Du, D., Bruno, R., Blizzard, L., Venn, A., Dwyer, T., Smith, K. J., … Gall, S. (2020). The metabolomic signatures of alcohol consumption in young adults. *European Journal of Preventive Cardiology*, *27*(8), 840–849. https://doi.org/10.1177/2047487319834767

The metabolomic signatures of alcohol consumption in young adults

Duc Du[1], Raimondo Bruno[2], Leigh Blizzard[1], Alison Venn[1], Terence Dwyer[1], Kylie J Smith[1], Costan G. Magnussen[1,3], Seana Gall[1]

[1]Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania, Australia

[2]School of Medicine, University of Tasmania, Tasmania, Australia

[3]Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland

Correspondence

Dr. Seana Gall

Menzies Institute for Medical Research, University of Tasmania

+61 3 6226 4728 (phone)

+61 3 6226 7704 (fax)

Seana.Gall@utas.edu.au

Abstract

Background: Metabolomic analysis may help us to understand the association between alcohol consumption and cardio-metabolic health. We aimed to: (i) replicate a previous study of alcohol consumption and metabolic profiles, (ii) examine associations between types of alcoholic beverages and metabolites, and (iii) include potential confounders not examined in previous studies.

Methods: Cross-sectional data of 1,785 participants (age 26-36 years, 52% women) from the 2004-06 Childhood Determinants of Adult Health (CDAH) study were used. Consumption of beer, wine and spirits were assessed by questionnaires. Metabolites were measured by a high-throughput nuclear magnetic resonance (NMR) platform and multivariable linear regression examined their association with alcohol consumption (combined total and types) adjusted for covariates including socio-demographics, health behaviours and mental health.

Results: Alcohol consumption was associated with 23 out of 37 lipids, 12 out of 16 fatty acids (FAs), and 6 out of 20 low-molecular-weight metabolites independent of confounders with similar associations for combined total alcohol consumption and different types of alcohol. Many metabolites (lipoprotein lipids in HDL subclasses, HDL cholesterol, apolipoprotein A-1, phosphotriglycerides, total FA, monounsaturated FA, omega-3 FA) had positive linear associations with alcohol consumption but some showed negative linear (LDL particle size, omega-6 FA ratio to total FA, citrate) or U-shaped (lipoprotein lipids in VLDL subclasses, VLDL triglycerides) associations.

Conclusions: Our results were similar to the only previous study. Associations with metabolites were similar for total and types of alcohol. Alcohol consumption in young adults is related to a diverse range of metabolomic signatures associated with benefits and harms to health.

Key words: alcohol, epidemiology, risk factors, metabolomics, fatty acids, metabolic profiling

Abstract word count: 250 words

Total word count: 4,484 words

Introduction

Recent studies have challenged the benefits of low-to-moderate consumption of alcohol for reducing cardiovascular disease (CVD) risk (1). Metabolomic analysis that profiles systemic metabolism may increase understanding of the association between alcohol consumption and cardio-metabolic health, particularly the contradictory effects of reducing risk of myocardial infarction but increasing risk of other cardiovascular diseases (1).

One previous study examined metabolites associated with alcohol consumption in 9,778 young adults aged 24-45 in three population-based cohorts in Finland. Higher alcohol consumption was associated with higher HDL cholesterol, monounsaturated fatty acids (MUFA), but lower omega-6 fatty acids, glutamine, citrate and lipoprotein particle size in three cohorts using an NMR platform (2). Most of these biomarkers are associated with inreased risk of CVD (3). Replication of results in other cohorts is important in this emerging field to understand generalisability. Further, it is not known whether the type of alcoholic beverage consumed influences relationships and important covariates such as diet, cardiorespiratory fitness and mental health have not been examined.

We aimed to (1) replicate previous findings between alcohol consumption and metabolic profiles; (2) examine the association of types of alcoholic beverages (beer and wine) with metabolites; and (3) consider covariates not previously examined in associations between metabolic profiles and alcohol consumption.

Methods

Participants

Participants were from the 2004-2006 Childhood Determinants of Adult Health (CDAH) study, a follow-up of the 1985 Australian Schools Health and Fitness Survey (ASHFS) (4). A representative sample of 8,498 Australian school children (51% male, aged 7 to 15 years) from 109 schools participated in the ASHFS. Of the 8,498 participants, 5,170 (60.8%) enrolled in the CDAH study and 2,410 (28.4%) attended one of 34 study clinics held across Australia when aged between 26 and 36 years (4). Of these participants, 1,785 had data available for alcohol consumption from questionnaires, gave a fasting blood sample in 2004-06, and had metabolomic profile measured by a serum NMR platform in 2014. A description of the cohort has been published elsewhere (5). The flow of participants from baseline to follow-up is described in the Supplementary Figure 1. The study was approved by the Tasmanian Health and Medical Human Research Ethics Committee. All participants gave informed written consent.

Measurements

Alcohol consumption

Participant reported frequency of intake (options: never or <1/month, 1-3 times/month, once/week, 2-4 times/week, 5-6 times/week, once/day, 2-3 times/day, 4-5 times/day, and >6 times/day) of ten alcoholic beverages (light/medium/full strength beer; red/white/sparkling wine; wine cooler, spirits/liqueurs, spirit-based mixed drinks, sherry/port, and other) over the last 12 months in a food frequency questionnaire. We assumed that one standard drink (10 grams of alcohol) was consumed at each occasion. We estimated alcohol consumed per day for each beverage by multiplying the frequency of drinking by the estimated grams of alcohol for each beverage: beer (light beer, medium beer and full strength beer) and wine (red and white wine). Spirits were infrequently consumed so were not examined in these analyses. Total alcohol consumed per week and per day was the sum of all ten types of beverages. Participants were classified into 5 groups according to daily alcohol intake: 0 g/day (Non-drinkers), >0-10 g/day (Light drinkers), >10-20 g/day (Moderate drinkers), >20-30 g/day

(Heavy drinkers) and >30 g/day (Very heavy drinkers), based on Australian guideline. This allowed comparison of our cohort to the general Australian population (6).

Metabolomics

Serum fasting blood samples collected in 2004-06 were stored at -80^{o}C for 11-13 years before analysis. The Computational Medicine metabolomic platform used high-throughput serum nuclear magnetic resonance (NMR) spectroscopy to quantify 223 key metabolic markers (7). Samples were subject to automated quality control, and values that could not be extracted reliably were excluded from the analysis. As per previous studies, we focused on 73 metabolic measures covering major biological pathways (7).

