2010; van der Merwe & Pepper, 2006). As a result, African women are at more risk for NAFLD than men.

Published articles by Onyekwere, Ogbera, & Balogun (2011) and Matsha et al. (2014) on NAFLD risk factors within the African population provide an indication that NAFLD is rising in sub-Saharan Africa. Fujita & Hata (2013) also showed a positive correlation of abdominal obesity and BP, and its association with stroke. Huang, Gusdon, & Qu (2013) additionally reported that NAFLD is increasing proportionately with the obesity epidemic. A number of groups also report women to be at greater risk for developing NAFLD and associated complications, which supports our findings of 72% of the suspected NAFLD group being women (Fujita & Hata, 2013; Ong et al., 2005). Although black South Africans are known for a favorable lipid profile, they are at more risk than white populations to develop NAFLD and CVD, possibly because of lifestyle changes associated with urbanization (Schutte et al., 2012). One important mechanism shown to contribute to NAFLD is increased lipolysis and lipid synthesis, as a result of insulin resistance and hyperglycemia associated with the metabolic syndrome. This is thought to be induced by changes to diet and physical activity and interventions to these are shown to correct this state (Huang et al., 2013).

Lastly, despite the non-alcohol users being characterized as a control group due to normal liver function and generally favorable cardiometabolic profiles, their mean BMI fell within the overweight range (26.0 [18.0-39.2] kg/m^2) in addition to 12% of this group using anti-hypertensive medication. This group also consisted mostly of women (84%) and their BP associated positively and independently with TG. Elevated TG is a marker of dyslipidemia, which is normally followed by hepatic accumulation of TG (Gaggini et al., 2013). Uric acid, which correlated well with DBP in these non-alcohol users, is elevated in obesity (Tsushima et al., 2013) and is associated with hypertension (Hwu & Lin, 2010). Palmer, Schutte, & Huisman (2010) found significant correlations between BP and uric acid, but these associations seem to be linked to obesity measures as they disappeared after adjustment for confounders.

Considering this, the high TG and the uric acid levels in the non-alcohol group may be indicative of an increased risk of hypertension and CVD. Moreover, as previously reported, metabolic syndrome-related elevations in uric acid is more common among blacks (DeBoer, Dong, & Gurka, 2012). The association of SBP with iron in our study, and its link to metabolic syndrome, is also supported by previous reports in the literature (DeBoer et al., 2012; Houschyar et al., 2012; Rajapurkar et al., 2012). There is also evidence for the higher serum iron levels, its association with oxidative stress (Muñoz-Bravo, Gutiérrez-Bedmar, Gómez-Aracena, García-Rodríguez, & Navajas, 2013), and the increased severity of atherosclerosis and coronary artery disease (Bagheri et al., 2013).

These results should be interpreted within the context of its strengths and limitations. One of the major strengths of this investigation was the use of African field workers to obtain reliable responses from the questionnaires. However, as we recruited all our participants from a low socioeconomic background, only from North West Province of South Africa, the results may not be a true reflection of the whole country. Moreover, the design of this study is cross-sectional and all results were based on associations. Although our results were consistent after multiple adjustments, residual confounding cannot be excluded. Finally, our study did not include the

association of HDL-C particle size and alcohol consumption, and this needs to be investigated by similar future studies.

In conclusion, we found distinctly different gender and cardiometabolic profiles for black South Africans with suspected NAFLD versus excessive alcohol users. The NAFLD group was comprised mainly of obese women with a dysmetabolic profile, while the alcohol-user group consisted mostly of lean men whose SBP and PP were independently positively associated with HDL-C. These disparate profiles may guide healthcare practitioners in primary healthcare clinics to identify individuals with elevated GGT levels who may suffer from NAFLD or alcohol overuse. These results follow similar calls of previous findings that emphasize the importance of modifiable risk factors as the main contributor of CVD in sub-Saharan Africa, and that lifestyle change should be the main focus of the national health systems and legislators in developing areas like sub-Saharan Africa. Additionally, it could further be investigated as to whether these disparate profiles may guide healthcare practitioners in primary healthcare clinics to identify individuals with elevated GGT levels who may suffer from NAFLD or alcohol overuse.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1. The comparison of the characteristics of African men and women.

	Men	Women	p-value
Socio-demographic profile			
N	144	194	
Age, years	48.1 ± 10.0	51.0 ± 12.0	**0.050**
Unemployed, n (%)	128 (89.0)	177 (91.0)	0.47
Education, n (%)	78 (54.0)	122 (63.0)	0.11
Weight, kg	57.4 ± 11.4	68.0 ± 20.0	**<0.001**
Body mass index, kg/m^2	20.0 (15.6 – 27.0)	26.4 (17.4 – 43.3)	**<0.001**
Waist circumference, cm	75.8 ± 8.90	82.4 ± 14.2	**<0.001**
Markers of alcohol			
Self-reported alcohol intake, n (%)	105 (73.0)	38 (20.0)	**<0.001**
Gamma-glutamyltransferase, U/L	131 (24.7 – 684)	50.0 (17 – 358)	**<0.001**
Carbohydrate deficient transferrin, %	3.77 (1.31 – 7.98)	1.81 (0.98 – 4.58)	**<0.001**

Bold p-values denote statistical significance where p≤0.05. Values are interpreted as arithmetic mean ± standard deviation; geometric mean (5th to 95th percentile interval); number of participants (%).

Supplementary Table 2. Pearson's correlations of blood pressure with cardiometabolic variables.

	Alcohol users (N=143)			Non-alcohol users (N=127)			NAFLD group (N=68)		
Independent Variable	**SBP, mm Hg**	**DBP, mm Hg**	**PP, mm Hg**	**SBP, mm Hg**	**DBP, mm Hg**	**PP, mm Hg**	**SBP, mm Hg**	**DBP, mm Hg**	**PP, mm Hg**
Age, years	**r=0.26;p=0.002**	r=0.10;p=0.26	**r=0.34;p=0.001**	**r=0.39;p<0.001**	r=0.17;p=0.057	**r=0.44;p<0.001**	**r=0.58;p<0.001**	**r=0.34;p=0.004**	**r=0.58;p<0.001**
Sex, 0 1	r=0.15;p=0.057	**r=0.29;p<0.001**	r=-0.06;p=0.44	r=0.10;p=0.29	r=0.13;p=0.15	r=0.03;p=0.77	r=0.17;p=0.17	r=0.24;p=0.055	r=0.07;p=0.56
BMI, kg/m^2	**r=0.24;p=0.004**	**r=0.27;p=0.001**	r=0.10;p=0.22	r=0.15;p=0.10	**r=0.20;p=0.029**	r=0.04;p=0.63	r=0.15;p=0.20	**r=0.35; p=0.004**	r=-0.03;p=0.78
WC, cm	**r=0.23;p=0.006**	**r=0.20;p=0.017**	**r=0.16;p=0.05**	r=0.14;p=0.11	r=0.14;p=0.11	r=0.08;p=0.35	r=0.11;p=0.37	**r=0.29; p=0.017**	r=-0.05;p=0.67
HbA1c, %	r=-0.05;p=0.58	r=-0.13;p=0.13	r=0.07;p=0.42	r=0.13;p=0.16	r=-0.03;p=0.76	r=0.17; p=0.062	r=-0.13;p=0.29	r=-0.16; p=0.20	r=-0.07;p=0.57
Iron, U/L	r=0.05;p=0.53	r=-0.04;p=0.67	r=0.05;p=0.53	r=0.15;p=0.09	r=0.14;p=0.13	r=0.11;p=0.24	**r=-0.29;p=0.016**	r=-0.19; p=0.12	**r=-0.28; p=0.020**
Uric acid, mmol/L	r=0.07;p=0.42	r=0.04;p=0.61	r=0.07;p=0.42	r=0.14;p=0.11	**r=0.20;p=0.023**	r=0.03;p=0.75	r=-0.03;p=0.79	r=0.09; p=0.47	r=-0.11;p=0.36
CRP, mg/L	r=-0.03;p=0.72	r=-0.04;p=0.62	r=-0.005;p=0.95	r=-0.07;p=0.41	r=-0.05;p=0.59	r=-0.07;p=0.45	r=0.20;p=0.09	r=0.14; p=0.27	r=0.19;p=0.12
HDL-C, mmol/L	**r=0.20;p=0.019**	r=0.15;p=0.07	**r=0.17;p=0.047**	r=-0.02;p=0.86	r=-0.009;p=0.99	r=-0.02;p=0.79	r=0.19;p=0.12	r=0.20; p=0.09	r=0.12;p=0.31
TG, mmol/L	r=0.08;p=0.35	r=0.07;p=0.42	r=0.06;p=0.49	**r=0.40;p=0.001**	**r=0.26;p=0.003**	**r=0.37;p=0.001**	r=0.12;p=0.33	r=0.11; p=0.39	r=0.09;p=0.46
Smoking, n (%)	r=-0.06;p=0.47	r=-0.11;p=0.18	r=0.03;p=0.75	r=-0.12;p=0.17	r=-0.02;p=0.81	r=-0.17;p=0.056	r=-0.05;p=0.69	r=-0.02;p=0.86	r=-0.09;p=0.47
AST/ALT	r=0.002;p=0.98	r=0.08;p=0.32	r=-0.09;p=0.26	r=-0.03;p=0.78	r=0.04;p=0.68	r=-0.004;p=0.97	r=-0.15;p=0.23	r=-0.15; p=0.21	r=-0.09;p=0.43
GGT, U/L	r=0.04;p=0.63	r=0.10;p=0.25	r=-0.04;p=0.63	r=0.07;p=0.44	r=0.05;p=0.60	r=0.06;p=0.49	r=-0.07;p=0.56	r=-0.05; p=0.70	r=-0.07;p=0.58
CDT, %	r=0.03;p=0.76	r=-0.05;p=0.59	r=0.09;p=0.24	r=-0.02;p=0.83	r=-0.07;p=0.46	r=0.03;p=0.71	r=0.22;p=0.070	**r=0.27; p=0.030**	r=0.13;p=0.31

Bold p-values denote statistical significance where p≤0.05. Sex (0, men; 1, women); SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; HbA1c, glycosylated haemoglobin; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; CDT, carbohydrate deficient transferrin.

Chapter 6

Alcohol intake, hypertension development and mortality in black South Africans

INSTRUCTIONS FOR AUTHORS *(Eur J Prev Cardiol)*

European Journal of Preventive Cardiology publishes papers on all aspects of clinical and public health disciplines that address the causes and prevention of cardiovascular disease, as well as cardiovascular rehabilitation and exercise physiology. For original articles, the journal requires a maximum of 5000 words, equivalent to 6 journal pages, with each table or figure reducing that word count by 250. A manuscript will be considered for publication on the understanding that it has not been published before and is not under consideration by another publication.

All contributing authors must have made a substantial contribution to the concept and design, data collection and analysis, and interpretation of results. They must also have drafted the article or revised it critically for important intellectual content, and finally approved the version to be published. Authors must state all possible conflicts of interest in the manuscript and a declaration thereof must be indicated at the end of the article after acknowledgements and before the references. All sources of funding should be acknowledged in the manuscript.

Patients or study participants have a right to privacy that should not be infringed without informed consent. When informed consent has been obtained it should be indicated in the published article. The study should also have been approved by the Ethics committee of the relevant institution, and statement thereof clearly stated in the methods.

Manuscript preparation and submission

The text should be double spaced throughout and be on 10 or 12 font. The pages must be numbered consecutively including title page. All original papers must be arranged in sections under the headings Title page, Abstract, Keywords, Text, Tables and Figures.

Title page: The title page should carry the full title of the paper, consisting of no more than 20 words, and should be clear and brief, conveying the message of the paper. All authors' names, the full first name and last name of each author should appear, the affiliations of all authors, the

sources of any support should appear in the title page. The names and full address of the corresponding author including email address should also be included.

Abstract and keywords: The abstract should not exceed 250 words and should be divided into sections: Background, Design, Methods, Results and Conclusions. The abstract should be followed by a list of 3-10 keywords which will assist the cross-indexing of the article.

Text: Full original papers should be divided into sections headed Introduction, Methods, Results and Discussion. Abbreviations should be written out on first mention followed by the abbreviation in parentheses.

Tables: Each table or figure should be typed on a separate sheet and have a caption and must be cited consecutively in the text. Abbreviations and any symbols should be defined in the order in which they appear in the table and must be spelled out in the table footnotes. Statistical measures of variation, standard deviation, standard error of the mean must be identified in the footnotes.

References: The reference numbers must have full points in the reference list. Publications must be referenced in the order in which they appear in the text and abbreviations of journals must follow the Index Medicus style. Only three authors must be listed. If more than that, then the first three authors and represent the rest with et al. Superscript numericals after punctuation should be used in text citations.

Examples:

1. Journal article: Le-Ha C, Beilin LJ, Burrows S, Huang R-C, et al. Oral contraceptives use in girls and alcohol consumption in boys are associated with increased blood pressure in late adolescence. Eur J Prev Cardiol 2012; 20:947-955.
2. Journal article published ahead of print: Matsha TE, Macharia M, Yako YY, et al. Gamma-glutamyltransferase, insulin resistance and cardiometabolic risk profile in a middle-aged African population. Eur J Prev Cardiol. Epub ahead of print 14 Aug 2013. Doi:10.1177/2047487313501967.
3. Book: Huff D and Black TL. Comprehensive statistics. In: Miller C and Smith H (eds) *How to lie with statistics*. 4th ed. London: Penguin, 1991, pp.51–55.

Alcohol intake, hypertension development and mortality in black South Africans

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ABSTRACT

Background: Excessive alcohol intake is a risk factor for cardiovascular disease (CVD) and predicts cardiovascular and all-cause mortality. We determined which alcohol marker (self-reported alcohol intake, gamma-glutamyltransferase (GGT) or percentage carbohydrate deficient transferrin (%CDT)) relates best with mortality and predicts hypertension development over 5 years in black South Africans.

Design: This was a longitudinal study as part of the PURE (Prospective Urban and Rural Epidemiology) study in the North West Province, South Africa.

Method: We included 2010 participants and followed 1471 participants. Over five years, 230 deaths occurred, of which 66 were cardiovascular-related. At enrolment, participants completed questionnaires on alcohol intake (yes, for former and current use; no, for alcohol never used). We measured blood pressure, collected blood samples and measured GGT and %CDT.

Results: When comparing hazard ratios (HRs) of self-report, GGT and %CDT, we found that only GGT predicted cardiovascular (HR=2.76 (1.49-5.12)), all-cause mortality (HR=2.47 (1.75-3.47)) and hypertension development ((HR=1.31 (1.06-1.62)). Participants self-reporting yes for alcohol intake had a 30% increased risk for developing hypertension [HR=1.30 (1.07-1.60)], but not for mortality. When adding both GGT and self-report in the prediction model for hypertension, only self-reporting of alcohol was significant (HR=1.24 (1.01-1.53)). The alcohol marker, %CDT, did not show any significant association with mortality or hypertension development.

Conclusion: GGT independently predicted cardiovascular and all-cause mortality, as well as hypertension development in black South Africans. Despite non-specificity to excessive alcohol consumption, GGT may be a useful general marker for hypertension development and mortality, also due to its significant association with self-reported alcohol intake.

Key words: Gamma-glutamyltransferase, percentage carbohydrate deficient transferrin, self-reported alcohol intake, hypertension, morbidity, sub-Saharan Africa, cardiovascular disease

INTRODUCTION

Cardiovascular disease (CVD) is a significant health threat in sub-Saharan Africa and is one of the main causes of morbidity and mortality.[1,2] Smoking and alcohol consumption are associated with the development of hypertension and CVD.[3,4] Alcohol abuse in particular is highly prevalent in South Africa[5] and is an important risk factor for hypertension,[6-9] stroke[6,10,11] and mortality.[12,13] Alcohol consumption is also linked to high blood pressure (BP) and addiction among adolescants – important risk factors for hypertension.[14] Furthermore, there is a J-shaped association between alcohol intake and mortality with heavy drinkers having higher mortality.[15] Some studies[16,17] also reported a linear association between alcohol consumption and BP.

An accurate estimation of alcohol intake is still a challenge and a biomarker of alcohol with sufficient sensitivity and specificity is yet to be identified. The known biochemical measures of alcohol include gamma-glutamyltransferase (GGT), percentage carbohydrate deficient transferrin (%CDT), ethyl glucuronide, ethyl sulphate and phosphatidylethanol. However, these biochemical measures are costly and therefore become a challenge to use in low resource settings.[18] While literature is sparse regarding other alcohol markers and the risk of mortality, GGT is the marker of alcohol that is known to predict cardiovascular and all-cause mortality, sometimes independent of alcohol intake.[13,19,20]

In the present study we included three estimates of alcohol consumption, namely self-reported alcohol intake, GGT and %CDT in a large African population cohort. We were able to follow-up 1471 participants over a period of five years, from which 230 were deceased. This study allowed us to determine which measure of alcohol intake related best to cardiovascular or all-cause mortality, and predicted the development of hypertension over five years in this population of black South Africans.

METHODOLOGY

Study population

This is a sub-study of the international Prospective Urban and Rural Epidemiology (PURE) study and the detailed methodology has been described elsewhere.[8,21] The sub-study is based in the North West Province of South Africa, where baseline data was collected in 2005 (*n*=2010), with a response rate of 69%. During the year 2010, we successfully followed 1471 participants (including 230 that were deceased) and 539 participants were lost to follow-up.

The study was approved by the Ethics Committee of the North-West University, South Africa, and complied with the Helsinki Declaration, as revised in 2013. All subjects were fully informed about the objectives and procedures of the study and gave written informed consent prior to voluntary participation. Field workers were available to translate information into the participant's native language. The participants were assured of the confidentiality and anonymity of the results. Participants found to have chronic illnesses such as hypertension and human immunodeficiency virus (HIV) were referred to their local clinics and hospitals.

Questionnaires

Structured demographic, socio-economic, lifestyle and physical activity questionnaires, developed and standardised for the international PURE study[21] were completed by the participants with the assistance of African fieldworkers. The questions on alcohol consumption included a 'yes' for current or former use and 'no' for alcohol never used.

Anthropometric measurements

Height, body weight and waist circumference (WC) were measured for each participant (Precision Health Scale, A & D company, Tokyo, Japan; Invicta Stadiometer, IP 1465, Leicester, UK; Holtain unstretchable metal tape) using standardised methods.[22]

Blood pressure

Blood pressure was taken after a 10-min rest period. Systolic BP (SBP), diastolic BP (DBP) and heart rate (HR) were measured in duplicate, five minutes apart, with the validated Omron HEM-757 apparatus (Omron Healthcare, Kyoto, Japan), while the participants were seated upright with the right arm at heart level. Appropriate sized cuffs were used for obese participants. Following the guidelines of International Society of Hypertension,[23] participants with a BP measurement of ≥140 and/or 90 mmHg at follow-up, were regarded as hypertensive.

