# Alcohol consumption and cardiovascular disease risk in an African population in transition: the Prospective Urban and Rural Epidemiology (PURE) study

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## Abstract

**Objective:** There is evidence showing a cardioprotective effect of light to moderate alcohol consumption in many populations. Whether alcohol consumption reduces cardiovascular disease (CVD) risk in an African population remains unclear. This study therefore assessed the associations between alcohol consumption (using reported alcohol intake and biological alcohol consumption markers) and CVD risk factors in an African population in transition.

**Design:** This cross-sectional epidemiological survey is part of the South African segment of the international 12-year Prospective Urban and Rural Epidemiology (PURE) study in which the health transition in urban and rural subjects is investigated.

**Setting:** A rural and urban site in the North West Province of South Africa.

**Subjects:** A total of 2 010 apparently healthy African volunteers (35 years and older) were recruited from a sample of 6 000 randomly selected households.

**Methods:** Alcohol consumption was assessed by a validated quantitative food frequency questionnaire (QFFQ) and two biological markers, percentage serum carbohydrate-deficient transferrin (%CDT) and gamma-glutamyl transferase (GGT). The cardiovascular risk factors included in this analysis were serum lipids and blood pressure. Complete data of 1 763 and 1 878 participants were available for %CDT and GGT respectively. The subjects were divided into quartiles on the basis of their reported alcohol consumption (QFFQ), %CDT and GGT values. Additionally, subjects were divided into self-reported drinkers and non-drinkers. Men and women were analysed separately.

**Results:** The two alcohol biomarkers %CDT and GGT had different associations with CVD risk factors in this population. The risk of CVD decreased with increasing %CDT level, in that high-density lipoprotein cholesterol (HDL-C) increased significantly with increasing %CDT concentrations for both men and women. There was no significant increase in blood pressure, triglycerides and total cholesterol with increasing %CDT concentrations, except for women, where blood pressure increased significantly with %CDT. Blood pressure, triglycerides and total cholesterol increased significantly with increasing GGT concentrations for both men and women. GGT was also positively associated with HDL-C for both men and women. Self-reported drinkers had a significantly higher HDL-C, blood pressure, %CDT, GGT and lower body mass index (BMI) values than self-reported non-drinkers for both men and women. No significant differences between self-reported drinkers and non-drinkers were seen for triglycerides and total serum cholesterol (even after adjusting for BMI and smoking) for both men and women.

**Conclusions:** In this population-based study, increased alcohol consumption was associated with higher HDL-C levels but also with increased blood pressure values, indicating that the cardioprotective effect of alcohol possibly may disappear because the increase in blood pressure offsets the benefits of the increase in HDL-C.

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## Introduction

The "French paradox" is a phenomenon that describes the low incidence of cardiovascular disease (CVD) risk in France, despite a general dietary pattern high in saturated fats.[1] Therefore, the low CVD mortality observed in French and Mediterranean populations has been attributed to an increased consumption of alcohol, particularly red wine. The debate of the effects of moderate to light alcohol consumption reducing the risk of CVD has continued for decades, with some suggesting this paradox to be an artefact of the way the French record their death statistics.[2] The South African Food-Based Dietary Guidelines advises "that if you drink, drink sensibly", due to the possible cardiovascular protective effects associated with light to moderate alcohol consumption.[3]

Epidemiological evidence suggests a J- or U-shaped relationship between alcohol consumption and CVD.[4–6] It has been established that the effect of alcohol consumption is primarily mediated by

its effects on lipid profile, particularly by increasing high-density lipoprotein cholesterol (HDL-C).[7–9] Additionally alcohol consumption may reduce blood coagulation.[10] Alcohol abuse has detrimental effects on the cardiovascular and lipoprotein system, and is often associated with stroke, hypertension, cardiomyopathy, arrhythmias, hypertriglyceridaemia and fatty liver, and in the long term with hypercholesterolaemia and decreased HDL-C.[11,12] Additionally alcohol abuse has been shown to affect many organs, mainly the liver, causing both acute and chronic liver disease. Three distinct pathological disorders, namely fatty liver, hepatitis and cirrhosis, are caused primarily by alcohol abuse.[12]

Circulating carbohydrate-deficient transferrin (CDT) and gamma-glutamyl transferase (GGT) are sensitive to high alcohol consumption and are the most suitable biomarkers available for identifying chronic alcohol consumption in most populations.[13,14] These biomarkers could be used in estimating or verifying reported alcohol consumption. %CDT, which measures the relative amount of CDT isoforms in proportion to total transferrin, has been shown to be a slightly better marker of chronic alcohol consumption compared to absolute CDT values[15–20] as well as in situations where there are variations in transferrin concentrations as experienced during pregnancy, anaemia and severe liver disease.[21] GGT, which is known to reflect liver function, is a membrane-bound glycoprotein enzyme which catalyses the transfer of the gamma-glutamyl moiety of glutathione to various peptide acceptors.[22] Alcohol is one of the agents shown to increase GGT levels in serum. In a study conducted on female Wistar rats, elevation of GGT in serum probably reflected its enhanced hepatic synthesis rate, increased transport to the liver plasma membranes, as well as liver injury.[23]

A validated quantitative food frequency questionnaire (QFFQ)[24] typically has a low rate of false-positive responses. The primary weakness in using this methodology for alcohol intake assessments is that people may not report their alcohol intakes accurately.[25] Under-reporting has been shown to be common among alcohol dependants.[26] Therefore, it could be more beneficial to use biological markers as indicators of alcohol consumption. %CDT and GGT were used to validate reported alcohol consumption. Reported alcohol intakes correlated significantly with both alcohol biomarkers for both genders.[27] Thus, %CDT and GGT were used in this study as the biological alcohol consumption markers.