Covariates

Full details of covariates are provided in the supplement. In brief, covariates were: age, sex, socio-economic status (SES) (high, medium-high, medium-low, or low), region of residence (major city/urban/rural areas), education level (university, vocational, or secondary school only), occupation (professional/manager, white collar, blue collar, or not in labour force), marital status (married or living as married versus other), smoking status (never, former, or current), total physical activity (minutes per week), cardiorespiratory fitness (CRF) (PWC170 uncorrelated with lean body mass), diet quality (Dietary Guideline Index, DGI) and depression and anxiety diagnosis in the previous 12 months (Composite International Diagnostic Interview, CIDI) (8).

Statistical analysis

We used multivariable linear regression to examine associations between alcohol consumption and each metabolic measure (β coefficients and 95% CIs). Alcohol consumption was examined as 1) total alcohol consumption (grams per week) and 2) beer or wine. Metabolic variables were scaled to standard deviation units and those with skewed distributions were log transformed. Results were presented graphically with numerical results also provided in the supplement.

The shape of significant linear metabolic pathways associated with alcohol consumption were examined using local quadratic regression fitting, with each smoothing function segment evaluated at 25 points through the range of alcohol intake. More complex shapes were examined using polynomial regression models, when needed.

Potential covariates associated with alcohol consumption and mortality and morbidity from a range of diseases (see 'Supplementary material') were included in the models in accordance with purposeful model building procedures (9). Models are presented adjusted for sex, age (model 1); model 1 plus region of residence, SES, educational level, occupation, marital status, smoking, dietary quality (DGI), physical activity, cardiorespiratory fitness, depression and/or anxiety (model 2). We included a multiplicative interaction term between each covariate and total alcohol consumption in models. We found no evidence for interaction by sex with all interactions terms $P>0.002$, therefore results were presented for men and women together.

Sensitivity analyses (see Supplementary methods) included: (1) total alcohol consumption in grams per day instead of grams per week; (2) to test whether drinking alcohol the day before the blood sample influenced the results; (3) exluding non-drinkers and those with alcohol use disorders.

We adjusted for multiple testing using the number of principal components that explained over 99% variance of the metabolomic data (2). Thirty-six principal components were identified the corrected significance threshold was $P\leq0.002$ (two-tailed).

Analysis was performed in RStudio 1.0.136 using the packages MASS, metafor, AER, RColorBrewer, and ggplot2 (R Core Team, 2016) and Stata 12.0.

Results

Characteristics of study population

There were 1,785 participants with complete data on alcohol consumption and metabolomic measures (Table 1). Participants with and without metabolomics data had similar characteristics (Table 1). Compared to the Australian population aged 25-34 years, the CDAH sample (n=1,785) has a similar prevalence current drinker (86% vs. 83%) and proportion living in each state but were more likely to be tertiary educated (26.1% vs. 22.3%) (10).

Associations of alcohol with lipoprotein lipids

Alcohol consumption was associated with 23 out of 37 lipoprotein and lipid measures (see Figure 1, Supplementary Table 1 and Table 2 for numerical results). In model 2, alcohol consumption was strongly associated with higher lipid concentrations for all high-density lipoproteins (HDL) subclasses, particularly medium-sized and large HDL particles. Concurrently, the HDL cholesterol, apolipoprotein A-1, phosphoglycerides and phosphatidylcholine concentrations were robustly elevated with higher alcohol consumption. In contrast, higher alcohol consumption was strongly associated with smaller low-density lipoproteins (LDL) particle size, lower levels of apolipoprotein B, lower levels of remnant cholesterol, intermediate (IDL) cholesterol and very-low-density lipoproteins (VLDL) cholesterol concentrations. Adjustment for demographic factors (Model 1) and other health behaviours (Model 2) mostly increased the magnitude of the associations compared to the unadjusted model (Supplementary Table 3). Similar results were observed when alcohol was examined per day instead of per week (Supplementary Figure 5).

In the fully adjusted model, beer and wine were positively associated with all HDL concentrations including large, medium and small-sized particles, HDL particle size, apoliporotein A-1, HDL cholestetol, phosphatidylcholine, and phosphoglycerides concentration while inversely associated with apoliporotein B, remnant cholesterol, VLDL cholesterol. Beer consumption was associated with greater lipid concentrations in the large, medium and small HDL subclasses, HDL particle size, HDL cholesterol, apolipoprotein A-1, phosphoglycerides and phosphatidylcholine concentrations compared to wine or total alcohol consumption.

HDL-related, phosphoglycerides, apolipoprotein A-1 measures were mainly linear across the range of alcohol consumption. Inverse linear associations were observed in the measures of LDL particle size, apolipoprotein B, remnant C and IDL cholesterol (Figure S1).

Non-linear associations were observed between alcohol consumption and lipid concentrations in the large and small HDL subclasses, larger HDL particle size, higher HDL cholesterol, higher apolipoprotein A-1, and higher phosphoglycerides and phosphatidylcholine concentrations (Supplementary Table 2).

Associations of alcohol with fatty acids

Alcohol consumption was associated with 12 out of 16 fatty acids measures (see Figure 2, Supplementary Table 1 and Table 2). Higher alcohol consumption was robustly associated with higher concentrations of total fatty acids (FA), saturated fatty acids (SFA), MUFA, omega-3 FA, DHA in absolute concentrations, and higher proportion of SFA, omega-3 FA and the proportion of DHA levels to total FA. In contrast, alcohol consumption was inversely associated with the omega-6 fatty acid ratio, polyunstaturated fatty acid (PUFA) ratio and linoleic acid ratio to total fatty acids. These results remained statistically significant after adjusting for potential covariates (Figure 3). Smoking, diet, physical activity and cardiorespiratory fitness (Model 2) increased the magnitudes of the associations (Supplementary Table 3).

In the fully adjusted models, similar results were observed with beer and wine consumption with fatty acids. Consumption of beer was associated with higher total FA, SFA, MUFA, PUFA, omega-6 FA, omega-3 FA, DHA and lower PUFA ratio, omega-6 FA ratio, linolenic acid ratio to total FA compared to wine or total alcohol consumption.