Blood, serum and plasma samples

Each participant was requested to fast for 10 hours before blood sampling commenced. A registered nurse took a blood sample from the ante-brachial vein branches. Samples were prepared according to appropriate methods. In the rural area, samples were immediately frozen and stored at -18°C for no longer than five days, transported to a laboratory facility and stored at -80°C until analysis.

Biochemical analyses

Liver enzymes (GGT, alanine aminotransferase (ALT), aspartate aminotransferase (AST)), high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs) and high-sensitivity C-reactive protein (hsCRP) were determined from the serum samples (Konelab20i auto analyser; Thermo Scientific, Vantaa, Finland) and glucose from sodium fluoride samples. Glycosylated haemoglobin (HbA1c) was determined in ethylenediaminetetraacetic acid (EDTA)-treated whole blood, using the D-10 Haemoglobin testing system (Bio-Rad Laboratories, Hercules, California, USA). Serum %CDT analyses were performed using an *in vitro* heterogeneous immunoassay with column separation followed by a turbidimetric measurement (Axis-Shield %CDT kit, Oslo, Norway). The coefficient of variance (CV) for all assays was <10%. The HIV status of each participant was determined using whole blood samples and following the protocol of the National Department of Health, South Africa, using the First Response (PMC Medical, India) rapid HIV card test. The test result was confirmed using the Pareeshak (BHAT Bio-tech, India) card test. Each participant received pre-test and post-test counselling.

In assessing the outcome, we obtained the cause of death through verbal autopsy coded by a physician according to the International Classification of Disease codes for the immediate and underlying causes, and from the family's death certificate. Cardiovascular mortality was defined as death from congestive cardiac failure, ischaemic heart disease, diabetes mellitus, stroke and myocardial infarction. All-cause mortality included cardiovascular and non-cardiovascular mortality.

Statistical analyses

We used Statistica version 12 (StatSoft, Inc., Tulsa, OK) for statistical analyses. Variables with a non-Gaussian distribution (body mass index (BMI) and all biochemical measurements (except HDL-C)) were logarithmically transformed. Means and proportions were compared by analysis of variance (ANOVA) and the Chi-square test, respectively. Multi-variable adjusted Cox regression analyses were used to assess the association of the selected alcohol markers with either all-cause, cardiovascular mortality, or hypertension development. We considered the following covariates for inclusion in the Cox regression models: age, sex, BMI, WC, baseline SBP, smoking, anti-hypertensive medication, AST/ALT ratio, HbA1c, glucose, hsCRP, low-density lipoprotein cholesterol, HDL-C, total cholesterol and TG. Finally, covariates included in the model were age, sex, smoking, anti-hypertensive medication, WC, baseline SBP, AST/ALT ratio, HbA1c, hsCRP, HDL-C, and TG. Statistical significance was set at $p \leq 0.05$.

RESULTS

We compared the characteristics of the participants that were successfully followed-up over five years to the deceased due to cardiovascular and other causes (Table 1). The cardiovascular mortality group was the oldest ($p<0.001$), had the highest BP and included the highest number of individuals on anti-hypertensive treatment (23%). The non-cardiovascular mortality group consisted mainly of men (53%) with a low BMI of 21 (15.0 - 32.2) kg/m^2 and the highest percentage of HIV infection (43%). Compared to the follow-up group, a larger proportion of the non-cardiovascular mortality group smoked (63% vs. 54%), consumed alcohol (self-reported alcohol, 60% vs. 42%) and had significantly elevated C-reactive protein (CRP) ($p=0.001$).

Table 1. A comparison of the baseline characteristics of the study participants.

	Follow-up group	Cardiovascular mortality	Non-cardiovascular mortality	p-value
Socio-demographic profile				
Number of participants	1241	66	164	
Age, years	51.0 ± 10.2[a]	60.0 ± 11.4[ab]	50.4 ± 11.1[b]	**<0.001**
Sex, women, n (%)	821/1240 (66.2)[a]	41/66 (62.1)[b]	77/164 (47.0)[ab]	**<0.001**
Body mass index, kg/m^2	24.1 (16.5-38.5)[a]	23.2 (15.3-40.0)[b]	21.0 (15.0-32.2)[ab]	**<0.001**
Waist circumference, cm	80.0 ± 13.0[a]	81.4 ± 17.0[b]	76.0 ± 11.0[ab]	**0.001**
Location, rural, n (%)	668/1241 (54.0)[a]	26/66 (39.4)[a]	81/164 (49.4)	**0.050**
HIV infected, n (%)	148/1234 (12.0)[a]	8/65 (12.3)[b]	70/162 (43.2)[ab]	**<0.001**
Smoking, n (%)	670/1235 (54.3)[a]	41/66 (62.1)	103/163 (63.2)[a]	**0.053**
Biochemical measurements				
Serum glucose, mmol/L	4.85 (3.50-6.90)	4.73 (3.20-6.10)	4.67 (3.80-6.10)	0.11
Glycosylated hemoglobin, mmol/L	5.63 (4.90-6.60)	5.58 (4.60-6.80)	5.52 (4.80-6.30)	0.15
HDL-C, mmol/L	1.54 ± 0.62	1.53 ± 0.64	1.40 ± 0.74	**0.040**
Triglycerides, mmol/L	1.15 (0.56-2.77)	1.20 (0.65-2.21)	1.11 (0.53-2.95)	0.51
C-reactive protein, mg/L	3.11 (0.25-39.4)[a]	4.12 (0.43-53.7)	5.59 (0.38-53.2)[a]	**<0.001**
Markers of alcohol intake				
Self-reported alcohol use, n (%)	513/1233 (42.0)[a]	29/66 (44.0)[b]	98/162 (60.1)[ab]	**<0.001**
ALT, IU/L	18.0 (7.49-50.0)	19.0 (7.00-77.0)	20.3 (7.00-69.0)	**0.009**
AST, U/L	28.0 (13.0-96.0)[a]	29.0 (12.0-98.0)	36.3 (12.2-159)[a]	**<0.001**
AST:ALT	1.60 (0.71-3.35)	1.52 (0.66-3.90)	1.78 (0.83-3.63)	**0.031**
Gamma-glutamyltransferase, U/L	53.5 (19.0-347)[ab]	80.0 (25.0-480)[a]	75.2 (21.0-489)[b]	**<0.001**
Carbohydrate deficient transferrin, %	2.64 (1.33-5.65)	2.74 (1.25-7.54)	2.95 (1.45-6.22)	**0.021**
Cardiovascular variables at baseline				
Systolic blood pressure, mmHg	133 ± 24.0[a]	147 ± 34.4[ab]	132 ± 25.4[b]	**<0.001**
Diastolic blood pressure, mmHg	88.0 ± 14.1	92.0 ± 13.1	87.0 ± 16.0	**0.030**
Pulse pressure, mmHg	47.0 ± 15.0[a]	54.4 ± 23.3[ab]	45.0 ± 14.2[b]	**<0.001**
Heart rate, bpm	78.4 ± 15.4[ab]	80.0 ± 20.0[a]	81.0 ± 19.2[b]	**<0.001**
Hypertension, n (%)	737/1231 (60.0)	47/66 (71.2)	95/163 (58.2)	0.15
Anti-hypertensive medication, n (%)	129/1239 (10.4)[a]	15/66 (23.0)[ab]	14/164 (8.54)[b]	**0.004**

Values with the same superscript letter differ significantly, and bold p-values denote statistical significance where p≤0.05. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CV, cardiovascular; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus. Values are interpreted as arithmetic mean ± standard deviation; geometric mean (5th to 95th percentile interval); number of participants (%). Bold p-values denote statistical significance.

Table 2 presents the characteristics of the participants that were all normotensive at baseline (N=582). Thirty-eight percent of these participants had BP in the hypertensive range, while 15% died after a 5-year period. The deceased group had a lower BMI [20 (15.0 - 32.0) kg/m^2] compared to other groups, with 49% infected with HIV and having elevated CRP levels (8.00 (0.45 - 54.0) mg/L).

They also reported the highest alcohol use (54%), which was significant and confirmed by the highest GGT levels ($p<0.001$). The group with BP in the hypertensive range had a higher WC (mean 79 cm vs. 75 cm) than the followed normotensive group. Finally, we observed a steady significant increase in self-reported alcohol use and liver enzymes, especially GGT, from the normotensive to the deceased group ($p<0.001$).

In Figure 1 the multi-variate adjusted hazard ratios (HRs) of each alcohol marker for the prediction of mortality and hypertension development are shown. We found that GGT was the only alcohol marker significantly predicting cardiovascular and all-cause mortality, as well as hypertension development (HR=2.76 (1.49 - 5.12); 2.47 (1.75 - 3.47) and 1.31 (1.06 - 1.62), respectively). Participants self-reporting yes for current and former alcohol intake had a 30% increased risk for developing hypertension over five years (HR=1.30 (1.07 - 1.59)). Percentage CDT did not show any significant association with either cardiovascular and all-cause mortality, as well as hypertension development.

Table 2. The baseline characteristics of participants that were normotensive at baseline (N=582), remaining normotensive, becoming hypertensive or deceased after 5 years.

	Followed-NT	Followed-HT	All-cause mortality	p-value
Socio-demographic profile				
Number of participants	275	219	88	
Age, years	48.1 ± 10.0	48.4 ± 9.49	51.0 ± 13.0	**0.008**
Sex, women, n (%)	183/275 (67.0)[a]	141/219 (64.4)[b]	42/88 (48.0)[ab]	**0.005**
Body mass index, kg/m^2	22.4 (16.0-35.0)[ab]	24.0 (17.3-36.2)[ac]	20.0 (15.0-32.0)[bc]	**<0.001**
Waist circumference, cm	75.0 ± 12.0[a]	79.2 ± 11.4[ab]	74.0 ± 11.0[b]	**<0.001**
Location, rural, n (%)	171/275 (62.2)	129/219 (59.0)	51/88 (58.0)	0.67
HIV infected, n (%)	54/275 (20.0)[ab]	25/217 (12.0)[ac]	42/86 (49.0)[bc]	**<0.001**
Smoking, n (%)	148/274 (54.0)[a]	117/217 (54.0)	57/87 (66.0)[a]	0.14
Biochemical measurements				
Serum glucose, mmol/L	5.00 (3.40-5.90)	4.80 (3.50-6.30)	4.61 (3.80-5.60)	0.16
Glycosylated haemoglobin, mmol/L	5.53 (4.40-6.20)	5.60 (4.90-6.50)	5.56 (4.80-6.30)	0.36
HDL-C, mmol/L	1.45 ± 0.56	1.46 ± 0.56	1.31 ± 0.73	0.11
Triglycerides, mmol/L	1.18 (0.53-2.21)	1.10 (0.58-2.77)	1.15 (0.63-2.58)	0.38
C-reactive protein, mg/L	2.31 (0.25-45.0)[a]	2.92 (0.24-40.0)[b]	8.00 (0.45-54.0)[ab]	**<0.001**
Markers of alcohol intake				
Self-reported alcohol use, n (%)	82/274 (30.0)[ab]	87/217 (40.0)[ac]	46/86 (54.0)[bc]	**<0.001**
ALT, IU/L	16.0 (7.00-39.2)[a]	16.0 (6.00-40.2)[b]	20.0 (6.20-68.3)[ab]	**0.005**
AST, U/L	26.0 (12.0-55.0)[a]	25.3 (12.3-84.0)[b]	33.3 (13.3-143)[ab]	**<0.001**
AST:ALT	1.61 (0.71-3.34)	1.63 (0.69-3.50)	1.71 (0.94-3.63)	0.64
Gamma-glutamyltransferase, U/L	40.0 (18.0-180)[ab]	48.4 (18.4-218)[ac]	74.0 (23.2-452)[bc]	**<0.001**
Carbohydrate deficient transferrin, %	2.55 (1.35-5.61)	2.61 (1.27-5.04)	2.82 (1.45-6.16)	0.26
Cardiovascular variables at baseline				
Systolic blood pressure, mmHg	113 ± 12.0[a]	117 ± 12.0[ab]	111 ± 13.1[b]	**<0.001**
Diastolic blood pressure, mmHg	74.2 ± 7.34[a]	76.0 ± 7.00[ab]	73.0 ± 8.14[b]	**<0.001**
Pulse pressure, mmHg	39.0 ± 9.13	41.0 ± 9.56	39.0 ± 10.1	**0.040**
Heart rate, bpm	72.2 ± 15.0[a]	73.0 ± 15.0[b]	83.1 ± 19.1[ab]	**<0.001**
Anti-hypertensive medication, n (%)	12/275 (4.36)[a]	18/219 (8.22)	9/88 (10.23)[a]	0.084

Values with the same superscript letter differ significantly, and bold p-values denote statistical significance where p≤0.05. ALT, alanine aminotransferase; AST, aspartate aminotransferase; Followed-NT, normotensive after 5 years; followed-HT, hypertensive after 5 years; HDL-C, high-density lipoprotein cholesterol; HIV, human immunodeficiency virus. Values are interpreted as arithmetic mean ± standard deviation; geometric mean (5th to 95th percentile interval); number of participants (%).

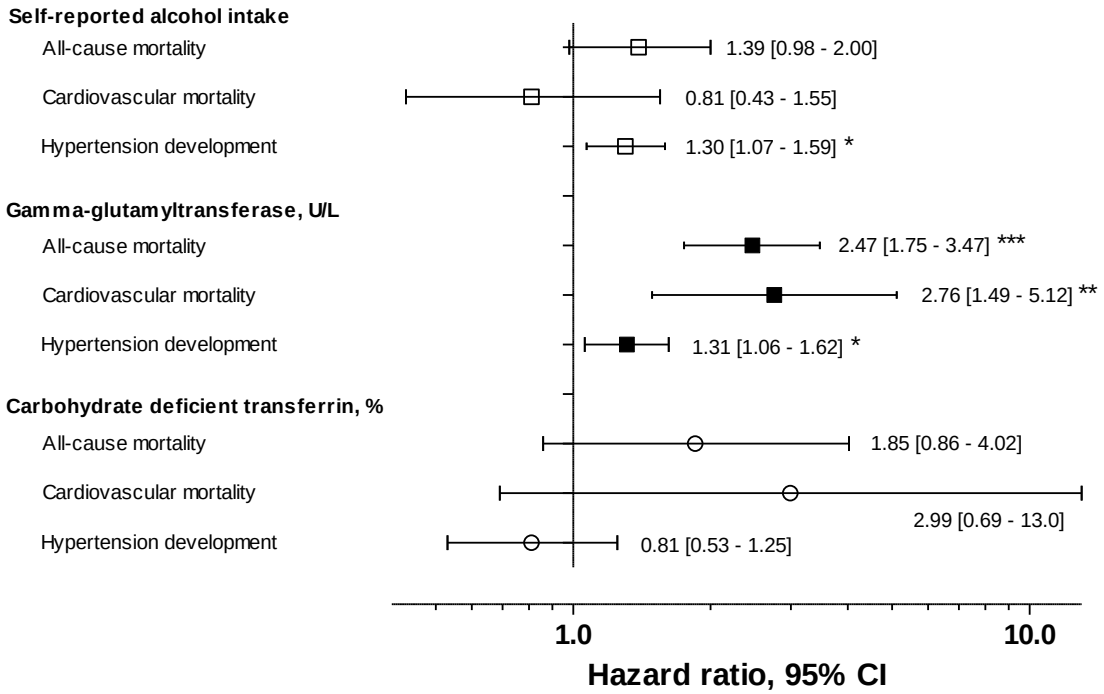

Figure 1. Standardized hazard ratios of alcohol markers in association with all-cause and cardiovascular mortality, and hypertension development over a 5-year period in 1471 individuals. Models included age, sex, smoking, anti-hypertensive medication, waits circumference, high-sensitivity C-reactive protein, AST:ALT ratio, high-density lipoprotein cholesterol, glycosylated haemoglobin, triglycerides, HIV infection, baseline systolic blood pressure. *, p≤0.05; **, p=0.001; ***, p<0.001.

Sensitivity analyses

Due to the known effects of HIV on mortality, we also included HIV infection as one of the independent variables (Supplementary Material Table S1). Our main results were robust regardless of the HIV status of our participants. HIV infection did not contribute significantly to hypertension development (HR=0.79 (0.61 - 1.05); p=0.11) or cardiovascular mortality (HR=1.31 (0.55 - 3.10); p=0.54) but contributed strongly to all-cause mortality (HR=3.58 (2.57-4.97); p=<0.001) (Supplementary Material Table S1). Furthermore, we tested if the association of GGT with the dependent variables in question was alcohol-related. In the Cox regression models, we respectively included self-reported alcohol intake or %CDT, with GGT. Our main findings remained the same. However, when self-reporting was included with GGT with hypertension development as dependent variable, only self-reported alcohol intake was significant (R^2=0.238; HR=1.24; p=0.043), and not GGT (R^2=0.238; HR=1.20; p=0.11) (Supplementary Material Table S2).

DISCUSSION

In this study we evaluated which measure of alcohol intake (self-reported alcohol intake, GGT or %CDT) related best to cardiovascular and all-cause mortality in a population of black South Africans over five years. We also assessed which alcohol markers predict the development of hypertension. Our main finding is that GGT was the only marker that significantly predicted the development of hypertension as well as cardiovascular and all-cause mortality. We also found that self-reported alcohol intake was useful since it showed a significant association with hypertension development.

GGT is a well-known marker of alcohol intake that is associated with both cardiovascular and all-cause fatal events. Several large cohort studies indicate a positive association between GGT and cardiovascular mortality,[24-26] and all-cause mortality,[27,28] with an increased total mortality risk in diabetic individuals[13] and coronary artery disease, independent of other cardiovascular risk factors.[29] The positive association between GGT and change in BP is an indication that high GGT levels are alcohol-related and a risk factor for hypertension.[8,30] However, it is also important to mention that GGT can predict hypertension development and mortality independent of alcohol intake.[31] Moreover, there is evidence of GGT linking with insulin sensitivity and metabolic syndrome without alcohol use.[32] GGT is also elevated in non-alcoholic fatty liver disease or non-alcoholic steatohepatitis (NASH) in the absence of alcohol intake.[33] Therefore, it is clear that GGT may not necessarily reflect only alcohol consumption.