The South African Food-Based Dietary Guidelines advise sensible drinking to those that do drink.[3] However, the South African population in transition experiences high levels of alcohol misuse and abuse, with many adverse consequences. This study therefore assessed the associations between alcohol consumption using biological alcohol consumption markers and CVD risk factors for an African population in transition.

## Materials and methods

### Study design and subjects

This cross-sectional epidemiological survey was the baseline assessment of the North West Province, South Africa (NWPSA) leg of the 12-year Prospective Urban and Rural Epidemiology study (PURE), which investigates the health transition in urban and rural subjects. Communities that took part in this study were not randomly selected but 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 also had to be part of the North West Province of South Africa. Additionally, both rural and urban areas had to be large enough communities to allow random selection of subjects. With regard to the rural areas, the selected communities had to be far away from cities, and still had to be under tribal law with as little urban influence as possible. In the urban areas, the selected communities had to show a true representation of urbanisation. All selected communities had to be logistically accessible to researchers. The baseline data for NWPSA were collected from October to December 2005. The rural community (A) was identified 450 km west of Potchefstroom on the highway to Botswana. A deep rural community (B), 35 km east from A and only accessible by gravel road, was also included. Both communities are still under tribal law. The urban communities (C and D) were chosen near the University in Potchefstroom. Community C was selected from the established part of the township next to Potchefstroom and D from the informal settlements surrounding community C. A total of 2 000 apparently healthy African volunteers (35 years and older) were recruited from a sample of 6 000 randomly selected households from all four communities.

*Selection of subjects.* A household census of number of people, their ages and health profile was done on the 6 000 households (1 500 in each community), starting from a randomly selected address in the communities. The head of each household gave signed consent to fill out the questionnaire. If a person refused or was not at home, the next house randomly selected was taken and a non-complier questionnaire was filled out. From the data obtained, a selection of possible subjects older than 35 years of age with no reported chronic diseases of lifestyle, tuberculosis (TB) or known HIV was made (n = 500). The final number of subjects chosen for each of the four selected communities (n = 500) was not based on a statistical power calculation, but it was rather the convenient cut-off number of subjects the budget and available resources could accommodate, keeping in mind that these subjects were to be followed up for 12 years. The cut-off number was considered large enough to compensate for the possible drop-outs during the 12-year follow-up. The final 2 000 recruited subjects were visited at home and after giving voluntary and informed consent, an extensive questionnaire regarding their physical and psychological health, socio-economic background, lifestyle practices and support systems available was completed.

*Ethical considerations and organisational procedures.* Permission to conduct the study in the above-mentioned communities, as well as advice on recruitment procedures, was obtained from the North-West Department of Health, tribal chiefs, community leaders, employers and mayors. The study was approved by the Ethics Committee of the North-West University, Potchefstroom, South Africa (ethics number: 04M10). All subjects were informed about the objectives and procedures of the study prior to participation. Subjects were asked to be in a fasted state for approximately 10 hours prior to sample collection. Trained (Setswana-speaking) field workers assisted and were available to provide information in the participants' language of preference. Confidentiality and anonymity of all results were assured and all participants signed an informed consent form. Prior to the study an agreement with clinics and hospitals serving the communities from which the subjects were recruited was reached, and newly identified subjects with HIV, abnormal blood pressure, lung dysfunction, tuberculosis and abnormal electrocardiogram (ECG) were referred to them together with a standardised referral letter, without compromising the confidentiality of their health status. Participants received remuneration for all the travelling expenses to and from the clinics.

*Questionnaires.* A total of 2 000 subjects were interviewed using structured demographic, socio-economic, lifestyle and physical activity questionnaires developed and standardised for the international PURE study.[28] Validated dietary questionnaires (QFFQ and 24-hour recalls) were used.[24] All questionnaires and home visits were done by 16 intensively trained fieldworkers recruited from the four different communities. 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 questionnaires included two sets of questions regarding alcohol consumption: the quantity and frequency question from the food frequency questionnaire and another from the 24-hour recall. In both sets of questions, intakes of different beverages were assessed separately. Average 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. These calculation conversions were based on the South African Food Composition Tables.[29] 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. The QFFQ was used to distinguish drinkers and non-drinkers.

*Blood pressure, ECG and anthropometric measurements.* From August until the end of November 2005, an appointment with each person who completed the questionnaire was made, and they were voluntarily taken by taxi to meet a team of expert researchers for the purpose of measuring blood pressure (using the Omron automatic digital blood pressure monitor, Omron HEM-757), blood glucose (using the Vitros DT6011 Chemistry Analyzer, Ortho-Clinical Diagnostics, Rochester, New York, USA) and anthropometric measurements (height, weight, waist and hip circumference, mid upper arm circumference, triceps skinfold, calf circumference, calf skinfold, supra spinal skinfold, upper flexed arm circumference) using the guidelines adopted at the NIH-sponsored Arlie Conference.[30] The blood glucose measurements were used as a screening tool for diabetes. ECG and lung function tests were done using spirometers.

*HIV testing.* All participants who gave informed consent for HIV testing were additionally given an option to know the outcome of the analysis. A rapid test was done according to the National Department of Health of South Africa's protocol. Everyone received pre-test counselling in groups of 10 before the blood sample collection as well as individual post-test counselling for those participants who tested positive and opted to know the outcome of the test.

*Blood samples.* Blood was drawn from the ante-cubital vein in the subject's right arm, using a disposable needle. A new sterile transfer pipette was used to aliquot each individual's collected blood sample for analyses to follow. Blood was centrifuged within two hours of collection. Once the blood was centrifuged and separated, it was stored at -70 °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 stored at -70 °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 -70 °C until analysis. Excessive use of tourniquets was avoided as this could lead to hemoconcentration and inaccuracies in analytical results. Contents of the tubes were mixed thoroughly by gently inverting each tube five times.