Similar results were observed when using alcohol per day instead of per week (Supplementary Figure 6).

Most associations were linear across the range of alcohol consumption. Inverse linear associations were observed for omega-6 FA ratio, linoleic acid ratio, PUFA to total fatty acids. In contrast, positive linear associations were observed for omega-3 FA, DHA or largely positive in measures of total FA, SFA, MUFA, PUFA, omega-6 FA concentrations, SFA and MUFA ratio to total FA where the slope modestly declined in light alcohol consumption but mainly increased across higher range of alcohol use (Figure S2).

Associations of alcohol with low-molecular-weight metabolites

Alcohol consumption was associated with 6 out of 20 low molecular weight metabolite measures (see Figure 3, Supplementary Table 1 and Table 2). The strongest associations were observed for glycine, isoleucine, valine, phenylalaine, and citrate which were all inversely associated with higher alcohol consumption (Figure 4). Demographic factors including sex, age, SES status (Model 1) and smoking, diet, physical activity and cardiorespiratory fitness (Model 2) accounted for most of the significant changes and increased the magnitude of the associations compared to the unadjusted model (Supplementary Table 3). Most of the small molecular metabolites were not strongly associated with alcohol consumption but subtle non-linear associations were evident for several measures, e.g. phenylanaline (Supplementary Table 2 and Figure S3). Similar results were observed when using alcohol per day instead of per week (Supplementary Figure 7).

In the fully adjusted model, similar results were observed with beer consumption and wine consumption except for the associations with albumin and acetate, which were positively associated with beer, but not wine, consumption (Figure 4).

Sensitivity analyses

Results excluding people with alcohol use disorders (see Supplementary Table 5) and non-drinkers (see Supplementary Table 6) were similar to the results reported in the main text.

Discussion

Diverse molecular processes were related to alcohol consumption, comprising both favourable and unfavourable effects in relation to the risk of cardio-metabolic diseases. Our results were largely similar to the previous study in Finland except for associations with some triglycerides, fatty acids, and several low-molecular-weight metabolites. We generally found limited differences in associations between types of beverages. Including diet and cardiorespiratory fitness, but not mental health, increased the magnitudes of the associations suggesting that inadequate control for confounders may have led to a misestimation of the associations between alcohol consumption and some of these measures in previous studies.

Associations of alcohol with lipoprotein lipids

Our findings on alcohol consumption and lipid and lipoprotein measures were mostly consistent with the only comparable study (2). This included that alcohol consumption was positively associated with measures associated with lower cardiovascular risk (large and small HDL subclasses, HDL particle size, HDL cholesterol and apolipoprotein A-1). Associations between alcohol consumption and phosphoglycerides, phosphatidylcholine, apolipoprotein A-1 were non-linear, with less positive effects at higher levels of alcohol consumption.

Consistent with the previous study (2), higher alcohol consumption was strongly associated with several lipid and lipoprotein measures (smaller LDL particle size, higher phosphoglycerides and phosphatidylcholine) associated with greater cardiovascular risk.

Alcohol consumption was not associated with serum total triglycerides, triglycerides in HDL contrasting to the previous study (2). One explanation is the differences in determinants of trigylcerides (e.g. diet and body weight (11)), however, we adjusted for these and no associations were evident in unadjusted analyses. Our participants fasted for 8-9 hours before sampling. In the Wurt et al. study (2), two cohort used fasting blood samples (NFBC-1966 and YFS-2001) while one used semi-fasting samples with participants fasted for 4 hours (FINRISK-1997). Non-fasting triglycerides were generally higher (12) however the most discordant finding was with the fasted samples. This suggests that further examination is needed to confirm the associations between alcohol consumption and triglycerides.

Beer and wine had similar associations with lipids and lipoproteins to total alcohol consumption. There was some evidence that beer consumption was associated with higher lipid concentrations in large, medium and small HDL subclasses, HDL particle size, HDL cholesterol, apolipoprotein A-1, phosphoglycerides and phosphatidylcholine concentrations than wine or total alcohol consumption. This may be influenced by demographic characteristics and health behaviours in beer drinkers as these factors accounted for large increases in the associations, suggesting negative confounding. These findings suggest that common components of alcohol may affect lipids which is supported by a meta-analysis of beer and wine consumption with cardiovascular events (13).

Adjusting for cardiorespiratory fitness and diet increased the magnitudes of the associations between alcohol and lipid concentrations in the large, medium and small HDL subclasses, HDL particle size, HDL cholesterol, apolipoprotein A-1, phosphoglycerides and phosphatidylcholine concentrations. This highlights the close interaction between cardiorespiratory fitness, diet and alcohol consumption (14) that might explain some of the pathways to cardiometabolic diseases through lipids and lipoproteins.

Numerous studies have indicated strong associations between higher alcohol consumption on elevated HDL cholesterol, adiponectin, apolipoprotein A-1 levels (15, 16). Alcohol may influence HDL cholesterol through cholesteryl ester transfer protein (CETP) activity (17) in combination with increased transport rate of apolipoproteins (18) and reduced hepatic lipase activity (19). In turn, HDL cholesterol moves excess cholesterol molecules from peripheral cells to the liver (20). Alcohol increases apolipoprotein A-1 concentration due to the increase of A-1 lipoprotein particle, which has been suggested to represent the antiatherogenic fraction of HDL (21). The underlying mechanisms by which alcohol affects LDL particle size, phosphoglycerides and phosphatidylcholine are not well established. However, there is evidence linking lower LDL particle size and plasma triglyceride-rich lipoprotein particles (e.g. phosphoglycerides and phosphatidylcholine) to coronary heart disease (22). These conflicting effects of alcohol consumption on lipids and lipoproteins coupled with findings on metabolic markers differentially predicting myocardial infarction and stroke (23) may explain the conflicting effects of alcohol on different cardiovascular events (1).

Associations of alcohol with fatty acids

The relationships between alcohol consumption and fatty acid subclasses were consistent with the previous study (2, 3), with mostly adverse effects in relation to cardiovascular risk. While total FA, SFA, MUFA, omega-3 FA concentrations, SFA ratio, omega-3 FA ratio, and

DHA ratio to total fatty acids displayed positive associations with alcohol intake, alcohol consumption was inversely associated with the omega-6 fatty acid ratio, PUFA ratio and linoleic acid ratio to total fatty acids. The predominantly adverse changes in fatty acids support higher risks of some cardiovascular diseases associated with alcohol consumption in recent studies (1) when considered alongside studies of metabolites and risk of cardiovascular events (23).