GGT is further influenced by ethnicity, sex, obesity, age, CRP, HbA1c and liver damage[34-38] and our finding that GGT is the strongest predictor of mortality and hypertension, may thus reflect GGT's representation of both alcohol- and non-alcohol related risk. However, a recent study based on the 2005-08 National Health and Nutrition Examination Survey (NHANES) showed that excessive current drinkers had an increased likelihood of 75-314% for men and 226% for women of having elevated GGT.[39] Moreover, we found a positive correlation of GGT and self-reported alcohol use ($r=0.44$; $p=0.001$) in the same study population.[9] In this study we also found that with regard to hypertension development, GGT may represent specifically alcohol use since it became

non-significant when included with self-reported alcohol intake in the same model, further confirming our previous finding.[9] In this instance, self-reported alcohol use was the significant predictor of hypertension development. Thus, the pattern of drinking in black South Africans seems to follow a linear association with BP or mortality risk with high GGT likely to increase in heavy binge drinking on weekends or traditional cultural occasions.[40,41]

We included %CDT in this study as a highly specific marker of alcohol intake but with low sensitivity.[42,43] Baros and colleagues[30] found that BP related positively with %CDT in alcohol users confirming %CDT as a marker of alcohol-related CVD and mortality. Recently it was confirmed that %CDT is a reliable biomarker to screen for alcohol abuse.[44] It is noteworthy that the presence of CDT in the vitreous humor of the eyes of alcoholics at post-mortem indicated that CDT is a marker for possible withdrawal-related death in alcoholics.[45] Although we found a positive association between self-reported alcohol intake and %CDT in the same study population,[9] %CDT did not predict hypertension development or mortality in this study, suggesting that it may not have sufficient sensitivity to use in population studies.

South Africa continues to be the country with the most HIV infections in the world.[46,47] As expected, in our study mortality was strongly related to HIV infection. Individuals infected with HIV showed a more than 3-fold risk of all-cause mortality. HIV infected individuals are likely to consume more alcohol as a form of coping mechanism and alcohol abuse is a risk factor for hypertension in an HIV infected population.[48] HIV infection is in turn likely to be attributable to excessive alcohol use[49] as alcohol overuse may lead to risky sexual behaviour.[50,51] Our study is consistent with the above as all-cause mortality is closely associated with a high proportion of alcohol intake and HIV infection.

Limitations of our study were that GGT may have been influenced by other factors as reported earlier. Self-reported alcohol intake may have been overestimated or underestimated as a result of different individual perceptions. Although our study population may have been honest in their reporting of alcohol intake, their low level of education may have limited their ability to accurately

report on other alcohol intake behaviour, e.g. the volume of alcohol intake.[9] However, the accurate reporting of questionnaire responses was enhanced by the presence of African field workers who would translate the questions into the participants' native language. This study included only participants from the North West Province of South Africa and similar broader investigations are encouraged. Finally, residual confounding cannot be excluded, although our results remained consistent after multiple adjustments. This study adds to the limited availability of longitudinal studies on lifestyle-related mortality in sub-Saharan Africa.

In conclusion, GGT was the alcohol marker that consistently predicted cardiovascular and all-cause mortality as well as hypertension development in a population of African ancestry. Despite the confounding factors that influence GGT, the associations between self-reported alcohol intake and hypertension development in this study suggests that high GGT levels were mainly due to alcohol overuse – confirmed by the non-significance of GGT when included with self-reported alcohol intake in the same model with hypertension development. Although self-reported alcohol intake is useful in low-resource primary healthcare settings, measurement of GGT is encouraged due to its predictive value for hypertension and mortality, and since it represents alcohol intake, NASH and obesity - all known to have severe cardiovascular consequences.

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Declaration of Conflicting Interests

None declared.

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Supplementary Material Table S1. Standardized hazard ratios of alcohol markers in association with all-cause and cardiovascular mortality, and hypertension development over a five year period in 1471 individuals, with HIV infection included as an independent variable.

Alcohol marker	R^2	Hazard Ratio	95% CI	p-value
All-cause mortality				
Self-reported alcohol intake, n (%)	0.488	1.31	0.93; 1.84	0.13
HIV infection	0.488	3.67	2.63; 5.13	**<0.001**
Gamma-glutamyltransferase, U/L	0.531	2.41	1.72; 3.41	**<0.001**
HIV infection	0.531	3.58	2.57; 4.97	**<0.001**
Carbohydrate deficient transferrin, %	0.485	1.94	0.90; 4.17	0.09
HIV infection	0.485	3.66	2.61; 5.14	**<0.001**
Cardiovascular mortality				
Self-reported alcohol intake, n (%)	0.548	0.82	0.43; 1.56	0.54
HIV infection	0.548	1.29	0.55; 3.07	0.56
Gamma-glutamyltransferase, U/L	0.609	2.75	1.47; 5.13	**0.002**
HIV infection	0.609	1.31	0.55; 3.09	0.54
Carbohydrate deficient transferrin, %	0.557	3.04	0.70; 13.3	0.14
HIV infection	0.557	1.33	0.56; 3.16	0.52
Hypertension development				
Self-reported alcohol intake, n (%)	0.243	1.31	1.07; 1.61	**0.009**
HIV infection	0.243	0.78	0.60; 1.02	0.07
Gamma-glutamyltransferase, U/L	0.239	1.29	1.05; 1.60	**0.020**
HIV infection	0.239	0.80	0.61; 1.05	0.11
Carbohydrate deficient transferrin, %	0.251	0.81	0.53; 1.25	0.34
HIV infection	0.251	0.83	0.63; 1.09	0.17

Bold p-values denote statistical significance where $p \leq 0.05$. Models included age, sex, smoking, anti-hypertensive medication, waits circumference, high- sensitivity C-reactive protein, AST:ALT ratio, high-density lipoprotein cholesterol, glycosylated haemoglobin, triglycerides, HIV infection, baseline systolic blood pressure.

Supplementary Material Table S2. Standardized hazard ratios of gamma-glutamyltransferase with all-cause and cardiovascular mortality, as well as hypertension development over a five year period in 1471 individuals, with self-reported alcohol intake or percentage carbohydrate deficient transferrin included as independent variables.

	R^2	Hazard Ratio	95% CI	p-value
All-cause mortality	0.411			
Self-reported alcohol intake, n (%)		1.15	0.80; 1.66	0.44
Gamma-glutamyltransferase, U/L		2.52	1.78; 3.56	**<0.001**
Cardiovascular mortality	0.608			
Self-reported alcohol intake, n (%)		0.66	0.34; 1.27	0.21
Gamma-glutamyltransferase, U/L		3.29	1.76; 6.15	**<0.001**
Hypertension development	0.238			
Self-reported alcohol intake, n (%)		1.24	1.01; 1.53	**0.043**
Gamma-glutamyltransferase, U/L		1.20	0.96; 1.50	0.11
All-cause mortality	0.400			
Carbohydrate deficient transferrin, %		1.54	0.71; 3.32	0.27
Gamma-glutamyltransferase, U/L		2.46	1.73; 3.49	**<0.001**
Cardiovascular mortality	0.600			
Carbohydrate deficient transferrin, %		2.20	0.49; 9.94	0.31
Gamma-glutamyltransferase, U/L		2.82	1.48; 5.36	**0.002**
Hypertension development	0.250			
Carbohydrate deficient transferrin, %		0.76	0.49; 1.17	0.21
Gamma-glutamyltransferase, U/L		1.28	1.02; 1.59	**0.030**

Bold p-values denote statistical significance where p≤0.05. Models included age, sex, smoking, anti-hypertensive medication, waits circumference, high-sensitivity C-reactive protein, AST:ALT ratio, high-density lipoprotein cholesterol, glycosylated haemoglobin, triglycerides, HIV infection, baseline systolic blood pressure.

Chapter 7

General Findings and Conclusions

1. INTRODUCTION

The summary of the main findings of the three manuscripts (Chapters 4, 5 and 6) will be presented in this chapter. The results of each research article will be discussed, interpreted and compared to the literature. Conclusions will be drawn and recommendations made regarding further prospective studies of alcohol intake and cardiovascular disease (CVD).

2. SUMMARY OF MAIN FINDINGS

The main results reported in the manuscripts of this thesis are presented below.

2.1 Self-reported alcohol intake is a better estimate of 5-year change in blood pressure than biochemical markers in low resource settings: the PURE study

The aim of this manuscript was to compare self-reported alcohol estimates with biochemical measures of alcohol [gamma-glutamyltransferase (GGT) and percentage carbohydrate deficient transferrin (%CDT)], considering their relationship with percentage change in brachial blood pressure (BP) (%bSBP, %bDBP) and central systolic blood pressure (SBP) over a 5-year period in black South Africans. The hypotheses were that GGT and %CDT are better estimates of alcohol consumption and have better association with BP change over time in prospective studies.

The results of this study showed, however, that self-reported alcohol intake was independently associated with a change in BP in black South Africans over 5 years. This association was not found for GGT and %CDT – biomarkers of alcohol known to relate positively with hypertension and CVD.[1,2] These results showed self-reporting as a suitable marker of alcohol intake in low resource settings in which honest reporting is expected. Both hypotheses were therefore rejected as self-reported alcohol intake proved a better alcohol marker in this population and also had a better association with a change in BP over the 5-year period of this study.

2.2 A comparison of the cardiometabolic profile of black South Africans with suspected non-alcoholic fatty liver disease (NAFLD) and excessive alcohol use

Since GGT is also influenced by other factors, it is important to understand its involvement in both NAFLD and alcoholic fatty liver disease (AFLD). The prevalence of NAFLD is higher in poverty, among the least educated,[3] in women and is also associated with central obesity in Africans.[4] African women, without alcohol consumption, have the highest prevalence of obesity in all ethnic and sex groups in South Africa.[5,6] In contrast, men are traditionally more active, have a higher prevalence of smoking and alcohol intake, all of which contribute to their lean body weight.[7] Finally, studies on NAFLD and CVD in Africans are sparse and the disease profile has not yet been studied in South Africa.[8]

This manuscript therefore compared the cardiovascular and metabolic characteristics of black South Africans with suspected NAFLD and excessive alcohol use; and their associations with BP in a cross-sectional analysis. Non-alcohol users were included in the study as control. It was hypothesized that different adverse cardiovascular and metabolic profiles are present in excessive alcohol users and those with suspected NAFLD.

We found that different sex and cardiometabolic profiles characterised excessive alcohol users and individuals suspected with NAFLD. For NAFLD obese participants and female sex with a dysmetabolic profile were the dominant characters, where diastolic blood pressure (DBP) associated positively with waist circumference (WC). In the alcohol user group, lean body mass in men characterised excessive alcohol use and their SBP and pulse pressure (PP) were independently associated with high-density lipoprotein cholesterol (HDL-C). All hypotheses set for this investigation were therefore accepted.

2.3 Alcohol intake, hypertension development and mortality in black South Africans

With this manuscript, the aim was to determine which alcohol marker (self-reported alcohol intake, GGT or %CDT), related best with cardiovascular and all-cause mortality, and predicted

the development of hypertension over 5 years in a population of black South Africans (Fig. 7.1). We hypothesised that excessive alcohol consumption is associated with hypertension development and total mortality, and that GGT, a well-known marker of both alcohol intake and NAFLD, is better associated with both mortality and hypertension development in a black population of South Africa, than the other alcohol markers.

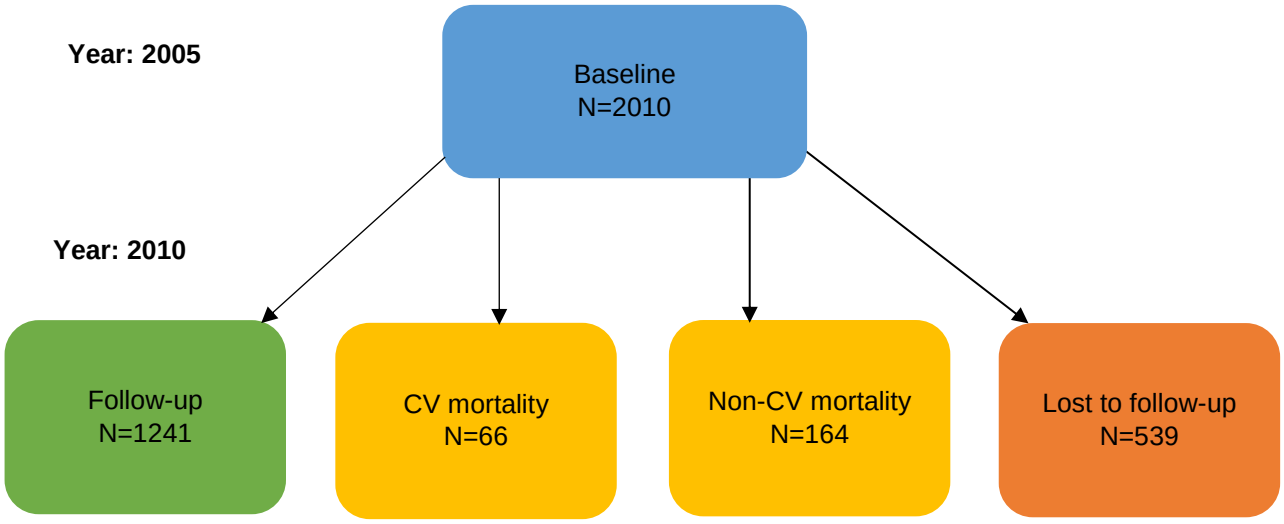

Figure 7.1. Outline of the study population.
CV, cardiovascular

The results indicated that excessive alcohol intake is an independent predictor of both hypertension development and total mortality based on the associations of GGT with cardiovascular and all-cause mortality, as well as hypertension development. Moreover, self-reporting of alcohol intake predicted a 30% increased risk of hypertension development, confirming that the association of GGT with cardiovascular and all-cause mortality, as well as hypertension development, was alcohol-related.

The third marker, %CDT - highly specific to alcohol intake - was not related to any health outcome variable. Also worth mentioning is the expected strong association between HIV infection and all-cause mortality, a result observed during sensitivity analysis. Participants with HIV infection also had the highest proportion of alcohol intake. All hypotheses set for this article were therefore accepted.

3. DISCUSSION AND THE COMPARISON OF MAIN FINDINGS WITH THE LITERATURE

It is important that the results of this study are compared with those found in the literature. Most of the results in this study confirmed published research findings while some were contradictory to those found in the literature.

I focused on the influence of alcohol overuse as a risk factor for CVD. The results were based on the association of alcohol biomarkers (self-reported alcohol use, GGT and %CDT) with hypertension development and mortality. Self-reporting of alcohol and GGT emerged as the prominent markers that independently predicted BP change, hypertension development and total mortality in this study. The results from this thesis therefore confirm literature findings that alcohol abuse has detrimental effects on the cardiovascular system, raising BP, leading to hypertension[9] and is a critical risk factor for both cardiovascular and all-cause mortality,[10] also in the South African setting.

The usefulness of self-reported alcohol intake

We found that self-reporting of alcohol predicted both the change in BP over 5 years, as well as incident hypertension. In the literature, there is sufficient evidence of self-reporting as a reliable tool to screen for alcohol abuse,[11-13] confirmed by positive correlations with known alcohol biomarkers (GGT and %CDT) in this thesis. The other self-reported markers of alcohol intake, namely, quantity, volume, duration of drinking and quantitative food frequency questionnaire (g/day) did not show any association with a change in BP over the study period. These self-reported estimates are perhaps more difficult to report correctly, also when different types of alcohol come into play (e.g. spirits vs. home-made beer intake). Moreover, they may also not have been sensitive enough to capture alcohol intake behaviour correctly. The low level of education of this population may have also contributed to having difficulties in estimating alcohol intake behaviour.[14,15] In the end a simple yes/no answer was easy to understand and to report on accurately. The screening of participants for alcohol use, quantity and frequency of alcohol consumption by questionnaires, and their significant association with GGT supported that self-reporting is a useful low cost tool to assess alcohol consumption.[16] Simple questionnaires to

estimate alcohol intake over an extended period of time have long been in use.[17] Based on findings of this thesis, in low income countries, self-reporting of alcohol may be a very useful low cost screening tool.

Alcohol abuse and cardiovascular risk

The results of this thesis showed that excessive drinking predicts hypertension development and mortality. According to published data, alcohol intake is only beneficial when it is consumed in low or moderate levels and seems to reduce the risk of CVD.[9,18,19] However, in the South African context, alcohol consumption follows a linear pattern with either none or heavy drinking dichotomy and heavy episodic drinking mainly on weekends or traditional ceremonies,[20,21] with no evidence of moderate drinking and its beneficial effects. This is perhaps the reason simple yes/no responses to alcohol consumption showed a positive correlation with a change in BP over other self-reported alcohol estimates. It would thus be advisable to recommend that drinking be avoided in South Africa given the linear association with the risk of CVD.

Our results that alcohol abuse is a risk factor for CVD and mortality found numerous support in the literature. The projected increase of GGT (about 314%) in excessive drinking men without history of liver damage confirmed the risk of developing CVD when alcohol is consumed heavily.[16,22] Tang and colleagues[23] found that excessive alcohol users have increased risk of having stroke while drinkers with hypertension are almost three times more susceptible [2.89 (1.55-5.39)] to stroke. These findings become especially more important in sub-Saharan Africa when considering that over two-thirds of stroke-related deaths occur in developing countries.[24] There was a significant association of a change in BP with high GGT levels over 4 years in drinkers in a prospective study by Lee and co-authors,[25] confirming that excessive alcohol intake contributes to elevated BP over time.

Studies exist that correspond well with hypertension development,[1,26] cardiovascular[10] and all-cause mortality[27] in alcohol-induced GGT elevation. The role of GGT in predicting hypertension development was further confirmed by the positive association of self-reported alcohol intake with

the development of hypertension in this study. In a large cohort study of more than 32 000 participants followed for 4 years, it was demonstrated that long-term alcohol use is an independent risk factor for hypertension development with a linear association between alcohol intake and incident hypertension.[28]

However, it is worth mentioning that GGT reflects both AFLD and NAFLD.[29] This suggests that the mortality associated with GGT may not necessarily be alcohol-related. GGT was in this study not associated with a percentage change in BP while it was an independent predictor for mortality and hypertension development over 5 years. This discrepancy could have been caused by the numerous confounding factors that influence GGT. There is also evidence of higher GGT levels in Africans compared with other ethnic groups.[30] Although the association of self-reporting of alcohol with elevated BP was confirmed later in the thesis (Chapters 4 and 6), GGT was emphasised as the useful marker since it has a predictive value with both hypertension and mortality, and possibly due to its representation of AFLD and NAFLD.