*Biochemical analyses.* The levels of GGT were measured by Sequential Multiple Analyzer Computer (SMAC), using the Konelab™ auto analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland). The same method (SMAC) was used for analysing serum HDL-C, total cholesterol and triglycerides. 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 measuring range of this test is 1.5 to 24 mg/L. The coefficient of variance (CV) for all assays was $< 10\%$.

*Statistical analyses.* Data were analysed using the SPSS (Statistical Package for Social Sciences, version 16) package. Means and standard deviations were calculated. Subjects of both genders were divided into quartiles on the basis of self-reported alcohol consumption (QFFQ), %CDT concentration and GGT activity. Additionally, drinkers and non-drinkers and men and women were analysed separately. Estimated significance was based on analysis of variance (ANOVA). A stepwise regression method was used to identify valid confounders in this particular population. Body mass index (BMI) and smoking were treated as confounding variables.

C-reactive protein (CRP) was not treated as a confounder when data were in GGT quartiles because no significant association was found between GGT and CRP, after adjusting for BMI and smoking for both genders.

## Results

The baseline characteristics of the PURE participants are shown in Table I. The serum concentrations of CVD risk factors for this population according to self-reported alcohol consumption quartiles (QFFQ), together with confounding factors BMI and smoking, are presented in Tables II and III for men and women respectively. As for Table III (women), the second quartile is not shown because none of the subjects fell in this category. Age was not a confounding factor in this study for both genders.

Tables II and III show a significant drop in BMI for both men and women with increasing self-reported alcohol consumption. Increasing self-reported alcohol consumption for both men and women showed a significant difference in blood pressure (systolic and diastolic) between the first and fourth quartiles (with the fourth quartiles having higher values than the first), and this remained significant even after adjusting for BMI and smoking. Serum HDL-C values were significantly higher with increasing reported alcohol consumption for both genders (see Tables II and III). There was no significant difference in serum total cholesterol between different self-reported alcohol consumption quartiles for both men and women. A significant increase in triglyceride concentrations with increasing reported alcohol consumption is shown for the women but not for the men. This association is maintained after adjusting for BMI and smoking. A positive association is shown between %CDT and GGT with increasing reported alcohol consumption in both genders, and this remains even after adjusting for BMI and smoking.

**Table I: Mean (SD) characteristics of all participants in the PURE study**

| Variables | Men (n = 716) | Women (n = 1192) |
|---|---|---|
| Age (years) | 49.75 (10.30) | 49.10 (10.37) |
| Reported alcohol intake g/day (QFFQ) | | |
| *Whole population* | 19.20 (28.00) | 7.70 (20.1) |
| *Self-reported drinkers* | 29.90 (30.00) | 23.30 (29.1) |
| *Self-reported non-drinkers* | 0.00 (0.00) | 0.00 (0.00) |
| Smoking cigarettes/day | 4.07 (4.71) | 2.61 (3.48) |
| Body mass index (kg/m$^2$) | 20.79 (4.04) | 26.90 (7.32) |
| Blood pressure (mmHg) | | |
| *Systolic* | 138.16 (31.34) | 134.17 (24.94) |
| *Diastolic* | 86.67 (14.37) | 88.31 (14.22) |
| Plasma lipids (mmol/L) | | |
| *HDL-C* | 1.58 (0.66) | 1.48 (0.62) |
| *TG* | 1.22 (0.86) | 1.34 (0.75) |
| *TC* | 4.81 (1.34) | 5.13 (1.39) |
| GGT (U/l) | 127.27 (241.78) | 79.58 (163.18) |
| %CDT | 3.49 (1.68) | 2.64 (1.12) |

n = number; QFFQ = quantitative food frequency questionnaire, SD = standard deviation, HDL-C = high-density lipoprotein cholesterol, TG = triglycerides, TC = total cholesterol, GGT = gamma-glutamyl transferase, %CDT = percentage carbohydrate-deficient transferrin

**Table II: Mean (SD) characteristics and CVD risk factors of men according to self-reported alcohol intake quartiles (QFFQ)**

| Variables | $Q_1$ (n = 156) 0 g | $Q_2$ (n = 51) > 0 ≤ 7.7 g/day | $Q_3$ (n = 94) > 7.7 ≤ 26.6 g/day | $Q_4$ n = 124) > 26.6 g/day |
|---|---|---|---|---|
| Age (years) | 50.29 (11.57) | 50.99 (10.35) | 49.04 (9.53) | 49.18 (8.98) |
| Smoking cigarettes/day | 2.44 (4.09) | 3.80 (4.00) | 5.29$^{ab}$ (5.70) | 5.28$^{ab}$ (4.37) |
| Body mass index (kg/m$^2$) | 21.96 (4.64) | 20.37$^{a}$ (3.64) | 20.51$^{a}$ (3.83) | 19.90$^{a}$ (3.30) |
| Blood pressure (mmHg) | | | | |
| *Systolic* | 134.07 (23.02) | 137.83 (22.17) | 141.19$^{a*}$ (23.90) | 140.35$^{a*}$ (3.30) |
| *Diastolic* | 83.56 (13.64) | 88.41$^{a*}$ (14.12) | 88.87$^{a*}$ (14.24) | 87.54$^{a*}$ (14.04) |
| Plasma lipids (mmol/L) | | | | |
| *HDL-C* | 1.36 (0.60) | 1.55$^{a}$ (0.60) | 1.60$^{a*}$ (0.63) | 1.88$^{abc*\#+}$ (0.69) |
| *TG* | 1.28 (0.85) | 1.38 (1.17) | 1.20 (0.87) | 1.11 (0.68) |
| *TC* | 4.93 (1.37) | 4.74 (1.31) | 4.80 (1.37) | 4.75 (1.28) |
| GGT U/l | 91.03 (296.49) | 117.80 (284.81) | 132.56$^{ab*\#}$ (188.58) | 190.14$^{abc*\#+}$ (187.37) |
| %CDT | 2.88 (1.44) | 3.39$^{a}$ (1.68) | 3.80$^{ab*\#}$ (1.63) | 4.09$^{ab*\#}$ (1.78) |

Superscript letters indicate significant differences ($p < 0.05$): [a] vs quartile 1; [b] vs quartile 2; [c] vs quartile 3 (not adjusted), while * vs quartile 1; [#] vs quartile 2; [+] vs quartile 3 indicate significant differences ($p < 0.05$) adjusted to smoking and body mass index. p-value derived from ANOVA test.
n = number; QFFQ = quantitative food frequency questionnaire, SD = standard deviation, CVD = cardiovascular disease, HDL-C = high-density lipoprotein cholesterol, TG = triglycerides, TC = total cholesterol, GGT = gamma-glutamyl transferase, %CDT = percentage carbohydrate-deficient transferrin.