Beer, wine and total alcohol consumption showed similar associations with fatty acids. There was evidence that beer consumption was associated with stronger associations with fatty acids compared to wine or total alcohol consumption, which might be due to residual confounding despite adjustment for covariates.

Demographic factors including sex, age, SES status and health behaviours (smoking, diet, physical activity) and cardiorespiratory fitness, but not mental health caused increases in the associations between total alcohol consumption and fatty acids measures compared to the unadjusted model. The interaction beween diet, fitness or physical activity and alcohol with fatty acids might be particularly important but the relationships are poorly understood.

Alcohol may influence fatty acids by mobilizing, uptake, synthesis and esterification of fatty acids from adipose tissue (24). SFA increase low-density lipoprotein (LDL) cholesterol, potentially increasing CVD risk (25). While dietary MUFAs protect against CVD (26), MUFA ratio to total fatty acids is a biomarker of higher cardiovascular risk (3). Likewise, the robust association of alcohol intake with lower proportion of omega-6 fatty acids has been related to higher cardiometabolic risk (27), noting recent findings with different effects of fatty acids on myocardial infarction and stroke (23). Omega-3 FA concentrations have been associated with lower cardiovascular risk (28). Within the omega-3 series, the long-chain docosahexaenoic acid (DHA) are associated with decreased coronary events, whereas the role of linolenic acid is less clear (29). In this cohort of young adults, the weight of evidence suggests that alcohol consumption is associated with mostly harmful effects on fatty acids that may increase cardiovascular risk.

Associations of alcohol with low-molecular-weight metabolites

In line with the previous finding, citrate and phenylalanine were strongly inversely associated with alcohol consumption but not glutamine. A strong linear association was observed for citrate, while phenylalanine initially decreased then levelled off as alcohol consumption increased. Beer and wine showed similar associations with low-molecular-weight metabolites as total alcohol consumption.

Alcohol may influence citrate through enzymes in oxidative pathways such as the citric acid or glyoxylate cycle, including succinate dehydrogenase (30). The effects of alcohol on phynylalanine might be through production of the 2-phenylethyl alcohol which is found in beer or ethyl alcohol (31). In turn, higher citrate levels have been linked with modestly lower risk for cardiovascular diseases (3). In constrast, higher phenylalanine has been associated with greater cardiovascular risk (3). In this cohort, these adverse changes in citrate and phenylalanine suggest higher cardiovascular risk related to higher alcohol consumption. Our finding that consumption of beer but not wine was significantly associated with higher albumin concentration is consistent with the previous study (32). Bioactive components of beer such as total phenols, flavanols, flavonoids and antioxidants may affect plasma albumin concentration and are associated with markers of atherosclerosis (32). Beer may be associated with acetate concentration as ethyl acetate is produced by yeast that is found in beer (33).

Strengths and limitations

A strength of our study is the comprehensive examination of linear and non-linear relationships between metabolite measures and alcohol consumption including types of alcohol (noting the limited power for spirit consumption due to its infrequent consumption). Other strengths include sensitivity analyses showing the robustness of results and exploration of confounding factors not examined in previous studies.

Limitations of the study include cross-sectional analyses excluding casual inferences. Our young and healthy cohort have few diseases and exclusion of those with alcohol use disorders addressed potential issues with reverse causation. Associations were unaltered when excluding non-drinkers, suggesting results were not influenced by those that stopped drinking for health reasons. Misclassification of alcohol consumption may have occurred with the FFQ. Self-reported alcohol intake by FFQ has been shown to be reliable and valid in young adults (34). Despite the large sample size, we had inadequate power to examine types of alcohol including types of wine. We had substantial loss to follow-up since childhood, which may affect the generalisability of our findings to other populations. However, as noted in the results, the CDAH sample with and without the metabolomics data were similar to the general population. Thus, the loss to follow-up does not appear to have greatly affected the results.

Conclusion

The metabolomic signatures associated with alcohol consumption in this young adult cohort were similar to the only existing study. They suggest a diverse range of molecular processes that are both beneficial and harmful to health are related to alcohol consumption with similar effects for total consumption and different types of alcohol.

Acknowledgements

We gratefully acknowledge the CDAH study project manager Ms Marita Dalton and sponsors (Target, Asics, Sanitarium and Veolia Environmental Services).

Funding

This study was supported by the National Health and Medical Research Council (Project Grant 211316, Senior Research Fellowship to AJV, Early Career Fellowship to KJS APP1072516); the National Heart Foundation (Project Grant GOOH 0578, Fellowships PH 11H 6047 and Future Leader Fellowship 100446 to SLG, and Future Leader Fellowship 100849 to CGM). Duc Du was supported by a Menzies Vietnamese Scholarship.

Conflict of Interest: none declared.

Authorship: DD, RB, TD, LB, KJS, CGM, AV and SG contributed to the conception or design of the work. DD, RB, TD, LB, KJS, CGM, AV and SG contributed to the acquisition, analysis, or interpretation of data for the work. DD and SG drafted the manuscript. DD, RB, TD, LB, KJS, CGM, AV and SG critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

References

1. Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T, et al. Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. Lancet. 2018;391(10129):1513-23.

2. Wurtz P, Cook S, Wang Q, Tiainen M, Tynkkynen T, Kangas AJ, et al. Metabolic profiling of alcohol consumption in 9778 young adults. International journal of epidemiology. 2016;45(5):1493-506.

3. Wurtz P, Havulinna AS, Soininen P, Tynkkynen T, Prieto-Merino D, Tillin T, et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. Circulation. 2015;131(9):774-85.

4. Gall SL, Jose K, Smith K, Dwyer T, Venn AJ. The childhood determinants of adult health study: a profile of a cohort study to examine the childhood influences on adult cardiovascular health2009.

5. Magnussen CG, Raitakari OT, Thomson R, Juonala M, Patel DA, Viikari JS, et al. Utility of currently recommended pediatric dyslipidemia classifications in predicting dyslipidemia in adulthood: evidence from the Childhood Determinants of Adult Health (CDAH) study, Cardiovascular Risk in Young Finns Study, and Bogalusa Heart Study. Circulation. 2008;117(1):32-42.