We were surprised that our third marker of alcohol intake, %CDT, did not show any association with elevated BP, hypertension development or total mortality. However, %CDT showed a strong correlation with both self-reported alcohol intake and GGT in this study - another confirmation that elevated BP and hypertension development were indeed mainly from alcohol abuse. Baros et al.[2] found that both GGT and %CDT correlated significantly with change in DBP but in their study only %CDT showed a positive association with SBP. But we found that %CDT, which is also expensive to use in low income countries, was not a useful marker in our African population perhaps due to its low sensitivity to alcohol consumption. The low sensitivity of CDT was also shown by Harasymiw and Bean[31] in which CDT test alone showed a sensitivity rate of 58% in contrast to the 88% sensitivity shown by Early Detection of Alcohol Consumption (EDAC) test in the same study. However, the sensitivity of CDT seems to improve significantly when used in combination with other markers of alchol consumption,[31,32] which may be useful in our study population.

HDL-cholesterol and alcohol abuse

Excessive alcohol consumption in this study associated with elevated HDL-C which correlated with increased SBP in heavy drinkers and these results are confirmed by others.[33,34] Despite the well-known protective role of HDL-C with moderate drinking,[35,36] evidence of elevated HDL-C in heavy alcohol consumption and its association with CVD exist.[35,37,38] The high HDL-C levels in excessive alcohol consumption and its risk for CVD seems to be influenced by the HDL-C particle size with some studies reporting larger particle size association with alcohol abuse[39,40] while there is evidence for alcohol-induced small HDL-C particle size.[41,42] However, there are studies that reported an inverse association between HDL-C larger particle size and CVD,[41,43] although the beneficial role of HDL-C seems to be linked to smaller HDL-C particle size.[35] Future studies in HDL particle size are recommended in order to better understand the significant association between HDL-C and alcohol overuse.

Alcohol intake and HIV infection

The significant association of heavy alcohol intake with HIV infection in this study is a finding well documented in the literature.[44,45] Although HIV and alcohol intake are not the main focus of this thesis, it is still important to mention it in the South African context, which continues to have the highest HIV infection prevalence in the world.[46] With the population under study mainly of low socio-economic background and with low level of education, it is possible that alcohol was consumed by HIV positive participants to cope with psychological stress and stigma associated with HIV infection.[47,48] Alternatively, alcohol abuse may lead to risky sexual behaviour that may raise the rate of HIV infection in poverty-stricken populations.[45,49] Studies from low-income populations reported similar results in which HIV prevalence was lower in the general population but significantly high in people who consume alcohol excessively mainly as a result of risky sexual behaviour with non-regular partners.[50-52]

The strong association ($p<0.001$) of all-cause mortality with HIV infection in this study suggests that heavy drinking in HIV infected individuals can lead to disease progression and re-infection with new partners.[53] Both excessive alcohol intake and HIV infection are associated with high

levels of CRP especially in the presence of smoking and low socio-economic status.[54] The mean CRP in this study, especially in the mortality group, was very high [8.00 (0.45-54.0)]. A CRP value of higher than 3.0 mg/L shows a high risk for disease, and the majority of participants in our study had values ranging from 3.0 to 8.0 mg/L. The high CRP levels may be indicative of advanced progression of disease from both alcohol intake and HIV infection – highly linked to mortality. The HIV infected participants in this sudy were newly diagnosed with HIV and did not yet receive antiretroviral therapy. This may have had an effect on the reported moratlity.

NAFLD and cardiovascular risk

We found the characteristics of NAFLD mainly in women presenting with a dysmetabolic profile. To our knowledge, literature confirming the distinct sex prevalences of NAFLD between African men and women is limited. But sex comparisons performed in other ethnic groups reported contrasting results.[55,56] It is well-known that there are distinct body composition profiles in African men and women, especially from poverty-stricken areas,[3] namely, obesity is more prevalent in African women while men have lean body weight. Malnutrition, either over- or under nutrition, and alcohol abuse may account for these body composition differences.[57] Finally, even the control group mainly comprised of women with no alcohol intake and body mass index (BMI) levels in the overweight and obese range. This may indicate that they are at risk to develop NAFLD, especially considering that they were younger (mean 49 years ± 12.3) than the NAFLD group (mean 51 years ± 11.9), which means they are likely to develop NAFLD as they age.

Alcohol abuse and mortality

In addition to hypertension development, we found that GGT independently predicted all-cause mortality in this study population as shown by Kaplan-Meier plots in Figure 7.2. While heavy alcohol consumption predicts total mortality, it is also an important contributing factor to stroke in sub-Saharan Africa.[58] The positive association of excessive alcohol intake with BP in lean males in this study is strongly related to the risk of stroke.[2,59] Stroke is the fourth leading cause of death in South Africa with obesity and heavy alcohol intake some of the main contributing factors.[60] As with hypertension development, the positive association of GGT with self-reported alcohol intake

in the earlier results confirmed the significant role of excessive alcohol consumption in total mortality.

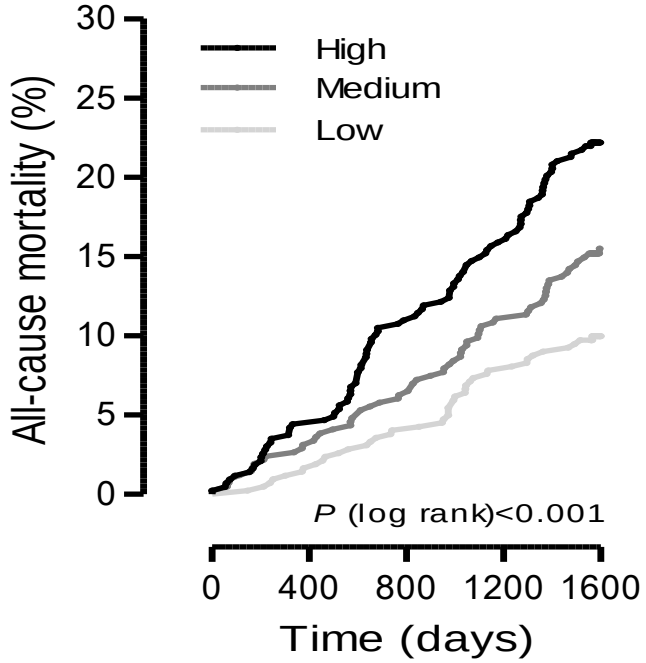

Figure 7.2. Kaplan-Meier plots of low, medium and high GGT levels and all-cause mortality.
GGT, gamma-glutamyltransferase

Non-cardiovascular mortality included accidents meaning that not all deaths were caused by disease. It is well-known that heavy alcohol intake is also responsible for major motor vehicle accidents,[61] criminal offences and suicide attempts.[62] Alcohol is actually a depressant, and when it is consumed excessively it is one of the main risk factors of depression and suicides.[63] Criminal offenses and suicides due to heavy drinking are not uncommon in low resourced impoverished areas of sub-Saharan Africa.[64] However, despite the increased risk of alcohol in injuring oneself, there appears to be a benefit to alcohol in mediating the body's response to injury and reducing in-hospital mortality.[65,66]

One study reported neuroprotective effects of alcohol through inhibition of N-methyl-D-aspartate receptors reducing intracellular calcium accumulation and hyperglycolysis, which is associated with reduced lesion in the brain.[67] However, whether this benefit is applicable to individuals with excessive alcohol use is unknown. Furthermore, given the state of poverty and unemployment,

this study population is likely to suffer from psychological distress which may enhance their hypertension development, with excessive drinking used as a coping mechanism further exacerbating the situation.[68] Finally, despite the above, the risk of excessive alcohol use in CVD and mortality is critically important and from the results of this thesis (Figure 7.3) early interventions that include lifestyle change should be an immediate consideration.

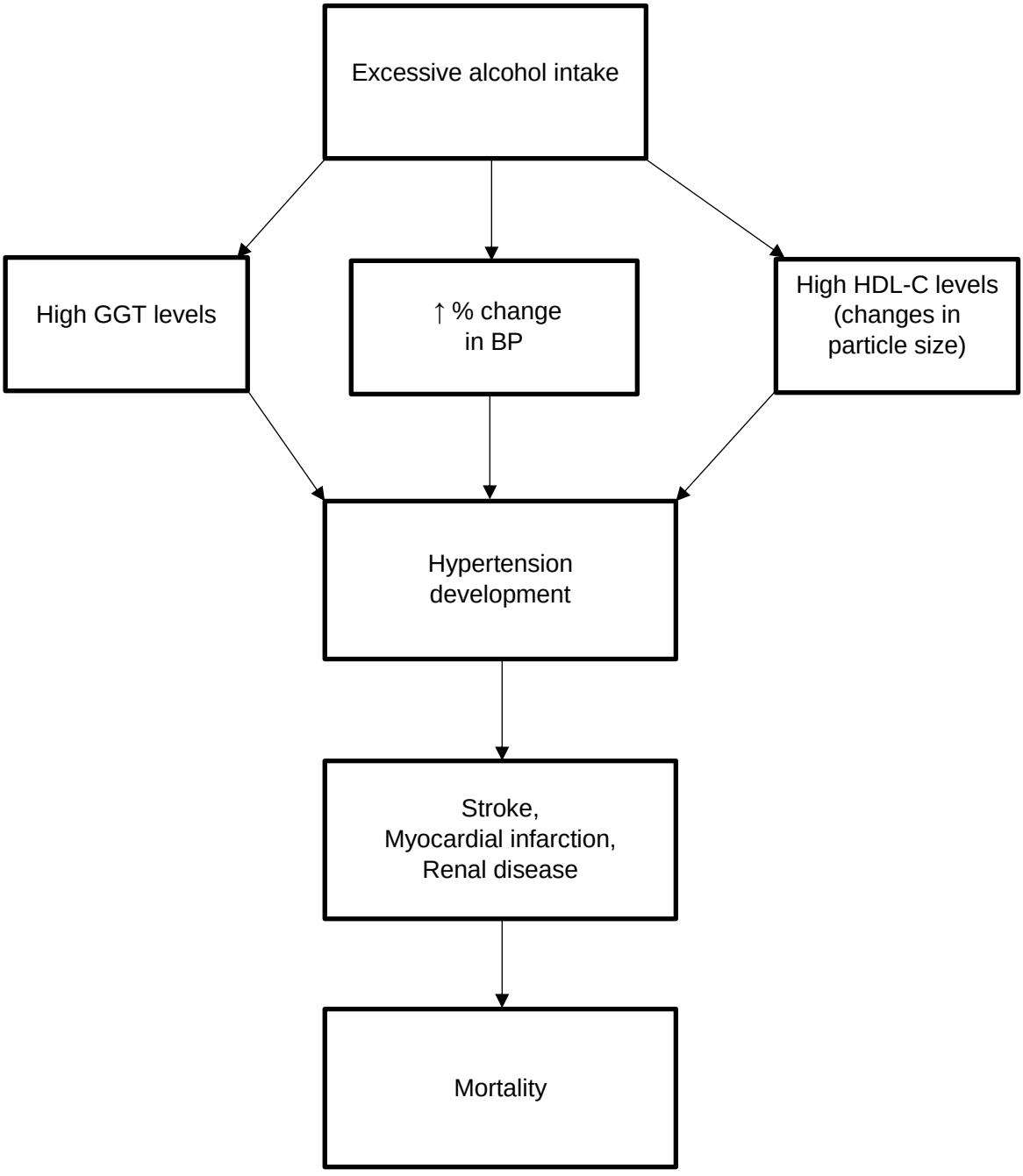

Figure 7.3. A simplified pathway on how alcohol intake leads to cardiovascular mortality. GGT, gamma-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; BP, blood pressure.[1,9,18,35]

4. CHANCE AND CONFOUNDING

It is important to note that several factors may have affected the results of this study. The methodological limitations could have resulted in some weaknesses and influenced various conclusions drawn from the results. Statistically, the results should be confirmed by future similar investigations as possibility of chance should be taken into account. Factors such as chronic illness, medication, physical activity, smoking, self-reported alcohol intake and socio-economic status were possibly affected by bias and cultural background. Although participants were requested not to eat or drink after 10:00 pm for the purpose of taking fasting blood samples, the number of participants who complied with this request could not be confirmed especially to chronic alcohol users who are likely to go to sleep late in the night. Nevertheless, an attempt was made to adjust for confounders relevant to these factors. By adjusting for relevant independent variables, there is a possibility that these covariates confounded associations between alcohol markers and the various variables investigated in this study.

The other self-reported markers of alcohol intake (e.g frequency of alcohol intake or volume of alcohol intake) were perhaps more difficult to report correctly, possibly due to low level of education. Despite the well-known confounders of GGT, ethnicity is one other confounder and Africans seem to have high GGT levels.[30] This may have affected some results in this study. Due to the prospective nature of this study, the number of participants at follow-up decreased as a result of migration to other places for various reasons while others decided not to participate further. Furthermore, some participants may have withdrawn to participate due to HIV testing required in the study because of the stigma associated with HIV infection in Africans.

5. WEAKNESSES AND STRENGTHS OF THIS STUDY

Weaknesses

- This study did not focus much on the influence of the rural or urban origin of the participants. In South Africa rural areas are no longer reflecting the true rural existence since the rise of urbanisation. There is evidence of increased sugar intake over a 5 year period in rural areas of this study population. This added sugar intake was associated with obesity and non-

communicable disease risk factors[69] – findings that are normally associated with urban existence. The urban existence found in many rural areas seems to be carried back by those who migrated to urban areas in search of better living conditions.

- The categorical nature of self-reported alcohol intake means all other factors associated with alcohol intake are ignored compared to biochemical markers which are able to quantify the amount of alcohol intake as they are continuous variables. This is critically important especially considering that, despite the recently proposed phosphatidyl ethanol as the most reliable alcohol marker, there is still no marker of alcohol specific enough to stand alone for alcohol consumption.
- The measurements used to quantify diet (e.g. micronutrients) and lifestyle factors such as smoking and physical activity in this large epidemiological study may not have been sensitive enough to capture the possible confounding effects of these behaviours accurately.
- In addition to risky sexual behaviour and HIV infection, high prevalence of pregnancy is not uncommon in poverty-stricken sub-Saharan population that consume alcohol excessively.[70] Children born to such families are likely to suffer from fetal alcoholic syndrome, which this study did not investigate.
- The population in this study comprised only of black South Africans from a low socio-economic background. Our results are not necessarily generalizable to other ethnic groups in sub-Saharan Africa.

Strengths

- The prospective nature of this study adds to the paucity of longitudinal studies on CVD in sub-Saharan Africa, especially the availability of the mortality data.
- The study population included black individuals from understudied environments, namely, from low resource settings, which are characterised by poverty and a low level of education.
- This thesis showed that elevated HDL-C levels in excessive alcohol consumption is not beneficial and are closely associated with increased CVD risk.

- The presence of extensively trained African field workers, who could speak both the participants' native language and English, ensured that the questionnaire responses were as accurate as possible.
- All the questionnaires were adapted and validated for the African population.

6. RECOMMENDATIONS

Due to the adverse effects of excessive alcohol consumption in CVD in sub-Saharan Africa, it is important that the following recommendations are taken into consideration in order to improve future studies regarding this topic.

- Similar longitudinal studies including data from several research centres throughout sub-Saharan Africa are needed to determine the influence of lifestyle, health behaviours and excessive drinking, in particular on CVD in the African population. This wider representative ethnic population should also consider the rural-urban existence of the study participants, where still applicable.
- Policy makers are encouraged to consider providing funding in order to improve the overall detection of alcohol abuse and education on its consequences in this black African population, which compares poorly with other ethnic groups.
- It is suggested that more advanced validated questionnaires be developed and investigated, relevant to recording other self-reported alcohol estimates accurately, especially in low-resource settings with low level of education.
- Prospective studies that focus specifically on the joint association of heavy alcohol consumption, HIV infection and CVD or mortality need to be explored further.
- The national Department of Health of South Africa is advised to use self-reporting as a tool in primary healthcare settings and to perhaps implement routine GGT assessment to identify individuals at increased CVD risk.
- Since fetal alcoholic syndrome is likely to be more of a concern in low resource settings because of alcohol abuse, poverty, stress and unemployment, it will be a significant advantage in these areas if prospective studies on excessive alcohol consumption and

pregnancy could be performed at the same time. Education on the effects of alcohol beyond CVD is therefore warranted.

- HDL-C particle size in excessive alcohol intake and its association with CVD needs more scientific investigations in South Africa.
- Since studies on alcohol abuse and CVD are scant in South Africa, similar investigations should be extended to other areas and ethnic groups in the country.
- Regarding those patients already on treatment from alcohol-related outcomes such as hypertension and stroke, proper management and timely dispensing of treatment in clinics with no delays in availability could help reduce mortality that is related to these health outcomes.

7. FINAL CONCLUSIONS AND PERSPECTIVES

In addition to smoking, physical inactivity and unhealthy diet, alcohol abuse continues to be a major risk factor for hypertension and CVD in sub-Saharan Africa. This study was able to validate these findings regarding excessive alcohol intake in South Africa. Self-reported alcohol intake was independently associated with change in BP and central SBP over 5 years. Furthermore, GGT independently predicted hypertension development and mortality over the same period. GGT elevation also showed significant correlations with different cardiometabolic profiles from excessive alcohol use to NAFLD. Both GGT and %CDT showed highly significant correlations with self-reported alcohol intake, suggesting that high levels of GGT and %CDT indeed reflect excessive alcohol use. Overall, these results suggest that heavy alcohol consumption is a critical risk factor for hypertension, CVD and total mortality. Early identification, education and prevention of alcohol abuse should be the main target in the low-income black South African population.

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Appendix A

PURE South Africa Adult Questionnaire

PURE/South Africa

We are very grateful to you for your participation in this study. All information given by you will be held in strict confidence, and will be used for the purpose of this study only after removing any personal identifying information.