**Table III: Mean (SD) characteristics and CVD risk factors of women according to self-reported alcohol intake quartiles (QFFQ)**

| Variables | $Q_1$ (n = 476) 0 | $Q_3$ (n = 54) > 0.1 ≤ 4.6 g/day | $Q_4$ (n = 155) > 4.6 g/day |
|---|---|---|---|
| Age (years) | 49.76 (11.00) | 47.25 (9.28) | 48.21 (8.90) |
| Smoking cigarettes/day | 2.26 (3.38) | 3.33$^{a}$ (3.45) | 3.51$^{a}$ (3.65) |
| Body mass index (kg/m$^2$) | 27.53 (7.20) | 27.11 (8.50) | 24.84 a (6.83) |
| Blood pressure (mmHg) | | | |
| *Systolic* | 133.30 (25.22) | 137.14 (24.86) | 135.92$^{a*}$ (23.79) |
| *Diastolic* | 87.48 (14.08) | 90.60$^{*}$ (14.91) | 89.87$^{a*}$ (14.12) |
| Plasma lipids (mmol/L) | | | |
| *HDL-C* | 1.43 (0.54) | 1.49 (0.70) | 1.65 ac$^{*+}$ (0.73) |
| *TG* | 1.30 (0.73) | 1.36 (0.75) | 1.45 a* (0.82) |
| *TC* | 5.19 (1.43) | 5.00 (1.31) | 5.02 (1.34) |
| GGT U/l | 50.72 (65.14) | 102.22$^{a*}$ (157.75) | 141.43$^{ac*+}$ (204.12) |
| %CDT | 2.46 (1.00) | 2.97$^{a*}$ (1.20) | 2.99$^{a*}$ (1.33) |

Superscript letters indicate significant differences ($p < 0.05$): [a] vs quartile 1; [c] vs quartile 3 (not adjusted), while * vs quartile 1; [+] vs quartile 3 indicate significant differences ($p < 0.05$) adjusted to smoking and body mass index. p-value derived from ANOVA test.
n = number; QFFQ = quantitative food frequency questionnaire, SD = standard deviation, CVD = cardiovascular disease, HDL-C = high-density lipoprotein cholesterol, TG = triglycerides, TC = total cholesterol, GGT = gamma-glutamyl transferase, %CDT = percentage carbohydrate-deficient transferrin. Quartile 2 is absent because there were no participants that fell into this group.

**Table IV: Mean ( SD) characteristics and CVD risk factors of men in %CDT quartiles**

| Variables | $Q_1$ (n = 177) < 2.3% | $Q_2$ (n = 175) 2.3–3.1% | $Q_3$ (n = 161) 3.2–4.5% | $Q_4$ (n = 162) >4.6% |
|---|---|---|---|---|
| Age (years) | 50.94 (11.95) | 49.69 (10.38) | 49.52 (9.83) | 48.44[a] (9.06) |
| Alcohol intake g/day | 8.20 (14.94) | 17.37[a*] (25.57) | 23.00[a*#] (31.93) | 29.03[abc*#] (32.44) |
| Smoking cigarettes/day | 2.82 (4.02) | 3.91 (4.42) | 4.61[a] (4.46) | 4.75[a] (5.54) |
| Body mass index (kg/m$^2$) | 22.15 (4.60) | 21.75 (4.52) | 19.66[ab] (3.47) | 19.45[ab] (2.57) |
| Blood pressure (mmHg) | | | | |
| *Systolic* | 137.05 (23.50) | 136.64 (24.39) | 140.72 (49.35) | 137.68 (23.20) |
| *Diastolic* | 84.97 (14.21) | 86.13 (14.48) | 84.47 (14.33) | 87.27 (14.26) |
| Plasma lipids (mmol/L) | | | | |
| *HDL-C* | 1.32 (0.49) | 1.38 (0.55) | 1.71[ab*#] (0.69) | 1.92[abc*#+] (0.72) |
| *TG* | 1.27 (0.68) | 1.34 (0.98) | 1.10[b] (0.63) | 1.16 (1.00) |
| *TC* | 4.81 (1.32) | 4.84 (1.28) | 4.82 (1.39) | 4.73 (1.34) |
| GGT U/l | 81.89 (114.23) | 122.59 (172.62) | 133.18 (184.80) | 182.73[ab*] (415.25) |

Superscript letters indicate significant differences ($p < 0.05$): [a] vs quartile 1; [b] vs quartile 2; [c] vs quartile 3 (not adjusted), while * vs quartile 1; [#] vs quartile 2; [+] vs quartile 3 indicate significant differences ($p < 0.05$) adjusted to smoking and body mass index. p-value derived from ANOVA test.
n = number; QFFQ = quantitative food frequency questionnaire, SD = standard deviation, CVD = cardiovascular disease, HDL-C = high-density lipoprotein cholesterol, TG = triglycerides, TC = total cholesterol, GGT = gamma-glutamyl transferase, %CDT = percentage carbohydrate-deficient transferrin.