6. Australian Government NHaMRC. Australian Alcohol Guidelines: Health Risks and Benefits. Canberra ACT: Commonwealth of Australia; 2001.

7. Soininen P, Kangas AJ, Wurtz P, Suna T, Ala-Korpela M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circulation Cardiovascular genetics. 2015;8(1):192-206.

8. Haro JM, Arbabzadeh-Bouchez S, Brugha TS, de Girolamo G, Guyer ME, Jin R, et al. Concordance of the Composite International Diagnostic Interview Version 3.0 (CIDI 3.0) with standardized clinical assessments in the WHO World Mental Health surveys. International journal of methods in psychiatric research. 2006;15(4):167-80.

9. Greenland S. Modeling and variable selection in epidemiologic analysis. American journal of public health. 1989;79(3):340-9.

10. Australian Bureau of Statistics. 2006 Census of Population and Housing. Community Profile Series. Cat. No. 2001.0. Canberra: ABS; 2007.

11. Klop B, do Rego AT, Cabezas MC. Alcohol and plasma triglycerides. Current opinion in lipidology. 2013;24(4):321-6.

12. Bhatt D, Tannock L. Risk of Fasting and Non-Fasting Hypertriglyceridemia in Coronary Vascular Disease and Pancreatitis. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, et al., editors. Endotext. South Dartmouth (MA)2000.

13. Costanzo S, Di Castelnuovo A, Donati MB, Iacoviello L, de Gaetano G. Wine, beer or spirit drinking in relation to fatal and non-fatal cardiovascular events: a meta-analysis. European journal of epidemiology. 2011;26(11):833-50.

14. Baumeister SE, Finger JD, Glaser S, Dorr M, Markus MR, Ewert R, et al. Alcohol consumption and cardiorespiratory fitness in five population-based studies. European journal of preventive cardiology. 2017:2047487317738594.

15. Gepner Y, Golan R, Harman-Boehm I, Henkin Y, Schwarzfuchs D, Shelef I, et al. Effects of Initiating Moderate Alcohol Intake on Cardiometabolic Risk in Adults With Type 2 Diabetes: A 2-Year Randomized, Controlled Trial. Annals of internal medicine. 2015;163(8):569-79.

16. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: systematic review and meta-analysis of interventional studies. Bmj. 2011;342:d636.

17. Jensen MK, Mukamal KJ, Overvad K, Rimm EB. Alcohol consumption, TaqIB polymorphism of cholesteryl ester transfer protein, high-density lipoprotein cholesterol, and risk of coronary heart disease in men and women. European heart journal. 2008;29(1):104-12.

18. De Oliveira ESER, Foster D, McGee Harper M, Seidman CE, Smith JD, Breslow JL, et al. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apolipoproteins A-I and A-II. Circulation. 2000;102(19):2347-52.

19. Riemens SC, van Tol A, Hoogenberg K, van Gent T, Scheek LM, Sluiter WJ, et al. Higher high density lipoprotein cholesterol associated with moderate alcohol consumption is not related to altered plasma lecithin:cholesterol acyltransferase and lipid transfer protein activity levels. Clinica chimica acta; international journal of clinical chemistry. 1997;258(1):105-15.

20. Hewing B, Moore KJ, Fisher EA. HDL and cardiovascular risk: time to call the plumber? Circulation research. 2012;111(9):1117-20.

21. Valimaki M, Laitinen K, Ylikahri R, Ehnholm C, Jauhiainen M, Bard JM, et al. The effect of moderate alcohol intake on serum apolipoprotein A-I-containing lipoproteins and lipoprotein (a). Metabolism: clinical and experimental. 1991;40(11):1168-72.

22. Rizzo M, Berneis K. Low-density lipoprotein size and cardiovascular risk assessment. QJM : monthly journal of the Association of Physicians. 2006;99(1):1-14.

23. Holmes MV, Millwood IY, Kartsonaki C, Hill MR, Bennett DA, Boxall R, et al. Lipids, Lipoproteins, and Metabolites and Risk of Myocardial Infarction and Stroke. Journal of the American College of Cardiology. 2018;71(6):620-32.

24. Baraona E, Lieber CS. Effects of ethanol on lipid metabolism. Journal of lipid research. 1979;20(3):289-315.

25. Mensink RP. Effects of the individual saturated fatty acids on serum lipids and lipoprotein concentrations. The American journal of clinical nutrition. 1993;57(5 Suppl):711S-4S.

26. Gillingham LG, Harris-Janz S, Jones PJ. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. Lipids. 2011;46(3):209-28.

27. Wu JH, Lemaitre RN, King IB, Song X, Psaty BM, Siscovick DS, et al. Circulating omega-6 polyunsaturated fatty acids and total and cause-specific mortality: the Cardiovascular Health Study. Circulation. 2014;130(15):1245-53.

28. Bowen KJ, Harris WS, Kris-Etherton PM. Omega-3 Fatty Acids and Cardiovascular Disease: Are There Benefits? Curr Treat Options Cardiovasc Med. 2016;18(11):69.

29. Lluis L, Taltavull N, Munoz-Cortes M, Sanchez-Martos V, Romeu M, Giralt M, et al. Protective effect of the omega-3 polyunsaturated fatty acids: Eicosapentaenoic acid/Docosahexaenoic acid 1:1 ratio on cardiovascular disease risk markers in rats. Lipids in health and disease. 2013;12:140.

30. Kokavec A, Crowe SF. Alcohol consumption in the absence of adequate nutrition may lead to activation of the glyoxylate cycle in man. Medical hypotheses. 2002;58(5):411-5.

31. Fabre CE, Blanc PJ, Goma G. Production of 2-phenylethyl alcohol by Kluyveromyces marxianus. Biotechnol Prog. 1998;14(2):270-4.

32. Gorinstein S, Caspi A, Libman I, Leontowicz H, Leontowicz M, Tashma Z, et al. Bioactivity of beer and its influence on human metabolism. International journal of food sciences and nutrition. 2007;58(2):94-107.

33. Graham GS. The Oxford Companion to Beer definition of ethyl acetate 2018 [Available from: https://beerandbrewing.com/dictionary/O1rjQz3DYu/.