Adult Questionnaire

INSTRUCTIONS

Please answer EACH question by marking an X in ONE BOX on each line: (unless otherwise instructed)

OR

By writing number(s) in the spaces provided:

OR

By specifying the answer on the line(s) provided

April 28, 2005

Adult Questionnaire

Subject Initials- F= first letter of first name
M= first letter of middle name
L= first letter of last name

3. **National I.D#**

If not applicable please mark the N/A box

Ethnicity Codes

01 - South Asian (India, Sri Lanka, Pakistan, Bangladesh)

02 - Chinese (China, Hong Kong, Taiwan)

03 - Japanese

04 - Malays

05 - Other Asian (Korea, Malaysia, Papua New Guinea, Thailand, Philippines, Indonesia, Nepal,Vietnam, Cambodia, Laos, Myanmar/Burma, Bhutan, Singapore)

06 - Persian

07 - Arab

08 - Black African

09 - Coloured African (Subsaharan African only)

10 - European

11 - Native North/South American or Australian Aborigine

12 - Latin American (Latino)

13 - Bantu/Semi Bantu

14 - Hemitic/Semi Hemitic

15 - Nilotic/Hausa

16 - Pygmie

17 - Swahili

18 - Other (any other ethnoracial group not listed above)

Subject ID

Centre #	Community#	Household #	Subject #

Subject Initials F M L

Today's date: year month day

1. **Name:** _____ _____
   Given name *Surname*

2. **Not applicable in South Africa**

3. **National identity # or equivalent:** _____ **N/A** ☐

4. **DOB:** year month day **OR** **Age** ☐☐ yrs

5. **Sex:** ☐ Female ☐ Male

6. **Marital status:** (check one only)

   ☐ Never married ☐ Currently married ☐ Common law/Living with partner
   ☐ Widowed ☐ Separated ☐ Divorced

7. **Ethnicity:** ☐☐ → (Please refer to facing page for codes)

8. **Caste/Tribe:** _____

9. **What level of formal education have you <u>completed</u>?** (check highest level only):

   ☐ None
   ☐ Primary
   ☐ Secondary/highschool/higher secondary
   ☐ Trade School
   ☐ College/University
   ☐ Unknown

Adult Questionnaire

11. Occupation

Group 1: Legislators, senior officials and managers

Legislators and senior officials
Corporate managers
General managers
Businessman

Group 2: Professionals

Physical, mathematical and engineering science professionals
Life science and health professionals
Teaching professionals
Other professionals

Group 3: Technicians and associate professionals

Physical, mathematical and engineering-science associate professionals/technicians
Life science and health associate professionals/technicians
Teaching associate professionals/technicians
Other associate professionals/technicians

Group 4: Clerks

Clerks
Customer service clerks

Group 5: Service workers and shop and market sales workers

Personal and protective services workers
Models, salespersons and demonstrators

Group 6: Skilled agricultural and fishery workers

Market-oriented skilled agricultural and fishery workers
Subsistence agricultural and fishery workers

Group 7: Craft and related trade workers

Extraction and building trade workers
Metal, machinery and related trades workers
Precision, handicraft, printing and related trades workers
Other craft and related trades workers

Group 8: Plant and machine operators and assemblers

Stationary plant and related operators
Machine operators and assemblers
Drivers and mobile plant operators

Group 9: Elementary occupations

Sales and services elementary occupations
Agricultural, fishery and related labourers
Labourers in mining, construction, manufacturing and transport

Group 10: Armed forces

Armed forces

Group 11: Homemaker

Housewife/Househusband

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (F M L)

10. Not applicable in South Africa

11a) Not applicable in South Africa

b) Please indicate which group best describes your main occupation.
(Please refer to facing page for definitions of groups and instruction manual for detailed definitions)

☐ Group 1 ☐ Group 2 ☐ Group 3 ☐ Group 4 ☐ Group 5
☐ Group 6 ☐ Group 7 ☐ Group 8 ☐ Group 9 ☐ Group 10 ☐ Group 11

c) Not applicable in South Africa

d) What is your main source of income? _____

If occupation is group 11 (homemaker) go to question 13

12. Are you currently employed?

☐ No → (answer 12a - 12b) ☐ Yes → Go to #13

a) Are you retired/stopped work from your primary occupation due to old age? ☐ No ☐ Yes

b) Have you stopped working due to illness? ☐ No ☐ Yes

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (F M L)

13. CURRENT DISABILITY:

		No	Yes
a)	Do you have any problems using your fingers to grasp or handle?	☐	☐
b)	Do you have any trouble walking about?	☐	☐
c)	Do you have any trouble bending down and picking up an object from the floor?	☐	☐
d)	Do you require a walking stick cane/walker to move about?	☐	☐
e)	Do you have any trouble reading or seeing the individual grains of rice/corn on your plate? (with glasses worn)	☐	☐
f)	Do you have trouble seeing a person from across the room? (12 feet/3.5 meters) (with glasses worn)	☐	☐
g)	Do you have trouble speaking and being understood?	☐	☐
h)	Do you have any trouble hearing what is said in a normal conversation?	☐	☐

<u>Subject Medical History</u>

14. Have you experienced any of the following in the last six months?

	No	Yes		No	Yes
a) Chest pain or tightness with usual activity	☐	☐	i) Vomiting	☐	☐
If Yes, ⟶ does the pain spread to the back, neck or inner border of arm	☐	☐	j) Loss of appetite	☐	☐
b) Breathlessness with usual activity	☐	☐	k) Painful or bleeding teeth/gums	☐	☐
c) Cough for at least 2 weeks	☐	☐	l) Jaundice	☐	☐
d) Any sputum while coughing	☐	☐	m) Burning while passing urine	☐	☐
e) Blood in sputum	☐	☐	n) Swelling of feet	☐	☐
f) Wheezing or whistling in the chest	☐	☐	o) Swelling of face	☐	☐
g) Early morning cough with chest tightness	☐	☐	p) Blood in urine	☐	☐
h) Loose stools/diarrhea for at least 3 days	☐	☐	q) Involuntary weight loss of > 3kg	☐	☐

15. Not applicable in South Africa

16a) Do you use glasses/spectacles/contact lenses at present? No ☐ Yes ☐

b) Do you use a hearing aid? No ☐ Yes ☐

Adult Questionnaire

Cancer Sites

1= Mouth
2= Esophagus
3= Stomach
4= Small intestine
5= Large intestine including rectum
6= Pancreas
7= Liver
8= Lung
9= Breast
10= Cervical/uterine/ovarian
11= Prostate
12= Head and neck
13= Other, specify

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

17. Have you ever been diagnosed with any of the following?(check all that apply)

	No	Yes	#of yrs since diagnosis
a) Diabetes	☐	☐	☐☐
b) Hypertension/ high blood pressure	☐	☐	☐☐
c) Stroke	☐	☐	☐☐
d) Angina/heart attack/ Coronary artery disease	☐	☐	☐☐
e) Heart failure	☐	☐	☐☐
f) Other heart disease	☐	☐	☐☐
h) Hepatitis/Jaundice	☐	☐	☐☐
g) Cancer	☐	☐	☐☐

Please refer to facing page for cancer sites → ☐☐ *site*

_____ *other, specify*

	No	Yes	#of yrs since diagnosis
i) COPD	☐	☐	☐☐
j) Asthma	☐	☐	☐☐
k) Tuberculosis	☐	☐	☐☐
l) Malaria	☐	☐	☐☐
m) Chagas	☐	☐	☐☐
n) HIV/AIDS	☐	☐	☐☐

Not answered ☐

18. Have you been taking any medications regularly (ie. at least once per week) in the last month? ☐ No → **go to 19** ☐ Yes

a) If yes, for what conditions:

	No	Yes
Blood pressure	☐	☐
Cholesterol lowering drugs	☐	☐
Stroke	☐	☐
Diabetes	☐	☐
Asthma	☐	☐
Chinese medicine	☐	☐
Others	☐	☐ → If Yes, specify _____
Unknown	☐	☐

18b) If name of medication is unknown, please list as unknown.

Subject ID

Centre #	Community#	Household #	Subject #

Subject Initials F M L

18b) List all the medications you are currently consuming at least once a week for the last month?

i) _____ ii) _____

iii) _____ iv) _____

v) _____ vi) _____

vii) _____ viii) _____

Men go to question #23

For Women Only (Questions 19 - 22)

19. Are you currently pregnant ? ☐ No ☐ Yes → Go to #21

20. Do you still have periods? ☐ No → (answer 20a) ☐ Yes → Go to #21

a) How many years since you stopped menstruating? ☐☐ years

21. Have you ever used an oral/ injectable contraceptive? ☐ No ☐ Yes

22a) How many live children have you given birth to? ☐☐ Boys ☐☐ Girls

b) Did you breast feed any of your children? ☐ No ☐ Yes

Adult Questionnaire

23. Accidents and Injuries

Location of Injury

1= Factory/industrial place
2= Office
3= Agriculture field/farm
4= Home
5= Road
6= Sport/game e.g. track, court, field, etc.
7= Public building
8= Mine/quarry
9= Construction site e.g. building, road-works, etc.
10 = Other

Type of Injury

1= Burns
2= Scalds
3= Fractures
4= Muscle and ligament sprains/tears
5= Cuts and lacerations
6= Bruises and abrasions
7= Suffocation
8= Head injury (where person did not lose consciousness)
9= Head injury (where person lost consciousness for some time)

Subject ID

Centre #	Community#	Household #	Subject #

Subject Initials F M L

23. During the past 12 months, have you had any injuries that were serious enough to limit your normal activities? (check all that apply)

☐ No → **Go to #24** ☐ Yes → **(answer 23a - 23s)**

If yes, please provide details:

Please refer to facing page for Location and Type Codes

Cause of injury			Location	Type	Absence from work or usual activities (Days)
a) Motor vechicle accident (as a passenger)	☐ No	☐ Yes →			
b) Motor vehicle accident (as a pedestrian)	☐ No	☐ Yes →			
c) Struck by an object	☐ No	☐ Yes →			
d) Explosion	☐ No	☐ Yes →			
e) Natural/environmental factors (gales/cyclones/lightning, etc.)	☐ No	☐ Yes →			
f) Suffocation	☐ No	☐ Yes →			
g) Poisoning	☐ No	☐ Yes →			
h) Snake/scorpion bite	☐ No	☐ Yes →			
i) Fall	☐ No	☐ Yes →			
j) Fire/flames, resultant fumes	☐ No	☐ Yes →			
k) Physical assault (gun, kidnapping, etc.)/violent crime	☐ No	☐ Yes →			
l) Domestic violence (beaten by a family member)	☐ No	☐ Yes →			
m) Drowning/submersion	☐ No	☐ Yes →			
n) Hot or corrosive liquids/floods/substances	☐ No	☐ Yes →			
o) Crush injuries (boulders, building materials, etc.)	☐ No	☐ Yes →			
p) Accident caused by machinery	☐ No	☐ Yes →			
q) Attempted suicide	☐ No	☐ Yes →			
r) Armed conflict	☐ No	☐ Yes →			
s) Other(specify) _____	☐ No	☐ Yes →			

Adult Questionnaire

Location of Fractures

1= Hip/pelvis
2= Thigh
3= Leg
4= Forearm
5= Wrist
6= Hand/finger
7= Vertebrae (back)
8= Other

Fractures: In situations where subjects are in a cast and cannot differentiate between ligament tear or fracture, include as fracture only if doctor confirmed it as a broken bone

25c) Tobacco: Regular use is defined as consuming at least one tobacco product per day.

Duration of use:

For those that have consumed tobacco for <1 year, please enter "0"

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

24. Have you ever fractured a bone? ☐ No (go to #25) ☐ Yes (if yes, answer a),b) and c)

a) Number of fractures

b) Years since last fracture (yrs)

c) Bone (s) broken in the most recent fracture(if more than 3, list most severe sites) (location) If other, specify

Please refer to facing page for fracture locations

<u>Tobacco</u>

25. Which best describes your history of tobacco use?

a) ☐ Formerly used tobacco products ☐ Currently use tobacco products ☐ **Never used tobacco products** → **Go to #26**

b) At what age did you start? yrs

c) Have you ever regularly used any of the following tobacco products? (check all that apply)

	Average amount/day		Past users only: Duration (years)	When Stopped (years ago)	If less than 1 yr (months ago)
(i) Cigarettes (all kinds)		number			
(ii) Beedies		number			
(iii) Cigars		number			
(iv) Pipes		number			
(v) Sheesha/water pipe Hookah		# of times			
(vi) Chewing tobacco		# of times			
(vii) Snuff		# of times			
(x) Other _____ *Specify*					

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (F M L)

Question 26 to be answered by non-smokers and former smokers only

26. During the past 12 months, have you been regularly (at least once per week) exposed to other people's tobacco smoke?
("Exposed" is defined as a minimum of 5 consecutive minutes, during which you inhale other people's smoke.)

☐ No ⟶ **Go to #27** ☐ Yes ⟶ Please answer questions **26a**

a) Over the past 12 months, what has been your typical exposure to other peoples smoke?
("Exposed" is defined as a minimum of 5 consecutive minutes, during which you inhale other peoples smoke)

Select ONE only

☐ 1-2 times/week ☐ 3-6 times/week ☐ at least once a day ☐ 2-3 times/day ☐ 4 or more times/day

27. Not applicable in South Africa

Adult Questionnaire

28c) **Alcoholic Beverage:** Regular use is defined as at least once a month.

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (F M L)

28. Which best describes your history of alcohol use?

a) ☐ Formerly used alcohol products ☐ Currently use alcohol products ☐ **Never used alcohol products** → **Go to #29**

b) At what age did you start? ☐☐ yrs

c) What forms of alcohol have you regularly used? (check all that apply)

Form of Alcohol	Approx. size of one "drink"	Frequency: Daily	Weekly	Monthly	Average # of drinks	Duration (years)	Past users only When Stopped (years ago)
(i) Spirits(rum,whisky, gin,vodka etc)	30ml	☐	☐	☐	☐☐	☐☐	☐☐
(ii) Wine	125ml	☐	☐	☐	☐☐	☐☐	☐☐
(vi) Beer	375ml	☐	☐	☐	☐☐	☐☐	☐☐
(vii) Country liquor/arrack/ sugar cane spirit	30ml	☐	☐	☐	☐☐	☐☐	☐☐

d) **At least once a month, do you consume >5 alcoholic drinks/day?** ☐ No → **Go to #29** ☐ Yes

↓ **If yes,(i,ii)**

i) How many times per month do you consume >5 alcoholic drinks in a day? ☐☐

ii) What is the average number of drinks that you consume each time? ☐☐

29 a) During your longest or nocturnal sleep period, what time do you normally go to bed? ☐☐ : ☐☐ (00:00-23:59)

b) During your longest or nocturnal sleep period, what time do you normally wake up? ☐☐ : ☐☐ (00:00-23:59)

c) Do you usually take naps/siestas? ☐ No ☐ Yes → *Total nap duration* ☐☐☐ mins

33. Civic organization: are defined as non-profit, voluntary organization societies, self help groups and clubs.

    Religious organization: are defined as different types of formal and informal groups set up on a religious basis.

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (F M L)

30. Are you a member of any of the following:

			How often do you participate in the activities of this group? Per Month	OR	Per Year
(i) Self help group, Co-operative, Social club, Sports club,	☐ No	☐ Yes →	☐☐		☐☐
(ii) Religious Group (e.g: church group, etc.)	☐ No	☐ Yes →	☐☐		☐☐
(iii) Other _____ *Specify*	☐ No	☐ Yes →	☐☐		☐☐

31. Please answer the following: (choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
(i) People are generally honest and want to help others.	☐	☐	☐	☐
(ii) If I do nice things for someone, I can anticipate that they will respect me and treat me just as well as I treat them.	☐	☐	☐	☐

32a) The television, radio, newspaper or magazine advertisements help me decide to buy the type of: (choose only one option for each)

					Not Applicable
(i) Cooking oil	☐	☐	☐	☐	☐
(ii) Flour	☐	☐	☐	☐	☐
(iii) Rice/ Maize meal	☐	☐	☐	☐	☐

b) The television, radio, newspaper or magazine advertisements influence whether I buy: (choose only one option for each)

(i) Soft drinks	☐	☐	☐	☐	☐
(ii) Snacks	☐	☐	☐	☐	☐
(iii) Cigarettes	☐	☐	☐	☐	☐
(iv) Alcohol	☐	☐	☐	☐	☐

33. In a difficult situation, whose help can you count on from? (Please see facing page for definitions)

(i) Civic organizations: specify _____

☐ none ☐ little ☐ moderate/average ☐ a great deal

(ii) Religious organizations: specify _____

☐ none ☐ little ☐ moderate/average ☐ a great deal

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (F M L)

34. Have you experienced any of the following events during the last 12 months?

	No response	No	Yes	
(i) Loss of job	☐	☐	☐	
(ii) Retirement	☐	☐	☐	
(iii) Loss of crop/business failure	☐	☐	☐	
(iv) Household break in	☐	☐	☐	
(v) Marital separation/divorce	☐	☐	☐	
(vi) Other major intra-family conflict	☐	☐	☐	→ Please specify _____
(vii) Major personal injury or illness	☐	☐	☐	
(viii) Violence	☐	☐	☐	
(ix) Armed conflict/war	☐	☐	☐	
(x) Death of a spouse	☐	☐	☐	
(xi) Death/major illness of another close family member	☐	☐	☐	
(xii) Other major stress	☐	☐	☐	→ Please specify _____
(xiii) Wedding of family member	☐	☐	☐	
(xiv) New job	☐	☐	☐	
(xv) Birth in the family	☐	☐	☐	
(xvi) Seperation from family	☐	☐	☐	
(xvii) Unavailability of food/ food insecurity	☐	☐	☐	

Subject ID

☐☐ ☐☐☐ ☐☐☐ ☐☐
Centre # Community# Household # Subject #

Subject Initials ☐☐☐
F M L

35. Please answer the following: (Choose only one option for each)

For the following question, stress is defined as feeling irritable or filled with anxiety, or as having sleeping difficulties as a result of conditions at work or at home.

	No response	Never Experienced Stress	Some Period of Stress	Several Periods of Stress	Permanent Stress
a) How often have you felt stress at work in the last 12 months? (Mark here if not applicable: i.e. no longer working ☐)	☐	☐	☐	☐	☐
b) How often have you felt stress at home in the last 12 months?	☐	☐	☐	☐	☐

36. What level of financial stress have you felt in the last 12 months?

☐ No response ☐ Little/none ☐ Moderate ☐ High/severe

37. During the past twelve months, was there ever a time when you felt sad, blue, or depressed for two weeks or more in a row?

☐ No ☐ Yes ⟶ **If yes, during those times, did you:**

	No response	No	Yes
a) Lose interest in most things like hobbies, work or activities that usually give you pleasure?	☐	☐	☐
b) Feel tired or low on energy?	☐	☐	☐
c) Gain or lose weight?	☐	☐	☐
d) Have more trouble falling asleep than you usually do?	☐	☐	☐
e) Have more trouble concentrating than usual?	☐	☐	☐
f) Think a lot about death (either your own, someone else's, or death in general)	☐	☐	☐
g) Feel down on yourself, no good or worthless?	☐	☐	☐

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (F M L)

38. **Please answer the following:** (Choose only one option for each)

	Strongly Disagree	Somewhat Disagree	Somewhat Agree	Strongly Agree
a) I can do most of my regular shopping (food, household necessities, etc.) at stores within easy walking distance (less than 15 minutes) of my home.	☐	☐	☐	☐
b) Walking or bicycling in my neighbourhood is difficult because of the speed and/or amount of traffic.	☐	☐	☐	☐
c) My neighbourhood is generally free from pollution (litter, air pollution and noise pollution).	☐	☐	☐	☐
d) My neighbourhood streets are well lit at night.	☐	☐	☐	☐
e) I can see other people when I am walking in my neighbourhood.	☐	☐	☐	☐
f) I can speak to other people when I am walking in my neighbourhood.	☐	☐	☐	☐
g) There is a high crime rate in my neighbourhood.	☐	☐	☐	☐
h) There is a problem with unattended dogs in my neighbourhood.	☐	☐	☐	☐

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (F M L)

38a) Please answer the following: (Please check all that apply)

i) Has your household been a victim of the following crime(s) in the last 12 months?