**Table V: Mean (SD) characteristics and CVD risk factors of women in %CDT quartiles**

| Variables | $Q_1$ (n = 310) < 1.9% | $Q_2$ (n = 285) 1.9–2.5% | $Q_3$ (n = 239) 2.6–3.2% | $Q_4$ (n = 254) ≥ 3.3% |
|---|---|---|---|---|
| Age (years) | 50.44 (11.86) | 49.43 (19.35) | 47.95[a] (9.14) | 47.98[a] (9.08) |
| Alcohol intake g/day | 5.27 (17.55) | 6.46 (18.29) | 6.90 (16.31) | 12.23[abc*#+] (25.46) |
| Smoking cigarettes/day | 2.23 (2.93) | 2.52 (3.23) | 2.35 (3.11) | 3.23[abc] (4.42) |
| Body mass index (kg/m$^2$) | 28.23 (7.61) | 27.16 (7.05) | 26.15[a] (6.89) | 24.67[abc] (6.40) |
| Blood pressure (mmHg) | | | | |
| *Systolic* | 134.75 (26.77) | 134.30 (24.95) | 132.07 (22.15) | 134.33[+] (24.44) |
| *Diastolic* | 87.85 (14.54) | 88.79 (14.23) | 86.44 (13.67) | 89.27[c*+] (14.62) |
| Plasma lipids (mmol/L) | | | | |
| *HDL-C* | 1.35 (0.56) | 1.44 (0.54) | 1.54[ab] (0.55) | 1.63[ab*#] (0.75) |
| *TG* | 1.38 (0.78) | 1.41 (0.77) | 1.29 (0.82) | 1.26[b] (0.63) |
| *TC* | 5.11 (1.32) | 5.16 (1.39) | 5.23 (1.33) | 5.06 (1.41) |
| GGT U/l | 54.76 (56.78) | 96.27[a*] (255.82) | 73.83 (124.06) | 89.79[a*] (129.04) |

Superscript letters indicate significant differences ($p < 0.05$): [a] vs quartile 1; [b] vs quartile 2; [c] vs quartile 3 (not adjusted), while * vs quartile 1; [#] vs quartile 2; [+] vs quartile 3 indicate significant differences ($p < 0.05$) adjusted to smoking and body mass index. p-value derived from ANOVA test.
n = number; QFFQ = quantitative food frequency questionnaire, SD = standard deviation, CVD = cardiovascular disease, HDL-C = high-density lipoprotein cholesterol, TG = triglycerides, TC = total cholesterol, GGT = gamma-glutamyl transferase, %CDT = percentage carbohydrate-deficient transferrin.

**Table VI: Mean (SD) characteristics and CVD risk factors of men in GGT quartiles**

| Variables | $Q_1$ (n = 175) < 36.7 U/l | $Q_2$ (n = 177) 36.7–58.2 U/l | $Q_3$ (n = 176) 58.3–123.6 U/l | $Q_4$ (n = 177) ≥ 123.7 U/l |
|---|---|---|---|---|
| Age (years) | 49.56 (11.06) | 49.77 (10.83) | 50.89 (10.24) | 49.28 (9.09) |
| Alcohol intake g/day | 10.33 (20.07) | 12.61 (20.63) | 19.86[ab*#] (28.19) | 33.61[abc*#+] (35.43) |
| Smoking cigarettes/day | 3.29 (4.45) | 4.10 (4.49) | 4.03 (4.41) | 5.09[a] (5.60) |
| Body mass index (kg/m$^2$) | 20.71 (3.47) | 21.07 (3.47) | 20.81 (4.52) | 20.55 (3.95) |
| Blood pressure (mmHg) | | | | |
| *Systolic* | 135.66 (27.36) | 137.66 (22.42) | 139.10 (48.41) | 139.83 (21.58) |
| *Diastolic* | 84.03 (15.76) | 85.79 (13.14) | 85.45 (13.26) | 90.59[abc*#+] (13.86) |
| Plasma lipids (mmol/L) | | | | |
| *HDL-C* | 1.28 (0.46) | 1.49[a*] (0.58) | 1.69[ab*#] (0.69) | 1.85[abc*#] (0.75) |
| *TG* | 1.10 (0.64) | 1.11 (0.52) | 1.27 (0.87) | 1.39[ab*#] (1.17) |
| *TC* | 4.36 (1.20) | 4.79[a*] (1.29) | 5.09[ab*] (1.43) | 5.00[a*#] (1.30) |
| %CDT | 2.96 (1.29) | 3.33[a] (1.70) | 3.63[a*] (1.71) | 4.05[abc*#] (1.83) |

Superscript letters indicate significant differences ($p < 0.05$): [a] vs quartile 1; [b] vs quartile 2; [c] vs quartile 3 (not adjusted), while * vs quartile 1; [#] vs quartile 2; [+] vs quartile 3 indicate significant differences ($p < 0.05$) adjusted to smoking and body mass index. p-value derived from ANOVA test.
n = number; QFFQ = quantitative food frequency questionnaire, SD = standard deviation, CVD = cardiovascular disease, HDL-C = high-density lipoprotein cholesterol, TG = triglycerides, TC = total cholesterol, GGT = gamma-glutamyl transferase, %CDT = percentage carbohydrate-deficient transferrin.