34. Turconi G, Bazzano R, Roggi C, Cena H. Reliability and relative validity of a quantitative food-frequency questionnaire for use among adults in Italian population. International journal of food sciences and nutrition. 2010;61(8):846-62.

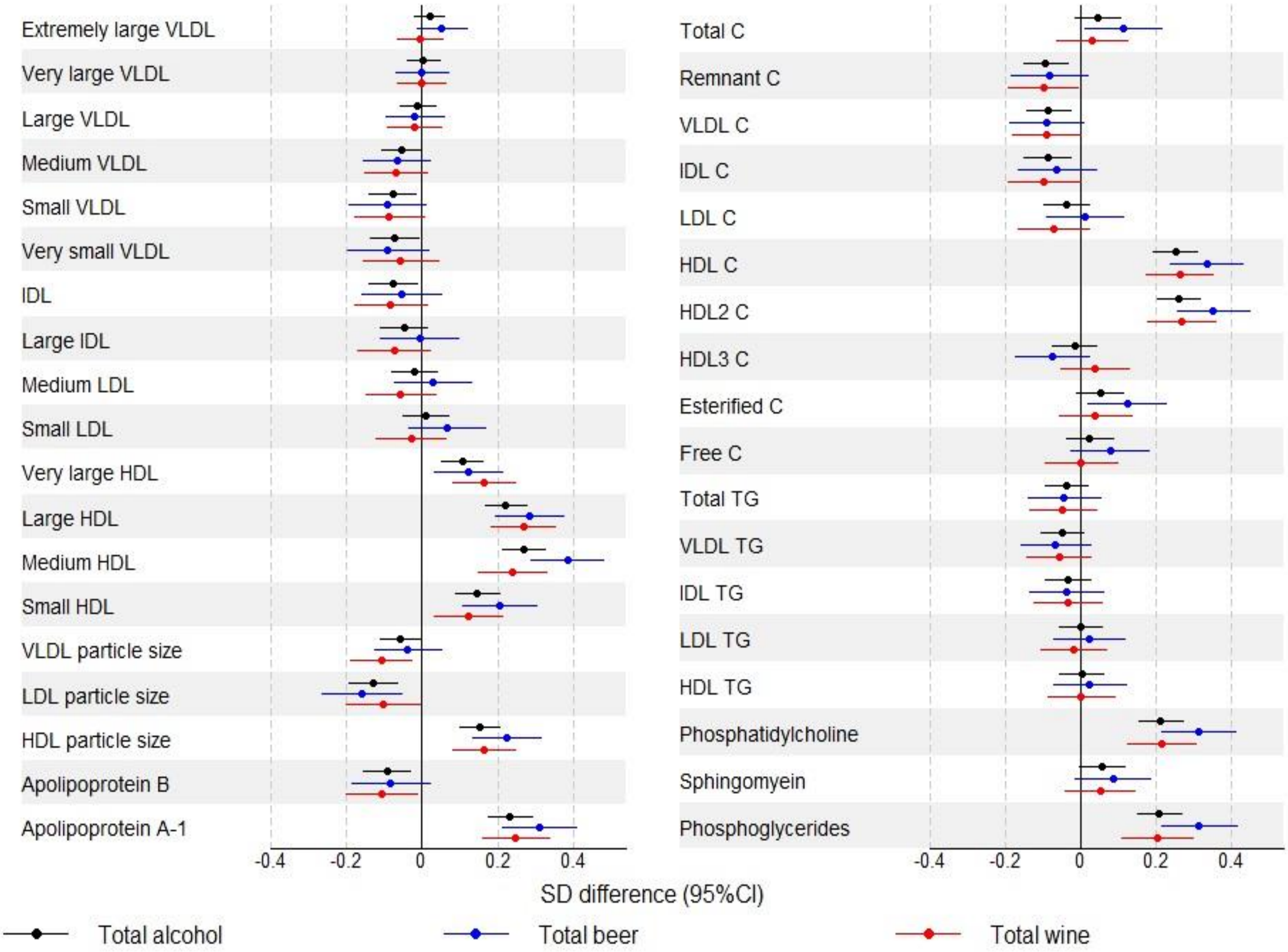


Figure 1. Cross-sectional associations between alcohol consumption as total alcohol, beer and wine consumed and lipoprotein lipid measures. All association were adjusted for age, sex, region of residence, SES, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety. Error bars denote 95% confidence intervals. Differences in metabolite concentration are expressed as standard deviation difference (95% CIs) per 100 grams of alcohol per week. Association magnitudes in absolute concentration units are listed in Supplementary Table1 and continuous shapes of the metabolic associations with alcohol intake are shown in Supplementary Figure S1.