	No	Yes
1. Armed robbery	☐	☐
2. Violent attacks	☐	☐
3. Murder	☐	☐
4. Vehicle hijacking	☐	☐
5. House breaking	☐	☐
6. Theft	☐	☐
7. Rape	☐	☐
8. Women abuse eg. (beat,swear-words,sexual) please specify _____	☐	☐
9. Child abuse eg. (burn,swear-words,rejection) please specify _____	☐	☐
10. Child sexual abuse	☐	☐
11. Other, please specify _____	☐	☐

ii) Do you think that crime in your area has increased in the past 5 years? ☐ No ☐ Yes

if yes, which of the following crime(s)?

- ☐ Armed robbery
- ☐ Violent attacks
- ☐ Murder
- ☐ Vehicle hijacking
- ☐ House breaking
- ☐ Theft
- ☐ Rape
- ☐ Women abuse
- ☐ Child abuse
- ☐ Child sexual abuse
- ☐ Other, please specify _____

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (F M L)

38b) Questions on HIV:

i) Do you know people who have HIV/AIDS? ☐ No ☐ Yes

if yes, which of these people: (please mark all that apply)

- ☐ Your children
- ☐ Your grandchildren
- ☐ Your spouse
- ☐ Your family members
- ☐ Your friends
- ☐ People in the community

ii) What would you consider the mean age of the people who are ill/have died of HIV/AIDS?

- ☐ Younger than 10 years
- ☐ Between 11-20 years
- ☐ Between 21-30 years
- ☐ Between 31-40 years
- ☐ Between 41-50 years
- ☐ Over 50 years

iii) If someone in your household is HIV positive, who is the primary caregiver?

- ☐ Spouse
- ☐ Parents
- ☐ Family member
- ☐ Child.children
- ☐ Friends
- ☐ Volunteer

38c) Do you care for any orphans in your family? ☐ No ☐ Yes

Adult Questionnaire

40b) Health History:

Cancer Sites

1= Mouth
2= Esophagus
3= Stomach
4= Small intestine
5= Large intestine including rectum
6= Pancreas
7= Liver
8= Lung
9= Breast
10= Cervical/uterine/ovarian
11= Prostate
12= Head and neck
13= Other, specify

Subject ID

Centre #	Community#	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials ☐☐☐ (F M L)

39. How long would it take you to get from your house to the nearest facility if you walked?

	Minutes	Don't know		Minutes	Don't know
i) grocery/convenience store	☐☐☐	☐	iv) video store	☐☐☐	☐
ii) bank	☐☐☐	☐	v) non-fast food restaurant	☐☐☐	☐
iii) post office	☐☐☐	☐	vi) fast food restaurant	☐☐☐	☐

40a) Total number of siblings ☐☐

b) Health History: Complete for all parents and siblings, alive or dead

	Father			Mother			Siblings			# of siblings with the condition
	Unknown	No	Yes	Unknown	No	Yes	Unknown	No	Yes	If yes →
Diabetes	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐☐
Coronary Heart Disease	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐☐
High Blood Pressure	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐☐
Stroke	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐☐
Cancer (Please refer to facing page for cancer sites)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐☐
if Yes, indicate site		☐☐			☐☐			☐☐		
		Other, Specify			Other, Specify			Other, Specify		

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of "0" into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

41. Physical Measurements

a) Right arm blood pressure (Sitting)

#1 Systolic Diastolic mmHg

#2 Systolic Diastolic mmHg

b) Heart Rate

#1 beats/min

#2 beats/min

c) Waist

#1 cm

#2 cm

→ ☐ minimal/no clothing ☐ full clothing

d) Weight kg → ☐ minimal/no clothing ☐ full clothing

e) Hip

#1 cm

#2 cm

→ ☐ minimal/no clothing ☐ full clothing

f) Height cm (without shoes)

42a) Circumference of mid upper right arm: cm

b) Circumference of right calf: cm

c) Head Circumference: cm

d) Upper flexed arm circumference cm

43a) Right arm triceps skinfold:

#1 mm

#2 mm

#3 mm

b) Right calf skinfold:

#1 mm

#2 mm

#3 mm

Subject ID

Centre # | Community# | Household # | Subject #

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c) Biceps skinfold
#1 . mm
#2 . mm
#3 mm

d) Subscapular skinfold
#1 . mm
#2 . mm
#3 mm

e) Supra spinal skinfolds
#1 . mm
#2 . mm
#3 mm

44 a) Humerous breadth . cm

b) Femur breadth . cm

45. Grip Strength (Maximal contraction):

a) Non-dominant hand:
#1 kg.
#2 kg.
#3 kg.

b) Dominant hand:
#1 kg.
#2 kg.
#3 kg.

Adult Questionnaire

If subject refuses to provide any of the measures, enter a value of "0" into each of the boxes for that question

For more detailed instructions please refer to the instruction manual

46. Spirometry:

American Thoracic Society criteria for acceptable spirograms:
Spirograms are acceptable if they are free from:

1. Cough during exhalation
2. Early termination or cut-off
3. Variable effort
4. Leaks
5. Obstructed mouth piece

Subject ID

Centre # | Community# | Household # | Subject #

Subject Initials F M L

46. Spirometry:

a) FEV1 (Litre): #1 ☐☐.☐☐ #2 ☐☐.☐☐ #3 ☐☐.☐☐

b) Does FEV1 obtained meet ATS criteria?

☐ No → (answer (i) to (iii) ☐ Yes → Go to c)

Reasons for not meeting the ATS criteria: (check all that apply)

i) Cough ☐

ii) Values not within 0.2L of each other ☐

iii) Less than 3 values ☐

c) FVC (Litre): #1 ☐☐.☐☐ #2 ☐☐.☐☐ #3 ☐☐.☐☐

d) Does FVC obtained meet ATS criteria?

☐ No → (answer (i) to (iii) ☐ Yes → Go to e)

Reasons for not meeting the ATS criteria: (check all that apply)

i) Cough ☐

ii) Values not within 0.2L of each other ☐

iii) Less than 3 values ☐

e) PEFR (Litre/min): #1 ☐☐☐ #2 ☐☐☐ #3 ☐☐☐

f) Does PEFR obtained meet ATS criteria?

☐ No → (answer (i) to (ii) ☐ Yes → Go to Q#47

Reasons for not meeting the ATS criteria: (check all that apply)

i) Cough ☐

ii) Less than 3 values ☐

Subject ID

Centre #	Community#	Household #	Subject #

Subject Initials F M L

47. Not applicable in South Africa

48. ECG obtained? No → Go to #49 Yes

a) 2 0 ____ (year) ____ (month) ____ (day) → Place ECG :File Label Here

b) Please print ECG label #: ____

49 a) Blood sample obtained? No → Go to #50 Yes

b) Fasting sample Non-fasting sample

c) 2 0 ____ (year) ____ (month) ____ (day) Time ____ : ____ (00:00-23:59) → Hours since any food/beverage consumed (excluding water) ____

d) Please print Blood label #: ____ Place Blood label here

50 a) Urine sample obtained? No → Go to #51 Yes

b) Fasting sample Non-fasting sample

c) Please print Urine label #: ____ Place Urine label here

51. Name of Interviewer: (please print) _____ (First Initial) _____ (Last Name) Interviewer Code: ____

Appendix B

PURE South Africa Quantitative Food Frequency Questionnaire (QFFQ)

PURE Quantitative Food Frequency Questionnaire

Subject ID

Centre #	Community #	Household #	Subject #
☐☐	☐☐☐	☐☐☐	☐☐

Subject Initials

F	M	L
☐	☐	☐

Today's date: ☐☐☐☐ (year) ☐☐ (month) ☐☐ (day)

1. Name: _____ _____

2. Not applicable in South Africa

3. National identity # or equivalent _____ **N/A** ☐

4. DOB: ☐☐☐☐ ☐☐ ☐☐ OR Age ☐☐ years

5. Sex: ☐ Female ☐ Male

Please think carefully about the food and drink you have consumed during the ***past month*** (four weeks). I will go through a list of foods and drinks with you and I would like you to tell me:

- If you eat the food
- How the food is prepared
- How much of the food you eat at a time
- How many times a day you eat it and if you do not eat it everyday, how many times a week or a month you eat it.

To help you to describe the amount of a food you eat, I will show you pictures of different amounts of the food. Please say which picture is the closest to the amount you eat, or if it is smaller, between the sizes or bigger than the pictures.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

FOOD FREQUENCY QUESTIONNAIRE

INSTRUCTIONS: Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

I shall now ask you about the type and the amount of food you have been eating in the last few months. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
PORRIDGE AND BREAKFAST CEREALS AND OTHER STARCH								
Maize-meal porridge	Stiff (pap)						3400	
Maize-meal porridge	Soft (slappap)						3399	
Maize-meal porridge	Crumbly (phutu)						3401	
Ting								
Mabella	Stiff						3437	
Mabella	Soft							
Oats							3239	
Other cooked porridge	Type: _____							
Breakfast cereals	Brand name of cereals at home now: _____ _____ _____ _____							

Do you pour milk on your porridge or cereal? Yes 1 No 2

If yes, what type of milk (whole fresh, sour, 1%, fat free, milk blend, etc) _____

If yes, how much milk								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Do you put sugar on your porridge or cereal?		Yes 1		No 2				
If yes, how much sugar							3989	
							3989	
							3989	
Samp	Bought Self ground						3250	
Samp and beans	Give ratio of samp:beans						3402 (1:1)	
Samp and peanuts	Give ratio of samp:peanuts						3250 (samp)	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
Pasta	Macaroni Spaghetti Other specify: _____ _____						3262	
Pizza	Home made: Specify topping _____ _____						3353 (base+ch)	
	Bought: Specify topping _____ _____						3353 (base+ch)	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		

You are being very helpful. Can I now ask you about meat?

CHICKEN, MEAT, FISH

How many times do you eat meat (beef, mutton, pork, chicken, fish) per week? _____

Chicken (codes with skin)	Boiled						2926	
	Fried: in batter/crumbs Eg Kentucky						3018	
	Fried: Not coated							
	Bought: Chicken Licken						2925	
	Bought: Nando's							
	Roasted / Grilled						2925	
	Other: _____							

Do you eat chicken skin? Always 1 Sometimes 2 Never 3

Chicken bones stew								
Chicken feet							2997	
Chicken offal								
Red meat	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed							
	Mince with tomato and onion						2987	
	Other:							
Beef Offal	Intestines: boiled nothing added						3003	
	Stewed with vegetables							
	Liver						2920	
	Kidney						2923	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other: Specify _____ _____							
Goat meat	Boiled						4281	
	Stewed with vegetables							
	Grilled / Roasted						4281	
What type of vegetables is usually put into meat stews? _____								
Wors / Sausage							2931	
Bacon							2906	
Cold meats	Polony						2919	
	Ham						2967	
	Vienna						2936	
	Other: Specify _____ _____ _____							
Canned meat	Bully beef							
	Other: Specify _____ _____							
Meat pie	Beef						2939	
	Steak and kidney						2957	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Dried beans/peas/lentils	Soup						3145	
	Salad							
Soya products eg. Toppers	Brands at home now: _____ _____						3196 (Toppers)	
Pilchards in tomato/chilli/brine	Whole						3102	
	Mashed with fried onion							
Fried fish	With batter/crumbs							
	Without batter/crumbs							
Other canned fish	Tuna						3056 (oil)	
	Pickled fish							
	Other: Specify _____							
Fish cakes	Bought: Fried						3080	
	Home made with potato						3098	
Fish fingers	Bought						3081	
Eggs	Boiled/poached						2867	
	Scrambled: milk + fat							
	Fried: Fat							
Now we come to vegetables and fruit VEGETABLES AND FRUIT								
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						3756	
	Boiled with potato and onion and fat							
	Fried, nothing added Fried in							
	Boiled, then fried with potato, onion							
	Other:							
	Don't know							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Spinach/morogo/ beetroot leaves other green leafy	How do you cook spinach?							
	Boiled, nothing added						3913	
	Boiled with fat added Type of fat							
	With onion, tomato, potato							
	With peanuts							
	Other:							
	Don't know							
Tomato and onion gravy	Home made with fat Type of fat							
	Without fat						3925	
	Canned						4192	
Pumpkin (yellow)	How do you cook pumpkin?							
	Boiled, nothing added						4164	
	Cooked in fat and sugar Fat							
	Boiled, little sugar and fat Fat							
	Other							
	Don't know							
Carrots	How do you cook carrots?							
	Boiled, nothing added						3757	
	Boiled, sugar and fat Fat							
	With potato and onion: Fat							
	Raw, salad						3709	
	Chakalaka							
	Other							
	Don't know							
Mealies/ Sweet corn	How do you eat mealies?							
	On cob – fat added Fat							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	On cob – no fat added						3725	
	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin						4155	
	Boiled/baked without skin						3737	
	Mashed							
	Roasted Fat							
	French fries (chips)						3740	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Mashed							
	Other: _____							
	Don't know							
Salad vegetables	Mixed salad: tomato, lettuce and cucumber						3921	
	Raw tomato						3750	
	Other salad vegetables: _____ _____							
Other vegetables, specify + preparation	_____ _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Do you like fruit?		Yes 1	No 2					
Apples							3592	
Pears							3582	
Oranges							3560	
Naartjie							3558	
Grapes							3550	
Peaches	Fresh						3565	
	Canned						3567	
Apricots	Fresh						3534	
	Canned						3535	
Mangoes							3556	
Guavas	Fresh						3551	
	Canned						3553	
Avocado							3656	
Wild fruit/berries	Specify type: _____							
Dried fruit	Types: _____							
Other fruit	_____							
If subject eats canned fruit: Do you have custard with the canned fruit?		Yes 1	No 2					
Custard	Home made: Milk							
	Commercial eg Ultramel						2716	
BREAD AND BREAD SPREADS								
Bread / Bread rolls	White						3210	
	Brown						3211	
	Whole wheat						3212	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Do you spread anything on the bread?			Always 1		Sometimes 2		Never 3	
Margarine	What brand do you have at home now?							
	Don't know _____							
Peanut butter							3485	
Jam/syrup/honey							3985	
Marmite / Fray bentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Type: _____ _____ _____							
Achaar								
Other spreads	Specify: _____							
Dumpling								
Vetkoek	White flour						3257	
	Whole wheat flour						3324	
Provita, crackers, etc							3235	
Mayonnaise / salad dressing	Mayonnaise						3488	
	Other: Specify _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
DRINKS								
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
Sugar/cup tea or coffee	Tea:						3989	
	Coffee:						3989	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Whole milk powder Brand: _____						2721 (powder)	
	Low fat milk powder Brand: _____						2825 (powder)	
	Skimmed milk powder Brand: _____						2825 (powder)	
	Milk blend Brand: _____						2770 (powder)	
	Whitener: type _____ _____							
	Condensed milk						2714	
	Evaporated milk						2715	
	None							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Milk as such	What type of milk do you drink milk as such?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Condensed milk						2714	
	Sour/maas						2787	
	Other: _____ _____							
Milk drinks	Nestle: _____							
	Milo: _____							
	Flavoured milk: _____							
	Other:							
Yoghurt	Drinking yoghurt						2756	
	Thick yoghurt						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar						3982	
	- artificially sweetener						3990	
	KoolAid						4027	
	Other: _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Fruit juice	Fresh/Liquifruit/Ceres						2866	
	Tropica (Dairy –fruit juice mix)						2791	
	Other: _____ _____ _____							
Fizzy drinks Coke, fanta, etc	Sweetened						3981	
	Diet							
Maueu/Motogo							4056	
Home brew								
Tlokwe							4039	
Beer							4031	
Spirits							4035	
Wine red							4033	
Wine White							4033	
Other specify	_____ _____ _____							
SNACKS AND SWEETS								
Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	
Cheese curls, Niknaks, etc							3267	
Raisins							3552	
Peanuts and raisins								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Chocolates	Name: _____ _____ _____							
Candies	Sugus, gums, hard sweets, etc						4000	
Sweets	Toffees, fudge, caramels						3991	
Biscuits/cookies	Type: _____ _____ _____							
Cakes and tarts	Type: _____ _____ _____							
Scones								
Rusks	Type: _____ _____							
Savouries	Sausage rolls						2939	
	Samoosas: Meat filling						3355	
	Samoosas: Vegetable filling						3414	
	Biscuits eg bacon kips							
	Other specify: _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Jelly							3983	
Baked pudding	Type: _____							
Instant pudding	Milk type: _____							
Ice cream							3483	
Sorbet							3491	
Other specify	_____ _____ _____							
SAUCES, GRAVIES AND CONDIMENTS								
Tomato sauce / Worcester sauce							3139	
Chutney							3168	
Pickles							3866	
Packet soups							3165	
Other:	_____ _____							
WILD BIRDS, ANIMALS OR INCECTS (hunted in rural areas or on farms)								
Wild fruit								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
MISCELLANEOUS: Please mention any other foods used more than once/two times a week which we have talked about:								
INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS Please tell me if you use any indigenous plants OR other indigenous foods like mopani worms, locusts ect to eat								
Specify								

Thank you very much for your cooperation and patience.

Good-bye!