**Table VII: Mean (SD) characteristics and CVD risk factors of women in GGT quartiles**

| Variables | $Q_1$ (n = 295) < 26.7 U/l | $Q_2$ (n = 293) 26.7–40.4 U/l | $Q_3$ (n = 294) 40.5–71.0 U/l | $Q_4$ (n = 291) ≥71.1 ≥U/l |
|---|---|---|---|---|
| Age (years) | 48.07 (10.73) | 49.61 (10.61) | 49.37 (9.79) | 48.47 (9.55) |
| Alcohol intake g/day | 2.27 (7.49) | 4.15 (16.30) | 6.18[a] (17.39) | 18.71[abc*#+] (27.86) |
| Smoking cigarettes/day | 2.10 (2.82) | 2.21 (3.23) | 2.81[a] (3.72) | 3.46[ab] (4.09) |
| Body mass index (kg/m$^2$) | 26.47 (6.45) | 27.31 (7.25) | 27.74[a] (7.56) | 25.49[bc] (7.48) |
| Blood pressure (mmHg) | | | | |
| *Systolic* | 128.67 (23.00) | 132.82[a] (25.81) | 135.26[a*] (25.49) | 138.70[ab*#] (24.20) |
| *Diastolic* | 85.02 (12.96) | 86.45 (14.62) | 88.36[a] (14.25) | 92.45[abc*#+] (14.21) |
| Plasma lipids (mmol/L) | | | | |
| *HDL-C* | 1.28 (0.47) | 1.39[a] (0.48) | 1.53[ab*#] (0.62) | 1.73[abc*#+] (0.76) |
| *TG* | 1.10 (0.58) | 1.27[a*] (0.60) | 1.40[ab*] (0.80) | 1.62[abc*#+] (0.90) |
| *TC* | 4.65 (1.19) | 5.20[a*] (1.43) | 5.32[a*] (1.38) | 5.37[a*] (1.43) |
| %CDT | 2.55 (1.07) | 2.48 (1.01) | 2.72[b] (1.13) | 2.79[ab] (1.23) |

Superscript letters indicate significant differences ( $p < 0.05$): [a] vs quartile 1; [b] vs quartile 2; [c] vs quartile 3 (not adjusted), while * vs quartile 1; [#] vs quartile 2; [+] vs quartile 3 indicate significant differences ($p < 0.05$) adjusted to smoking and body mass index. p-value derived from ANOVA test.
n = number; QFFQ = quantitative food frequency questionnaire, SD = standard deviation, CVD = cardiovascular disease, HDL-C = high-density lipoprotein cholesterol, TG = triglycerides, TC = total cholesterol, GGT = gamma-glutamyl transferase, %CDT = percentage carbohydrate-deficient transferrin.

**Table VIII: Mean (SD) characteristics and CVD risk factors between self-reported drinkers and non-drinkers**

| Variable | Men | | ANOVA test | | Women | | ANOVA test | |
|---|---|---|---|---|---|---|---|---|
| | Drinkers | Non-drinkers | Significance | | Drinkers | Non-drinkers | Significance | |
| | Mean (SD) | Mean (SD) | P | Pa | Mean (SD) | Mean (SD) | P | Pa |
| Age (years) | 49.47 (9.49) | 50.29 (11.57) | NS | * | 47.96 (9.00) | 49.76 (11.00) | NS | NS |
| Body mass index ($kg/m^2$) | 20.24 (3.59) | 21.96 (4.64) | *** | - | 25.44 (7.36) | 27.53 (7.20) | ** | - |
| Smoking cigarettes/day | 5.01 (4.87) | 2.44 (4.09) | *** | - | 3.47 (3.60) | 2.26 (3.38) | *** | - |
| Systolic blood pressure (mmHg) | 140.20 (34.76) | 134.07 (23.0) | * | ** | 136.23 (24.05) | 133.30 (25.22) | *** | *** |
| Diastolic blood pressure (mmHg) | 88.25 (14.12) | 83.56 (13.64) | ** | *** | 90.06 (14.31) | 87.48 (14.08) | *** | *** |
| %CDT | 3.83 (1.71) | 2.88 (1.44) | *** | *** | 3.99 (1.30) | 2.46 (1.00) | *** | *** |
| GGT (U/l) | 152.53 (211.87) | 91.03 (296.49) | *** | *** | 131.50 (193.97) | 50.72 (65.14) | *** | *** |
| HDL-C (mmol/L) | 1.69 (0.67) | 1.36 (0.60) | *** | *** | 1.61 (0.73) | 1.43 (0.54) | *** | *** |
| Total cholesterol (mmol/L) | 4.76 (1.32) | 4.93 (1.37) | NS | NS | 5.02 (1.32) | 5.19 (1.43) | NS | NS |
| Triglycerides (mmol/L) | 1.20 (0.87) | 1.28 (0.85) | NS | NS | 1.43 (0.80) | 1.30 (0.73) | ** | ** |

Pa-, p-value adjusted to body mass index and smoking. Significance is based on ANOVA.
* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$
n = number; SD = standard deviation, CVD = cardiovascular disease, HDL-C = high-density lipoprotein cholesterol, GGT = gamma-glutamyl transferase, %CDT = percentage carbohydrate-deficient transferrin, NS = not significant.

The serum concentrations of CVD risk factors for this population in %CDT quartiles together with alcohol consumption (habitual intake) from the QFFQ and confounding factors (BMI and smoking) are presented in Tables IV and V, while the same data in GGT quartiles are presented in Tables VI and VII. Reported alcohol consumption increased with increasing %CDT and GGT levels for both men and women (see Tables IV to VIII). Statistically significant increases are shown as the quartiles ascend with or without adjusting for BMI and smoking. Mean habitual intakes of self-reported drinking men and women were 29.9 (SD 30.0) and 23.3 (SD 29.1) g of pure alcohol per day (see Table I). Self-reported habitual intakes of the whole population correlated positively and significantly with both %CDT ($R = 0.32$) and GGT ($R = 0.433$). After controlling for possible confounding factors (BMI and smoking), these relationships became weaker ($R = 0.19$ and 0.31 respectively) but remained highly significant.

Table IV shows a significant drop in BMI for men as %CDT concentrations increase but no significant change is shown for BMI when data is grouped in GGT quartiles (see Table VI). For women, BMI decreased significantly with increasing %CDT concentrations (see Table V) and with increasing GGT concentrations. BMI increased in quartiles 2 and 3 and was then lower in quartile 4 (see Table VII). Table VIII shows a significant difference in BMI between self-reported non-drinkers and drinkers (with drinkers having a lower BMI) for both men and women.