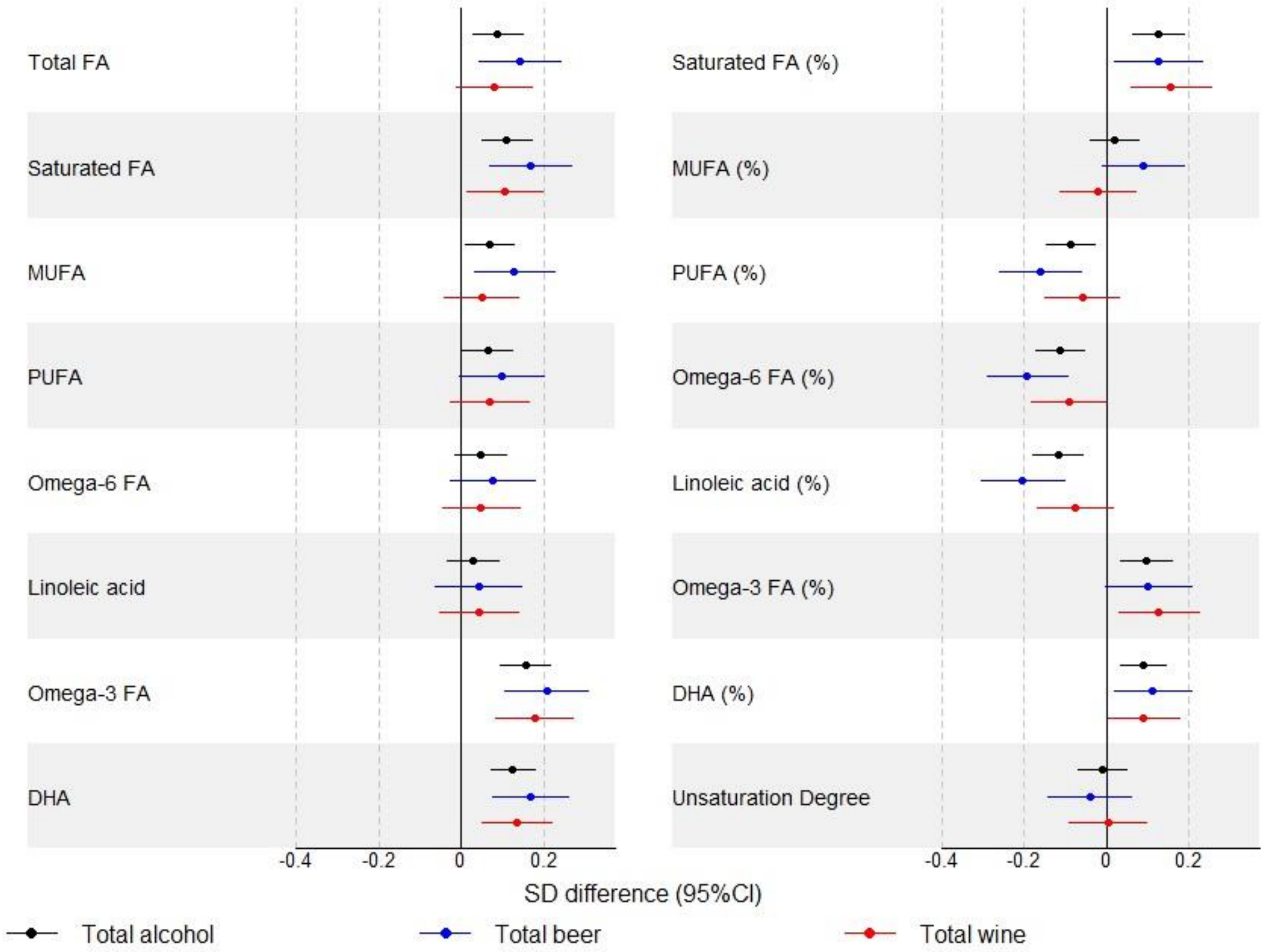


Figure 2. Cross-sectional associations between alcohol consumption as total alcohol, beer and wine consumed and fatty acids. All association were adjusted for age, sex, region of residence, SES, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety. Error bars denote 95% confidence intervals. Differences in metabolite concentration are expressed as standard deviation difference (95% CIs) per 100 grams of alcohol per week. Association magnitudes in absolute concentration units are listed in Supplementary Table 1 and continuous shapes of the metabolic associations with alcohol intake are shown in Supplementary Figure S2.

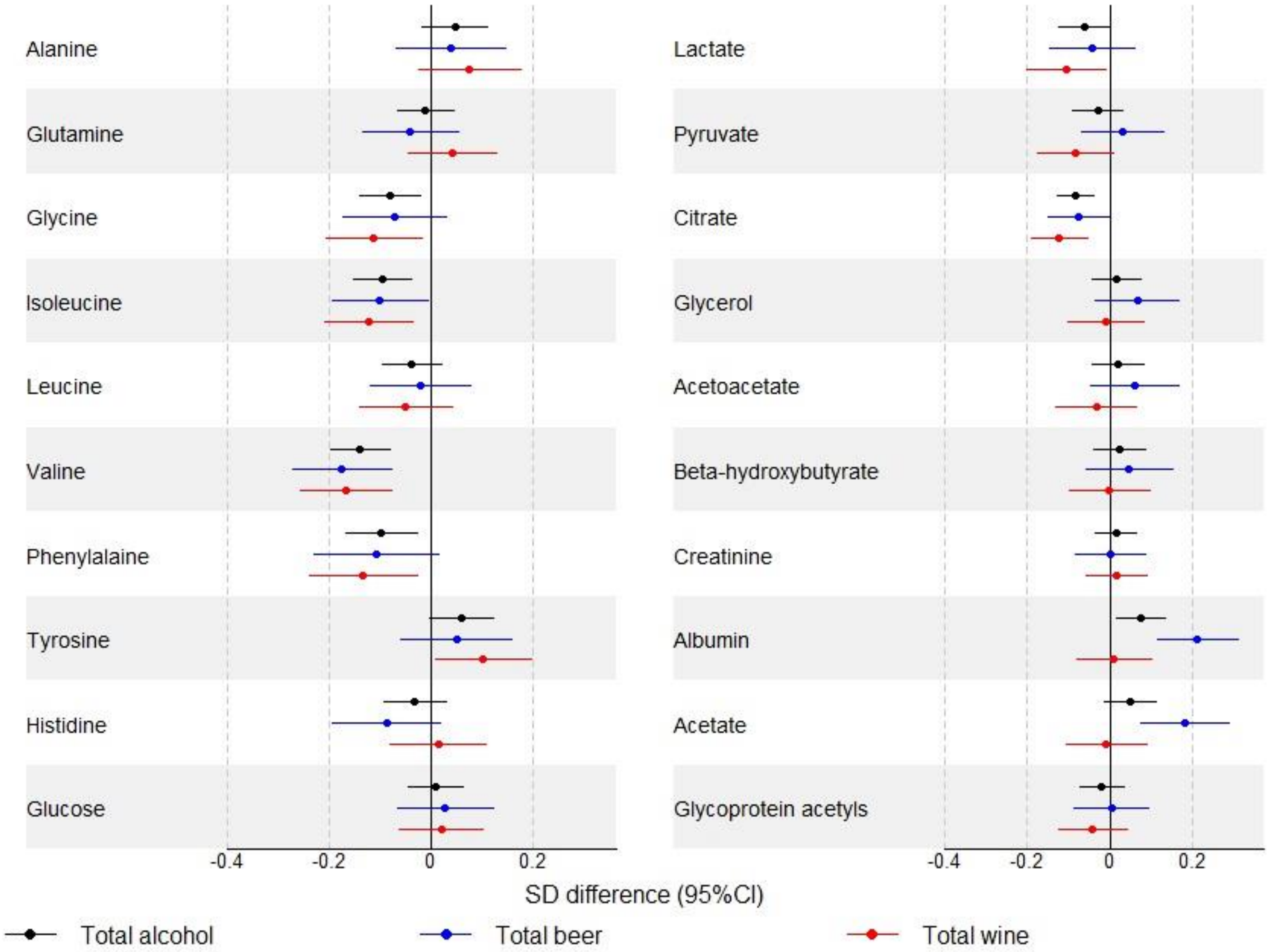


Figure 3. Cross-sectional associations between alcohol consumption as total alcohol, beer and wine consumed and low-molecular-weight metabolites. All association were adjusted for age, sex, region of residence, SES, educational level, occupation, marital status, smoking, dietary intakes, physical activity, cardiorespiratory fitness, depression and/or anxiety. Error bars denote 95% confidence intervals. Differences in metabolite concentration are expressed as standard deviation difference (95% CIs) per 100 grams of alcohol per week. Association magnitudes in absolute concentration units are listed in Supplementary Table 1 and continuous shapes of the metabolic associations with alcohol intake are shown in Supplementary Figure S3.