PURE Salt intake questionnaire

Subject ID Initials

Centre # | Community # | Household # | Subject #

Subject

F M L

Today's date: year | month | day

1. Name: _____

_____

2. Not applicable in South Africa

3. National identity # or equivalent _____ ☐
N/A

4. DOB: ☐☐☐☐ ☐☐ ☐☐ **OR** **Age** ☐☐ **years**

5. Sex: ☐ Female ☐ Male

NUTRITIONAL AND LIFESTYLE HABITS The following questions are about your dietary and life-style habits. All your answers will be strictly confidential							***Office use***	
During the **PAST 7 days (1 week)** did you eat any of the following? IF YES, ASK HOW OFTEN (*if no, circle never*) [DO NOT PROMPT THE ANSWER OPTIONS BELOW]								
Food item	***NEVER***	NOT EVERY DAY		EVERY DAY				
		1-3 times per **week**	4-6 times per **week**	1 time a **day**	2 times a **day**	3+ times a **day**		
White bread/ white bread rolls	0	1	2	3	4	5		4
Brown/wholewheat bread/ Rolls	0	1	2	3	4	5		
Breakfast Cereal (processed)	0	1	2	3	4	5		
Breakfast Cereal (weetbix, muesli)	0	1	2	3	4	5		
Crackers (ProVita etc)	0	1	2	3	4	5		
Cookies, biscuits,rusks	0	1	2	3	4	5		
Cake/scone/ muffin/ puddings/pancake/fruit pie/koeksister	0	1	2	3	4	5		
Roti/ samoosa/springroll/doughnut	0	1	2	3	4	5		
Pizza	0	1	2	3	4	5		
Pasta/noodle dishes with cheese sauces (macaroni cheese, lasagne, noodle salad etc.)	0	1	2	3	4	5		
Popcorn	0	1	2	3	4	5		

NUTRITIONAL AND LIFESTYLE HABITS

The following questions are about your dietary and life-style habits. All your answers will be strictly confidential

Office use

During the **PAST 7 days (1 week)** did you eat any of the following? IF YES, ASK HOW OFTEN (*if no, circle never*) [DO NOT PROMPT THE ANSWER OPTIONS BELOW]

Food item	***NEVER***	NOT EVERY DAY		EVERY DAY			
		1-3 times per **week**	4-6 times per **week**	1 time a **day**	2 times a **day**	3+ times a **day**	
Crisps (Simba and Niknaks etc.)	0	1	2	3	4	5	
Sausage (wors)	0	1	2	3	4	5	
Polony/salami/bacon/salami/pork suasages (processed meat, cooked, smoked and canned)	0	1	2	3	4	5	
Meat or chicken pies/sausage rolls	0	1	2	3	4	5	
Chicken - battered (KFC etc). and chicken burger only	0	1	2	3	4	5	
Meat and meat dishes (steaks, minced meat, cottage pie, mince, meatballs, stew, bobotie, etc.)	0	1	2	3	4	5	
Gravy, made with stock or gravy powder	0	1	2	3	4	5	
Biltong/dry wors/bokkems	0	1	2	3	4	5	
Milk (all types, also dairy fruit juice, malted milk, milk shakes)	0	1	2	3	4	5	
Maas	0	1	2	3	4	5	
Cheese	0	1	2	3	4	5	
Yoghurt	0	1	2	3	4	5	
Eggs	0	1	2	3	4	5	
Tinned fish (pilchards/tuna, etc.)	0	1	2	3	4	5	
Other fish and seafood	0	1	2	3	4	5	
Potato chips/french fries and potato salad	0	1	2	3	4	5	
Canned vegetables, incl. Baked beans, tomato paste, sweetcorn, etc.	0	1	2	3	4	5	
Soup (all types)	0	1	2	3	4	5	
Salad dressing/mayonnaise	0	1	2	3	4	5	
Ice cream (all types)	0	1	2	3	4	5	
Margarines, all types, also butter	0	1	2	3	4	5	
Chutney / atchar/chakalaka / Worcester sauce	0	1	2	3	4	5	
Savoury sauces (mushroom, monkey gland, white,cheese)	0	1	2	3	4	5	
Tomato sauce	0	1	2	3	4	5	
Salt	0	1	2	3	4	5	
Aromat / Fondor /mustard	0	1	2	3	4	5	
Peanuts	0	1	2	3	4	5	
Peanut butter	0	1	2	3	4	5	
Marmite/Bovril	0	1	2	3	4	5	
Chocolate sweets and sauce	0	1	2	3	4	5	
Beer and cider	0	1	2	3	4	5	

20

PURE 24-hour recall dietary intake

Subject ID

Centre # | Community # | Household # | Subject #

Subject Initials

F M L

Today's date: year month day

1. Name: _____ _____

2. Not applicable in South Africa

3. National identity # or equivalent _____ **N/A** ☐

4. DOB: ☐☐☐☐ ☐☐ ☐☐ **OR** Age ☐☐ years

5. Sex: ☐ Female ☐ Male

6. What day was yesterday? (tick correct one)

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

7. Would you describe the food that you ate yesterday as typical of your usual food intake?

Yes	1

No	2

Greetings!

Thank you for giving up your time to participate in this study. I hope you are enjoying it so far. Here we want to find out what people living in this are eat and drink. This information is important to know as it will tell us if people are eating enough and if they are healthy.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

I want to first ask you a few general questions about your food intake, the preparation of food and the type of food that you use in your home.

Instruction
Circle the subject's answer.

8. What type of pot do you usually use to prepare food in? (may answer more than one)

Iron pot ..... ☐ 1

Stainless steel pot ..... ☐ 2

Aluminium pot ..... ☐ 3

Glass ware ..... ☐ 4

Other (specify) ..... ☐ 5

9. Do you eat maize meal porridge? Yes 1 No 2

If YES, what type do you have at home now?

Brand name: _____

Don't know: _____ 2

Grind self: _____ 3

If brand name is given, do you usually use this brand? Yes 1 No 2 Don't know 3

Where do you get your maize meal from? (may answer more than one)

Shop ..... ☐ 1

Employer ..... ☐ 2

Harvest and grind self ..... ☐ 3

Other (specify) ..... ☐ 4

Don't know ..... ☐ 5

10. Do you eat fat/margarine or use it in the preparation of food? Yes 1 No 2

If YES, what type do you have at home now?

Brand name: _____

Don't know: _____ 2

If brand name is given, do you usually use this brand? Yes 1 No 2 Don't know 3

11. Do you use oil in the preparation of food? Yes 1 No 2

If YES, what type do you have at home now?

Brand name: _____

Don't know: _____ 2

If brand name is given, do you usually use this brand? Yes 1 No 2 Don't know 3

What type of oil do you buy for deep frying?

Brand name: _____

Do you use the same oil more than once? Yes 1 No 2

If yes, how many times will you use the same oil? _____

12. What type of salt do you use?

Give brand names _____

Do you add salt to food while it is being cooked?

Always 1	Sometimes 2	Never 3	Don't know 4

Do you add salt to your food after it has been cooked?

Always 1	Sometimes 2	Never 3

Do you like salty foods eg salted peanuts, crisps, chips, fritos, biltong, dried sausage, etc

Very much 1	Like it 2	Not at all 3

13. Do you use any of the following:

	Name of product	**Amount per day**
Vitamins/vitamins and minerals		
Tonics		
Health foods		
Body building preparations		
Dietary fibre supplement		
Other: Specify _____ _____ _____		

I want to find out about everything you ate or drank yesterday, including water or food you pick from the veld. Please tell me everything you ate from the time you woke up yesterday up to the time you went to sleep. I will also ask you where you ate the food and how much you ate.

To help you to describe the amount of a food you eat, I will show you pictures and examples of different amounts of the food. Please say which picture or example is the closest to the amount you eat, or if it is smaller, between the sizes or bigger than the pictures.

Time	Place	Description of food and preparation method	Amount	Amount in gram	Code (off use)

Appendix C

Published Research Article (Chapter 4)

Original Article

Self-reported alcohol intake is a better estimate of 5-year change in blood pressure than biochemical markers in low resource settings: the PURE study

Mandlenkosi C. Zatu[a,d], Johannes M. van Rooyen[a], Du Toit Loots[b], Edelweiss Wentzel-Viljoen[b], Minrie Greeff[c], and Aletta E. Schutte[a]

Background: Despite criticism of self-reported alcohol intake, it is a valuable tool to screen for alcohol abuse as a risk factor for cardiovascular disease. We aimed to compare various self-reported estimates of alcohol use with γ-glutamyltransferase (GGT) and percentage carbohydrate deficient transferrin (%CDT) considering their relationship with blood pressure changes (%BP) over a 5-year period in black South Africans.

Method: We recruited 1994 participants and collected 5-year followed up data ($N = 1246$). Participants completed questionnaires on alcohol intake indicating their former and current alcohol use ('yes' response and 'no' if alcohol was never used). We assessed alcohol intake (in g) using a quantified food frequency questionnaire. We collected blood samples and measured GGT and %CDT. Brachial BP (bBP) was measured at baseline and follow-up and central BP (cBP) at follow-up only.

Results: Self-reported alcohol intake was significantly associated with the 5-year change in bBP before and after adjusting for confounders (%bSBP: $R^2 = 0.263$, $\beta = 0.06$, $P = 0.023$; %bDBP: $R^2 = 0.326$, $\beta = 0.08$ $P = 0.005$), as well as cSBP ($R^2 = 0.286$, $\beta = 0.09$, $P = 0.010$) and central pulse pressure ($R^2 = 0.254$, $\beta = 0.06$, $P = 0.020$). GGT and %CDT correlated well with self-reported alcohol intake ($r = 0.44$; $P = 0.001$; $r = 0.34$ $P = 0.001$), but did not associate significantly with %bBP or cBP at follow-up.

Conclusion: Self-reported alcohol use was strongly associated with a 5-year increase in BP in Africans with a low socio-economic status. This was not found for biochemical measures, GGT and %CDT. Self-reported alcohol intake could be an important measure to implement in primary healthcare settings in middle to low income countries, where honest reporting is expected.

Keywords: blood pressure, cardiovascular disease, γ-glutamyltransferase, hypertension, low socio-economic status, percentage carbohydrate deficient transferrin, self-reported alcohol intake

Abbreviations: %CDT, percentage carbohydrate deficient transferrin; %DBP, percentage change in DBP; %SBP, percentage change in SBP; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BP, blood pressure; cPP, central pulse pressure; CRP, C-reactive protein; cSBP, central SBP; CVD, cardiovascular disease GGT, γ-glutamyltransferase; HbA1C, glycosylated haemoglobin; HDL-C, high-density lipoprotein cholesterol; PURE study, Prospective Urban and Rural Epidemiology study; TC, total cholesterol

INTRODUCTION

Cardiovascular disease (CVD) is a major challenge in developing countries and is one of the main causes of morbidity and mortality [1,2]. Rapid urbanization in sub-Saharan Africa (SSA) drives many lifestyle changes, including unhealthy high fat diets, stressful lifestyle and chronic excessive alcohol abuse [3,4], the latter of which is regarded as a critical risk factor for hypertension, stroke and coronary heart disease [5,6]. Although the detrimental consequences of alcohol abuse are constantly emphasized [7,8], excessive alcohol consumption remains a significant concern in SSA [9]. Apart from the devastating cardiovascular consequences of alcohol abuse in urbanizing SSA, it poses additional threats in the form of premature deaths through malnutrition, stress, violence and crime [10].

Biochemical markers for alcohol use are accepted as superior to self-reported estimates. Although self-reporting is the easier and cheaper method for assessing alcohol intake, its potential inaccuracy may lead to an underestimation or overestimation of alcohol intake, especially when comparing participants from different ethnical backgrounds with differing social perception to alcohol consumption [11]. The biochemical markers that are commonly used in screening for alcohol abuse are γ-glutamyltransferase (GGT) and percentage carbohydrate deficient

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transferrin (%CDT). GGT is the most widely used marker of excessive drinking [12], but high GGT levels could also be associated with ageing, obesity and liver damage [13,14]. Percentage CDT, on the contrary, is another well known marker of alcohol intake and is characterized by a higher specificity and lower sensitivity to alcohol consumption, when compared with GGT [12].

Due to limited resources in SSA to test for biochemical measures of alcohol abuse, we aimed to compare self-reported estimates with these biochemical measures (GGT and %CDT), considering their relationship with percentage change in brachial blood pressure (%bSBP, %bDBP) and central SBP over a 5-year period, in a black South African population.

METHODOLOGY

Study design and participant selection

This study is based on the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study that investigated the health transition in urban and rural individuals in the North West Province. This is an ongoing investigation that involves a series of follow-up studies over a 12-year period. The baseline data used in the present study were collected in 2005, with the first follow-up data obtained in 2010. To keep track of participants over the 5-year follow-up period, trained field workers contacted the participants every 3 months. A total of 2021 African volunteers (age 30 years and older) from 6000 households were originally recruited from urban and rural areas. The recruited participants were visited at their homes and gave voluntary informed consent.

The study was approved by the Ethics committee of the North-West University, South Africa. The provincial Department of Health of the North West Province, community leaders, tribal chiefs and mayors also gave permission to conduct the study. All participants were informed about the objectives and procedures of the study and were asked to fast for 10 h prior to sample collection. Field workers were available to do the translation in the participant's native language. Confidentiality and anonymity of all the results were assured, and individuals identified with chronic illnesses such as hypertension and HIV were referred to their local clinics and hospitals. Participants received remuneration for travelling expenses during the study, in addition to the necessary counselling.

Questionnaires

The participants were also interviewed using structured demographic, socio-economic, lifestyle and physical activity questionnaires developed and standardized for the international PURE study [15]. The questionnaires had questions on alcohol consumption behaviour including a no/yes question on alcohol use (yes, current or former use; no, never used), the quantity, frequency of intake and the type of alcoholic beverage. In these questions, intakes of different beverages were assessed separately.

In addition, a validated [16,17] food frequency questionnaire was completed for each participant by trained fieldworkers. The food intake was coded and analysed using the South African Food Composition database [17,18]. Alcohol consumed was expressed as pure alcohol in grams per day. Beer, home-made brews, spirits and wine were considered to contain 3.6, 3, 36 and 9.4 g of pure alcohol per 100 g of beverage, respectively.

Cardiovascular measurements

An appointment was secured with each participant to measure the cardiovascular variables. After a 10-min rest period, blood pressure (systolic, diastolic) and heart rate were measured in duplicate, 5 min apart, with the validated Omron HEM-757 apparatus (Omron Healthcare, Kyoto, Japan) while the participants were seated upright with the right arm at heart level. Additional cardiovascular measurements, namely central SBP (cSBP) and central pulse pressure (cPP) were taken at follow-up in 2010 using the Omron HEM-9000AI (Omron HealthCare) whilst the participant was in a sitting position.

Anthropometric measurements

Height, weight, waist and hip circumferences were measured (Precision Health Scale, A & D Company, Tokyo Japan; Invicta Stadiometer, IP 1465, Leicester, UK; Holtain unstretchable metal tape) using standardized methods [19].

Blood, serum and plasma samples

After fasting for approximately 10 h, a registered nurse took a blood sample from the ante-brachial vein branches. Samples were prepared according to appropriate methods and stored at −80°C until analyses. Blood samples were immediately frozen and stored at −18°C for no longer than 5 days, transported to a laboratory facility and stored at −80°C until analysis.

Biochemical analyses

From the blood samples, we measured GGT, alanine aminotransferase (ALT), aspartate aminotransferase (AST), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglycerides, C-reactive protein (CRP) and serum glucose (Konelab20i auto analyser; Thermo Scientific, Vantaa, Finland). Furthermore, glycosylated haemoglobin (HbA1c) was determined in EDTA-treated whole blood using the D-10 Haemoglobin testing system (Bio-Rad Laboratories, Hercules, California, USA) and serum %CDT analyses were performed by using an in-vitro heterogeneous immunoassay with column separation followed by a turbidimetric measurement (Axis-Shield %CDT kit, Oslo, Norway). The coefficient of variance (CV) for all assays was less than 10%.

The protocol of the National Department of Health, South Africa, was followed in determining the HIV status of all the participants. HIV status was determined in 2005 by using the First Response (PMC Medical, Daman, India) rapid HIV card test with whole blood. The test was repeated by using the Pareeshak (BHAT Bio-tech, Bangalore, India) card test. Everyone received pretest counselling before the test was done and then post-test counselling for those who tested positive and opted to know their results.

Statistical analyses

Statistica version 11 (StatSoft, Inc., Tulsa, Oklahoma, USA) was used to compare the means and proportions in this study. Variables with a non-Gaussian distribution were logarithmically transformed. Analysis of variance (ANOVA) was used to compare the variables when the 2005 characteristics of the participants were divided into three groups: those who were successfully followed from 2005 to 2010; those lost during the follow-up period; and those who passed away. Chi-square tests were used to compare proportions for categorical variables. Dependent *t*-tests and the McNemar test were used to compare baseline and follow-up variables. Single and partial linear regression analyses were employed to correlate alcohol markers with each other and with the cardiovascular variables. The multiple regression models employed percentage change in brachial SBP (%bSBP), DBP (%bDBP), and cSBP and cPP (taken at follow-up) as dependent variables with a single alcohol marker, and the following variables were included as independent variables: age, rural/urban, sex, BMI, HbA1c, TC to HDL ratio (TC:HDL), CRP, smoking, physical activity, hypertension medication at baseline and HIV status.

RESULTS

Table 1 presents baseline characteristics of those followed, lost to follow-up and those who passed away. Over this 5-year follow-up study, we were able to collect data from 1473 (73%) of the original 1994 recruited participants, of whom 227 (11.3%) passed away. At baseline, participants who passed away had a significantly lower BMI and higher proportion than the other groups reported that they use alcohol, confirmed by all the biochemical markers of alcohol abuse at baseline ($P < 0.001$). Those participants lost to follow-up were generally younger and showed the shortest duration of alcohol consumption. The three groups were comparable in terms of blood pressure.

Table 2 indicates the changes in characteristics of the follow-up group ($n = 1246$) over a period of 5 years. Obesity markers (weight, BMI, waist circumference), metabolic markers (glucose, HbA1c, TC:HDL, CRP) and BP increased despite a lowering in reported alcohol intake and serum liver enzymes.

To determine whether self-reported alcohol measures are related to the biochemical measures, we compared the correlations of self-reported and biochemical alcohol markers in Table 3. Self-reported alcohol intake (no/yes) showed highly significant associations with all the biochemical markers ($P \leq 0.001$) and showed a stronger correlation with GGT ($r = 0.44$; $P = 0.001$) and %CDT ($r = 0.34$; $P = 0.001$) than the intercorrelation between %CDT and GGT ($r = 0.23$; $P < 0.001$).