Increasing %CDT concentrations for men did not show any relationship with both systolic and diastolic blood pressures, with or without adjusting for BMI and smoking (see Table IV). A statistically significant increase in diastolic (and not in systolic blood pressure) with increasing GGT concentrations for men is shown in Table VI. For women, systolic blood pressure did not differ significantly between %CDT quartiles except for the two upper quartiles, but significant differences are shown for diastolic blood pressure with the last quartile having the highest value (see Table V). A statistically significant increase in diastolic and systolic blood pressure, with increasing GGT concentrations, for women is shown in Table VII; this is maintained after adjusting for BMI and smoking. A significant difference in blood pressure (systolic and diastolic) between self-reported drinkers and non-drinkers (with drinkers having higher blood pressure) is shown in Table VIII. Both drinking men and women had higher blood pressure values than non-drinkers even after adjusting for BMI and smoking.

Serum HDL-C values were significantly higher with increasing %CDT and GGT concentrations for both men and women (see Tables IV to VII). These results remained significant even after adjusting for BMI and smoking. The same result was shown when self-reported drinkers and non-drinkers were compared, with drinkers having a significantly higher HDL-C concentration than self-reported non-

drinkers, before and after adjusting for confounders for both men and women ($p < 0.001$) (see Table VIII).

There was no significant difference in serum total cholesterol and triglycerides between different %CDT quartiles for both genders, with the exception of a significant difference in triglyceride concentration between the second and third quartiles for men and the second and forth quartiles for women, but this disappears after adjusting for BMI and smoking (see Tables IV and V). Serum total cholesterol and triglycerides significantly increased with increasing GGT concentrations for both men and women. Some of the differences between the quartiles disappeared after adjusting for BMI and smoking (see Tables VI and VII). Table VIII, which compares serum concentrations of CVD lipid risk factors between self-reported drinkers and non-drinkers, shows that among men there was no statistically significant difference between the two groups while female self-reported drinkers had a significantly higher triglyceride concentration than female self-reported non-drinkers, and this remained the same after adjusting for BMI and smoking ($p < 0.01$). There was no significant difference in serum total cholesterol between self-reported drinkers and non-drinkers for both genders, before and after adjusting for BMI and smoking (see Table VIII).

For both men and women, GGT activity significantly increased with increasing %CDT quartiles (see Tables IV and V); however, %CDT concentrations did not increase significantly with increasing GGT quartiles for women after adjusting for BMI and smoking. A significant difference in GGT activity and %CDT concentrations between self-reported drinkers and non-drinkers is shown in Table VIII. Self-reported drinkers (both men and women) had a significantly higher %CDT and GGT value than their non-drinking counterparts. However, the correlation between %CDT and GGT was low ($R=0.211$ $p<0.01$). After controlling for BMI and smoking, this correlation became weaker ($R = 0.11$ p , 0.05).[27]

## Discussion

### Reported alcohol consumption

Alcohol consumption guidelines from developed countries indicate that daily alcohol consumption should not exceed 5% of total energy intake, or daily intakes of absolute alcohol should be approximately 20 g and 15 g for men and women respectively.[31,32] The QFFQ was used to assess alcohol consumption. Both men and women drinkers in this population reported high mean intakes of 29.9 and 23.3 g/day respectively (see Table I), which is far greater than the recommended general guidelines described above. Almost two-thirds of the men in this sample reported to be drinkers compared to only a third of the women.[27] Self-reported alcohol intakes are usually underestimated.[25,26] Because of this, biological alcohol consumption markers were used in the evaluation of CVD risk related to alcohol consumption. In this study, %CDT and GGT were used to validate reported alcohol consumption. The habitual alcohol intakes correlated significantly with the alcohol biomarkers as indicated earlier.

This is the first study to our knowledge carried out to assess the relation between reported alcohol consumption and biological alcohol markers (%CDT and GGT) with CVD risk factors in an African population in transition. This study shows that %CDT had a positive association with HDL-C, an inverse association with BMI, and no association with blood pressure, triglycerides and total cholesterol (except for a positive association with blood pressure for women). GGT showed a positive association with HDL-C, blood pressure, triglycerides, total cholesterol and an inverse association with BMI (for women). For both men and women, HDL-C and blood pressure increased significantly with increasing reported alcohol consumption; however, total cholesterol remained statistically unchanged in both genders. Triglyceride levels for women increased with increasing reported alcohol consumption, but not for men.

### Relation between reported alcohol consumption, biological alcohol markers (%CDT and GGT) and CVD risk factors

The two alcohol biomarkers %CDT and GGT had different associations with CVD risk factors in this population. The risk of CVD decreased with increasing %CDT, in that HDL-C increased significantly with increasing %CDT concentrations, and there was no significant increase in blood pressure, triglycerides and total cholesterol. Only for women did blood pressure increase with increasing %CDT. High GGT levels were positively associated with increases in all risk factors (blood pressure, triglycerides and total cholesterol) though it also was positively associated with HDL-C. Several studies have shown that moderate alcohol consumption decreases the risk of CVD.[33–36] The primary mechanism proposed for this cardioprotective effect of alcohol is that alcohol has positive effects on lipid metabolism and the haemostatic system. The latter was not investigated in this present study. It has been shown that moderate drinkers have higher HDL-C concentrations than non-drinkers.[37,38] A polymorphism in the gene for alcohol dehydrogenase type 3 alters the rate of alcohol metabolism.[39] A case control study illustrated that in moderate drinkers who where homozygous for the slow oxidising alcohol dehydrogenase type 3 allele had higher HDL-C levels and a decreased risk of developing CVD.[39] This study confirms that drinkers have higher HDL-C concentrations than non-drinkers, with self-reported drinkers having a significantly higher HDL-C level than self-reported non-drinkers. However, the need to encourage current abstainers to consume moderate alcohol in order to increase HDL-C as a public health strategy against CVD is questionable. The non-drinkers baseline mean levels of HDL-C were within normal protective ranges for both men and women. The cut-off points used for being normal were values of $\geq 1.0$ and $\geq 1.2$ mmol/L for men and women respectively.[40] This may serve as a possible explanation as to why African communities are thought to be protected against ischaemic heart disease,[41] though this gap is increasingly changing due to increased exposure and adoption of an atherogenic western lifestyle. This increase in triglyceride levels is in agreement with other studies but not consistently so. However, this can only be speculated on at this stage, as this study's focus was to provide sound relationships between exposure and the selected outcomes only.