Table 4 shows the independent associations of alcohol markers with the 5-year percentage change in BP (%SBP or %DBP), and cSBP or cPP. Self-reported alcohol intake was the only alcohol measure that was associated with %bSBP, %bDBP, cSBP and cPP at follow up, independent of the

TABLE 1. Descriptive characteristics at baseline of the study population who were followed-up, lost or passed away by 2010

	Follow-up ($n = 1246$)	Lost to follow-up ($n = 548$)	Passed away ($n = 227$)	*P*
Socio-demographic profile				
Age (years)	50.0 ± 10.16[a,b]	46.4 ± 9.39[a,c]	52.6 ± 11.82[b,c]	**<0.001**
BMI (kg/m^2)	25.1 ± 7.10[a]	24.98 ± 7.12[b]	22.2 ± 6.14[a,b]	**<0.001**
Sex, women, *n* (%)	824/1246 (66.1)[a]	324/537 (60.3)[a,b]	116/227 (51.1)[a,b]	**<0.001**
Location, rural, *n* (%)	669/1246 (53.7)[a]	238/547 (43.5)[a]	108/227 (47.6)	**<0.001**
HIV infected, *n* (%)	148/1239 (12.0)[a,c]	97/548 (18.0)[a,b]	75/224 (33.5)[b,c]	**<0.001**
Smoking, *n* (%)	674/1241 (54.3)[a]	303/533 (56.9)	142/226 (62.8)[a]	**0.053**
Biochemical measurements				
Serum glucose (mmol/l)	4.85 (3.50–6.90)	4.80 (3.50–6.20)	4.69 (3.40–6.10)	0.098
Glycosylated haemoglobin (%)	5.63 (4.90–6.60)[a]	5.51 (4.80–6.30)[a]	5.54 (4.70–6.60)	**0.001**
Total cholesterol (mmol/l)	5.10 ± 1.34[a]	4.97 ± 1.45[b]	4.61 ± 1.33[a,b]	**<0.001**
TC:HDL	3.81 ± 2.43	3.90 ± 2.55	4.26 ± 4.42	**<0.001**
C-reactive protein (mg/l)	3.10 (0.25–39.4)[a]	2.60 (0.27–31.8)[b]	5.18 (0.32–53.6)[a,b]	**<0.001**
Markers of alcohol intake				
Self-reported alcohol use, *n* (%)	517/1239 (41.7)[a]	241/533 (45.2)	124/225 (55.1)[a]	**0.001**
Alcohol intake (g/day)[d]	11.4 ± 23.2[a]	11.2 ± 24.1	15.7 ± 25.3[a]	**<0.001**
Daily frequency of alcohol use, *n* (%)	167/473 (35.3)[a]	66/228 (29.0)[a]	38/118 (32.2)	**0.050**
>5 drinks/day at least once/month, *n* (%)	90/366 (25.0)	30/171 (18.0)	23/82 (28.0)	0.102
Started drinking age (years)	26.1 ± 8.90	26.0 ± 8.58	25.5 ± 9.18	0.779
Duration of drinking (years)	23.0 ± 10.9[a,b]	20.5 ± 9.81[a,c]	26.2 ± 13.2[b,c]	**<0.001**
Volume of alcohol intake (l)	3.28 ± 8.00	4.58 ± 17.3	3.24 ± 6.91	0.352
Alanine aminotransferase (IU/l)	17.5 (7.71–49.7)[a]	18.7 (8.00–53.0)	19.6 (7.00–69.0)[a]	**<0.001**
Aspartate aminotransferase (U/l)	28.0 (13.0–96.1)[a]	29.1 (12.0–102.0)[b]	34.0 (12.0–146.1)[a,b]	**<0.001**
γ-glutamyltransferase (U/l)	53.9 (19.0–350)[a]	53.7 (17.8–347)[b]	74.0 (21.8–488)[a,b]	**<0.001**
Carbohydrate deficient transferrin (%)	2.92 ± 1.37[a]	2.94 ± 1.49[b]	3.26 ± 1.53[a,b]	**0.006**
Cardiovascular variables				
SBP (mmHg)	133 ± 23.7	132 ± 24.15	136 ± 29.1	0.163
DBP (mmHg)	87.8 ± 14.1	87.1 ± 14.9	88.4 ± 16.3	0.515
Pulse pressure (mmHg)	45.7 ± 15.0	45.3 ± 14.1	47.8 ± 18.0	0.108
Heart rate (bpm)	73.4 ± 15.4[a]	72.4 ± 15.6[b]	80.5 ± 19.2[a,b]	**<0.001**

Values are interpreted as arithmetic mean ± standard deviation; geometric mean (fifth to 95th percentile interval); number of participants (%). Bold *P*-values indicate p<0.05.
[a,b,c]Values with the same superscript letter differed significantly ($P < 0.05$).
[d]Alcohol intake as analysed using the quantified food frequency questionnaire (QFFQ).

TABLE 2. Comparison of the study population over 5 years ($N = 1246$)

	Baseline (2005)	Follow-up (2010)	*P*
Anthropometric measurements			
Weight (kg)	64.0 ± 17.1	65.2 ± 17.9	**<0.001**
Height (m)	1.60 ± 0.08	1.59 ± 0.08	**<0.001**
BMI (kg/m^2)	24.1 (16.5–38.5)	25.0 (16.4–39.8)	**<0.001**
Waist circumference (cm)	80.0 ± 13.0	82.0 ± 13.2	**<0.001**
Biochemical measurements			
Serum glucose (mmol/l)	4.86 (3.50–6.90)	5.10 (3.90–7.30)	**<0.001**
Glycosylated haemoglobin, %	5.63 (4.90–6.60)	6.02 (5.10–7.30)	**<0.001**
TC:HDL	3.47 (1.85–6.61)	3.65 (1.90–6.95)	**<0.001**
C-reactive protein (mg/l)	3.10 (0.25–39.4)	3.51 (0.33–34.0)	**0.013**
Markers of alcohol intake			
Self-reported alcohol use, *n* (%)	517/1239 (41.7)	430/1169 (36.8)	**<0.001**
Alcohol intake (g/day)[a]	13.5 (1.07–85.1)	11.0 (1.00–107)	**<0.001**
>5 drinks/day at least once/month, *n* (%)	143/619 (23.1)	–	–
Alanine aminotransferase (U/l)	17.5 (7.71–49.7)	16.8 (7.70–48.9)	**0.013**
Aspartate aminotransferase (U/l)	27.9 (12.6–96.1)	25.7 (13.5–79.7)	**<0.001**
γ-glutamyltransferase (U/l)	53.9 (19.0–350)	45.1 (12.1–310)	**<0.001**
Carbohydrate deficient transferrin, %	2.63 (1.32–5.62)	–	–
Cardiovascular measurements			
SBP (mmHg)	133 ± 23.8	135 ± 24.1	**0.017**
DBP (mmHg)	87.8 ± 14.1	88.4 ± 13.6	0.159
Central SBP (mmHg)	–	147 ± 24.4	–
Central pulse pressure (mmHg)	–	58.3 ± 18.4	–

Values are arithmetic mean ± standard deviation; geometric mean (fifth to 95th percentile interval); number of participants (%). Bold *P*-values indicate p<0.05.
[a]Alcohol intake as analysed using the quantified food frequency questionnaire (QFFQ).

various confounders. Furthermore, %bDBP associated positively with the volume of alcohol intake, whereas the duration of drinking was negatively associated with cPP.

Sensitivity analyses

Due to weak associations between %BP and alcohol markers, we wanted to determine whether baseline BP would yield similar results. When substituting %BP with baseline BP (Supplemental Tables S1 and S2, http://links.lww.com/HJH/A315), we found that SBP and DBP correlated significantly with baseline self-reported alcohol intake (both $r = 0.17$; $P < 0.001$), liver enzymes and %CDT, also after adjusting for age, sex and BMI ($P \leq 0.001$).

We additionally excluded all individuals who changed reporting from 'No' to 'Yes' or from 'Yes' to 'No' in 2005 vs. 2010. After repeating the original multiple regression

TABLE 3. Single linear regression analysis of markers of alcohol intake at baseline ($N = 1246$)

	Daily frequency of alcohol use, *n* (%)	Volume of alcohol (l)	>5 drinks/day at least once/month, *n* (%)	Duration of drinking (years)	Alcohol intake (g/day)	ALT (U/l)	AST (U/l)	GGT (U/l)	CDT, %
Self-reported alcohol, *n* (%)					$r = 0.42$	$r = 0.20$	$r = 0.32$	$r = 0.44$	$r = 0.34$
					$P =$ **0.001**	$P <$ **0.001**	$P =$ **0.001**	$P =$ **0.001**	$P =$ **0.001**
Start drinking age (years)	$r = 0.15$	$r = -0.09$	$r = 0.04$	$r = -0.57$	$r = -0.01$	$r = -0.10$	$r = -0.18$	$r = -0.06$	$r = -0.06$
	$P =$ **0.001**	$P = 0.06$	$P = 0.44$	$P =$ **0.001**	$P = 0.87$	$P =$ **0.03**	$P <$ **0.001**	$P = 0.17$	$P = 0.24$
Daily frequency of alcohol use, *n* (%)		$r = 0.15$	$r = 0.02$	$r = -0.14$	$r = -0.23$	$r = -0.11$	$r = -0.15$	$r = -0.13$	$r = -0.05$
		$P =$ **0.001**	$P = 0.72$	$P =$ **0.003**	$P <$ **0.001**	$P =$ **0.02**	$P =$ **0.002**	$P =$ **0.006**	$P = 0.30$
Volume of alcohol (l)			$r = 0.23$	$r = 0.10$	$r = -0.02$	$r = -0.07$	$r = -0.04$	$r = 0.03$	$r = -0.07$
			$P =$ **0.001**	$P =$ **0.04**	$P = 0.69$	$P = 0.14$	$P = 0.44$	$P = 0.55$	$P = 0.15$
>5 drinks/day at least once per month, *n* (%)				$r = -0.09$	$r = 0.04$	$r = 0.08$	$r = 0.11$	$r = 0.15$	$r = -0.01$
				$P = 0.10$	$P = 0.50$	$P = 0.12$	$P =$ **0.05**	$P =$ **0.006**	$P = 0.82$
Duration of drinking (years)					$r = -0.03$	$r = -0.02$	$r = 0.02$	$r = -0.02$	$r = -0.04$
					$P = 0.55$	$P = 0.71$	$P = 0.62$	$P = 0.74$	$P = 0.41$
Alcohol intake (g/day)[a]						$r = 0.12$	$r = 0.21$	$r = 0.25$	$r = 0.19$
						$P =$ **0.03**	$P =$ **0.001**	$P =$ **0.001**	$P <$ **0.001**
ALT (U/l)							$r = 0.62$	$r = 0.60$	$r = 0.12$
							$P =$ **0.001**	$P =$ **0.001**	$P <$ **0.001**
AST (U/l)								$r = 0.60$	$r = 0.21$
								$P =$ **0.001**	$P <$ **0.001**
GGT (U/l)									$r = 0.23$
									$P <$ **0.001**

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CDT, carbohydrate deficient transferase; GGT, γ-glutamyltransferase. Bold *P*-values indicate p<0.05.
[a]Alcohol intake as analysed using the quantified food frequency questionnaire (QFFQ).

TABLE 4. Multiple regression analyses with blood pressure as dependent variables, and one marker of alcohol intake as the main independent variable

	% Brachial SBP[a]				% Brachial DBP[b]		
Alcohol marker	***N***	***R^2***	**β value (SE)**	***P***	***R^2***	**β value (SE)**	***P***
Self-reported alcohol, *n* (%)	1239	0.263	0.06 (0.03)	0.023	0.326	0.08 (0.03)	0.005
Alcohol intake (g/day)[e]	1221	0.252	0.03 (0.03)	0.36	0.318	0.03 (0.03)	0.30
Daily frequency of alcohol intake, *n* (%)	473	0.239	0.03 (0.04)	0.57	0.307	−0.03 (0.04)	0.50
>5 drinks/day at least once/month, *n* (%)	366	0.232	0.01 (0.05)	0.81	0.301	−0.03 (0.05)	0.56
Started drinking age (years)	472	0.238	0.01 (0.05)	0.83	0.306	0.003 (0.044)	0.94
Duration of drinking (years)	472	0.239	−0.04 (0.06)	0.43	0.306	0.02 (0.05)	0.76
Volume of alcohol intake (l)	457	0.242	0.07 (0.04)	0.13	0.314	0.08 (0.04)	0.040
ALT (U/l)	1163	0.251	−0.02 (0.03)	0.41	0.320	−0.04 (0.03)	0.17
AST (U/l)	1164	0.252	−0.04 (0.03)	0.21	0.320	−0.04 (0.03)	0.14
GGT (U/l)	1164	0.251	0.01 (0.03)	0.76	0.317	0.01 (0.03)	0.75
CDT, %	1156	0.251	0.01 (0.03)	0.83	0.318	0.04 (0.03)	0.17
			Central SBP[c]			Central pulse pressure[d]	
Self-reported alcohol, *n* (%)	1239	0.286	0.09 (0.03)	0.010	0.254	0.06 (0.03)	0.020
Alcohol intake (g/day)*	1221	0.283	0.03 (0.03)	0.30	0.247	0.03 (0.03)	0.27
Daily frequency of alcohol intake, *n* (%)	473	0.270	0.005 (0.04)	0.89	0.235	0.04 (0.04)	0.35
>5 drinks/day at least once/month, *n* (%)	366	0.264	0.004 (0.05)	0.93	0.228	0.03 (0.05)	0.52
Started drinking age (years)	472	0.272	0.05 (0.05)	0.32	0.236	0.06 (0.05)	0.22
Duration of drinking (years)	472	0.273	−0.08 (0.06)	0.16	0.254	−0.12 (0.06)	0.033
Volume of alcohol intake (l)	457	0.275	0.07 (0.04)	0.083	0.234	0.04 (0.04)	0.41
ALT (U/l)	1163	0.282	−0.01 (0.03)	0.65	0.250	0.02 (0.03)	0.48
AST (U/l)	1164	0.282	−0.03 (0.03)	0.28	0.246	−0.004 (0.03)	0.86
GGT (U/l)	1164	0.282	0.023 (0.03)	0.40	0.247	0.03 (0.03)	0.23
CDT, %	1156	0.282	−0.008 (0.03)	0.77	0.248	−0.05 (0.03)	0.12

N, number of participants. Independent variables included in each model: a measure of alcohol intake, age, rural/urban, sex, BMI, glycosylated haemoglobin, total cholesterol to HDL ratio (TC:HDL), C-reactive protein (CRP), smoking, physical activity, hypertension medication at baseline and HIV status. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CDT, carbohydrate-deficient transferase; GGT, γ-glutamyltransferase.
[a]Adjusted for baseline SBP.
[b]Adjusted for baseline DBP.
[c]Adjusted for baseline SBP.
[d]Adjusted for baseline pulse pressure.
[e]Alcohol intake as analysed using the quantified food frequency questionnaire (QFFQ).

analyses (Table S3, http://links.lww.com/HJH/A315), we found similar results to those reported in Table 4.

As there is a possibility that changes in body composition over 5 years may associate with the %BP, we performed an additional sensitivity analysis by including change in BMI (%BMI) in our multiple regression models instead of baseline BMI (Table S4, http://links.lww.com/HJH/A315). However, this did not change our main findings.

DISCUSSION

This study evaluated self-reported estimates of alcohol intake, comparative to the accepted laboratory-based biochemical assays, considering their association with BP elevations over 5 years in a black South African population. We found that self-reported alcohol intake significantly predicted the change in BP, which was not seen for the biochemical alcohol markers, GGT and %CDT. Self-reported alcohol intake also associated significantly with central SBP and pulse pressure at follow-up, also not found for the biochemical markers. Given the honesty of the participants, self-reported alcohol intake may perhaps be the most accurate indicator of alcohol use, when considering that biochemical variables are known to be influenced by age, obesity, liver damage and sex [13,14,20,21]. This may especially be of benefit in low resource settings in which biochemical markers are an expensive option in the public health sector, and not readily available in poor resource settings.

Our results confirm findings from previous studies that self-reported alcohol intake correlates well with other measured biochemical markers (such as GGT and %CDT) and BP. Although self-reported intake has for long been regarded as reliable [22,23], effective screening methods may be complicated by cultural differences in response to questions such as frequency of drinking [24]. The reliability of self-reporting is undermined by under-reporting of alcohol abuse, which is common in high socio-economic populations, especially among young adults having to report excessive drinking [25,26]. Black South African school teachers with a high socio-economic status (SES) recently reported low use of alcohol but demonstrated high levels of GGT [27]. This places doubt on the honesty of self-reported alcohol intake especially in adults in the high income bracket [24], who are also likely to be involved in excessive drinking [28].

In 1991, Glovannucci *et al.* [29] indicated that a simple self-administered questionnaire can provide useful estimates of alcohol intake over an extended period of time. Furthermore, large studies have successfully employed self-reported alcohol intake to assess, for example, the association between alcohol consumption and CRP concentration [30], or BP. Tsai *et al.* [8] recently found that self-reported excessive drinkers also reflected high GGT concentrations in both men and women after adjustment for confounders,

confirming the honesty and reliability of self-reported alcohol use. Furthermore, the projected increase of GGT (about 314%) in excessive drinking men without history of liver damage confirmed the risk of developing CVD in individuals abusing alcohol [31].

These findings from Tsai *et al.* [8] and a study in a Chinese population in Hong Kong [32] support the importance of biochemical markers such as GGT as a risk marker for hypertension and CVD. Furthermore, GGT prevailed as the only marker associated with stroke when used with self-reported alcohol use [33], further exposing the underestimation of reporting alcohol intake in high socio-economic populations. However, our study failed to show an association between elevation in BP and GGT presumably because of other factors such as liver damage, obesity and old age [13,14] that elevated GGT levels irrespective of alcohol intake. Moreover, Africans are known to have elevated GGT levels, independent of alcohol intake [27]. This could also have impacted on the lack of association between GGT and change in BP in this study population.

The strengths of our study are that the participants were from a low socio-economic environment with an average age of 50 ± 10.16 years, which is a group likely to report honesty regarding alcohol intake in an attempt to improve their health. Moreover, the presence of African field workers who would translate the questions into the participants' home language ensured that clear responses were obtained. Honest alcohol reporting is arguably the most accurate measure of alcohol intake, as GGT levels may be influenced by other conditions [13,14]. Although our study population may have reported honesty on alcohol intake, their low level of SES may have limited their ability to accurately report on other alcohol intake behaviour, e.g. the volume of alcohol intake. This study also adds to the limited availability of longitudinal studies on lifestyle and CVD in SSA. This study included participants from the North West Province (South Africa), and although these results may have wider application in other populations, this needs to be investigated. It is therefore recommended that consistency of self-reported use of alcohol be explored in other populations and income groups. Finally, residual confounding cannot be excluded, although our results remained consistent after multiple adjustments.

We conclude that self-reported alcohol intake is independently associated with the 5-year change in BP of black South Africans with low SES. This association was not found for GGT and %CDT, which are biochemical alcohol markers known to relate to CVD [8,29]. We suggest that self-reported alcohol intake may be a highly useful and affordable measure of alcohol to use in populations wherein honest reporting is expected, such as in those with low SES. The early identification of excessive drinking through self-reporting may contribute in reducing the burden on the national health system regarding high incidence of noncommunicable diseases.

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Conflicts of interest

There are no conflicts of interest.

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Reviewer's Summary Evaluation

Referee 2

In this paper the authors conclude that self-reported alcohol intake can be an important measure to implement in primary healthcare settings in low to middle income countries, where honest reporting is expected. Self-reported alcohol use (yes/no) decreased during the 5-year study period, and also self-reported alcohol intake, as calculated from another questionnaire, decreased, but the upper limit of the range dramatically increased from 85 to 107 g/day. In my opinion, the latter questionnaire should be validated for classes of drinkers.