Our observations agree with an earlier study showing a positive association between GGT and HDL-C,[42] although negative correlations have been reported.[43–45] In this present study, GGT was positively associated to triglyceride levels for both men and women. Reported alcohol consumption values were also positively associated with triglyceride levels, but only for women. This increase in triglyceride levels is in agreement with other studies[37] but not consistently so.[46,47] GGT is known to be related to increased levels of very low density lipoprotein (VLDL) reflected by increased triglycerides.[42] An additional detrimental effect of alcohol among the women is, in part, explained by alcohol's effect on lipid metabolism. Among the women, drinkers had significantly higher triglyceride concentration than their non-drinking counterparts, and this remained after adjusting for BMI and smoking.

It has been shown that alcohol consumption increases blood pressure, even after low consumption.[48] The direct association between alcohol consumption and blood pressure is in agreement with other studies.[47,49] This current study provides additional information, in that a significantly higher blood pressure (both systolic and diastolic) in self-reported drinkers is shown, with the relationship becoming stronger after adjusting for BMI and smoking, suggesting an independent contribution of alcohol to increased blood pressure.

A positive association between GGT and the risk of CVD has been reported previously.[50] Unlike %CDT concentration, which showed no association with total cholesterol and triglycerides for both genders, GGT activity was positively associated with higher total cholesterol and higher triglycerides for both genders. This association between GGT and total cholesterol and triglycerides may be due to a deranged lipid metabolism, possibly associated with fatty liver formation.[43] The possible mechanisms for the different associations between GGT and %CDT and total cholesterol and triglycerides, for both genders, may be due to the different mechanisms by which alcohol influences them. These may include %CDT being a marker for earlier phases of alcohol consumption, and GGT reflecting toxic effects of ethanol on hepatic lipid metabolism.[51] It has additionally been suggested that %CDT reflects drinking frequency whereas GGT is more influenced by drinking intensity.[52] The correlation between GGT and %CDT was low, suggesting that the responses of CDT and GGT levels to alcohol consumption occur by different mechanisms.[53] Additionally, the results indicated a positive association between reported alcohol consumption and triglyceride levels for women, but not for men (see Table VIII). There are indications that elevated triglycerides could be an independent CVD risk factor,[44] and considering these results, women consuming alcohol in this particular transitional community seem to be at greater risk for developing CVD than drinking men.

In this study, a decrease in BMI is associated with increased %CDT and GGT, although this was not shown for men in GGT quartiles. This may explain the favourable effect on the lipid profile, but, after dividing the population into self-reported drinkers and non-drinkers, the significant difference in BMI between the male drinkers and non-drinkers ($p < 0.001$) may indicate that BMI influenced the relationship between CVD risk factors and alcohol consumption. The male drinkers showed a mean BMI of 20.2 kg/m$^2$, indicating a higher probability of being underweight. Correlations of CVD risk factors to GGT and %CDT for both men and women do not necessarily have the same outcome. This may be explained by the underlying mechanistic differences of how alcohol consumption influences %CDT and GGT.[27]

**Effect of different types of alcoholic beverages**

Additionally it has been debated whether the cardioprotective effect of light to moderate alcohol consumption is due to ethanol or some other substances in alcoholic beverages, e.g. polyphenols. Although there is evidence suggesting the positive attributes of light to moderate consumption of alcohol,[6] the mechanistic contribution of different alcoholic beverages remains debatable. Because alcohol is addictive, it is important to find out whether ethanol, auxiliary compounds or metabolic end-products of ethanol contribute significantly and by what amount to this cardioprotective effect. From this point of view, the protective effect of alcohol also may be associated to the type of drink and drinking pattern.[54] In the present study, all reported alcoholic beverages were translated to pure alcohol consumed using South African Food Composition Tables,[29] thus the effects of different types of alcoholic beverages on CVD risk factors were not examined.

**Public health perspective**

In this population-based study, increasing alcohol consumption was associated with higher HDL-C but also with increased blood pressure values. The cardioprotective effect of alcohol on CVD risk probably disappears because the increase in blood pressure offsets the benefits of the increase in HDL-C. The South African food-based dietary guidelines advise those who drink to drink sensibly due to the possible cardiovascular protective effects associated with light to moderate alcohol consumption.[3] The South African food-based dietary guidelines do not advise non-drinkers to start drinking, and the findings of this study in relation to the effects of alcohol on blood pressure, and the already high levels of HDL-C in the abstainers, would appear to be supportive of the approach. Additionally, abuse can also lead to addiction and dependency. From a public health perspective, it is suggested that there is not enough scientific evidence within the research domain to allow or warrant promotion of alcohol consumption, even in moderation, for those who do not drink. Furthermore, much disparity and inconsistency still exists among researchers, and the promotion of moderate consumption may in turn also lead to alcohol abuse (if a little is good, then more is better). Thus, for public health policy makers, especially in populations undergoing a health transition, the dilemma still exists whether to recommend moderate alcohol intake or not, illustrating that further research is still required on the topic.

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