Table 1. Characteristics of the study population

Characteristic	Participants with metabolomics data (N=1,785)	Participants without metabolomics data (N=1,078)	P_{value}
Number of participants (men/women)	811/974	465/613	0.231
Age (years)	31.3 (2.6)	32.0 (2.6)	<0.001
Body mass index (kg/m^2)	25.6 (4.8)	25.6 (5.0)	0.953
Systolic blood pressure (mmHg)	118 (12)	119 (13)	<0.05
Total cholesterol (mmol/l)	4.9 (1.0)	5.0 (1.0)	<0.05
HDL cholesterol (mmol/l)	1.4 (0.3)	1.4 (0.3)	0.226
Triglycerides (mmol/l)	0.9 (0.6-1.3)	0.9 (0.6-1.4)	0.513
Plasma glucose (mmol/l)	5.0 (4.7-5.2)	5.0 (4.7-5.3)	0.507
Insulin (IU/l)	6.0 (4.3-8.6)	5.9 (4.2-8.2)	0.487
HOMA-IR	1.3 (0.9-1.9)	1.3 (0.9-1.9)	0.489
cMSy	-0.01 (0.7)	0.03 (0.7)	0.198
Smoking prevalence, n (%)	369 (22)	121 (22)	0.994
Total alcohol consumption (g/week)	41.0 (15.0-87.5)	36.8 (11.8-82.5)	0.480
Total alcohol consumption (g/day)	5.9 (2.1-12.5)	5.3 (1.7-11.8)	0.480
Total beer (g/day)	1.1 (0.0-4.5)	0.7 (0.0-4.3)	0.487
Total wine (g/day)	2.1 (0.0-4.3)	1.1 (0.0-4.3)	0.473
Total spirits (g/day)	0.7 (0.0-1.7)	0.7 (0.0-1.7)	0.485
Alcohol consumption status, n (%)			0.065
Non-drinkers (0 g/day)	246 (14)	187 (17)	
Light drinkers (>0-10 g/day)	974 (55)	563 (52)	

Moderate drinkers (>10-20 g/day)	386 (22)	209 (19)	
Heavy drinkers (>20-30 g/day)	88 (5)	55 (5)	
Very heavy drinkers (>30 g/day)	91 (5)	64 (6)	
Self-rated health status in 1985, n (%)†			0.122
Excellent	477 (36)	346 (37)	
Very good	604 (45)	393 (42)	
Good/Fair	246 (18)	193 (19)	
Self-rated health status in 2002-04, n (%)*			0.456
Excellent	285 (16)	149 (15)	
Very good	719 (41)	396 (40)	
Good	573 (33)	357 (36)	
Fair/Poor	140 (8)	85 (8)	

Abbreviation: HDL, High-Density Lipoprotein; HOMA-IR, Homeostatic Model Assessment-Insulin Resistance; cMSy, Continuous Metabolic Syndrome Risk Scores. Data are shown as mean (± standard deviation) or median (interquartile range) for normally distributed or skewed continuous variables, respectively; and number (percentage) for categorical variables.

† Data were from the 1985 baseline survey of 7 to 15 years olds; * Data were from 2002-2004 when participants were re-traced to enrol in the 2004-06 follow-up (this study).

Supplementary material

Supplementary methods

Covariates

The following covariates were considered: age, sex, socio-economic (SES) quartile base on area of residence (high, medium high, medium low, or low), region of residence (major city/urban/rural areas), education level (university, vocational, or secondary school only), occupation (professional/manager, white collar, blue collar, or not in labour force), marital status (married or living as married versus other), and smoking status (never, former, or current), collected from questionnaires. A total physical activity score (minutes per week) was calculated from the duration, intensity, and frequency of physical activity in the past week by the International Physical Activity Questionnaire (IPAQ) (1). Cardiorespiratory fitness (CRF) was estimated as physical work capacity (PWC) at a heart rate of 170 bpm (PWC170) on a bicycle ergometer pedalled at 60 rpm (2). CRF was then adjusted for lean body mass to create an index uncorrelated with lean body mass because of the relation between absolute workload achieved and muscle mass (3). Dietary intakes were assessed using a food frequency questionnaire (FFQ) assessing usual frequency of intake of food excluding alcohol intake beverages over the last 12 months, and then calculated as a Dietary Guideline Index (DGI) score (4), based on recommendations in the 2003 Dietary Guidelines for Australian Adults (5) and the Australian Guide to Healthy Eating (6). Other covariates included childhood alcohol consumption experimentation (non-drinkers or drinkers at childhood), health-related quality of life (HRQoL) (SF-12 physical and mental component scores), and depression and anxiety diagnosed in the previous 12 months by the Composite International Diagnostic Interview (CIDI) (7). These covariates has been shown to be associated with alcohol consumption (8-10), jointly contribute mortality and morbidity from a range of diseases (11, 12).

Supplementary data analysis methods

Exclusion of those with alcohol use disorders (AUDs): Sensitivity analyses were performed and compared to the results reported in the main text by excluding those with AUDs diagnosed in the previous 12 months by the CIDI (7) to address potential issues with reverse causation on the associations between alcohol consumption and metabolite measures that might be infuenced by those who had drinking problems at the time of the study.

Exclusion of non-drinkers: Sensitivity analyses were performed and compared to the results reported in the main text by excluding those were non-drinkers to prevent reverse causation that might be influenced by those that stopped drinking for health reasons at the time of the study.

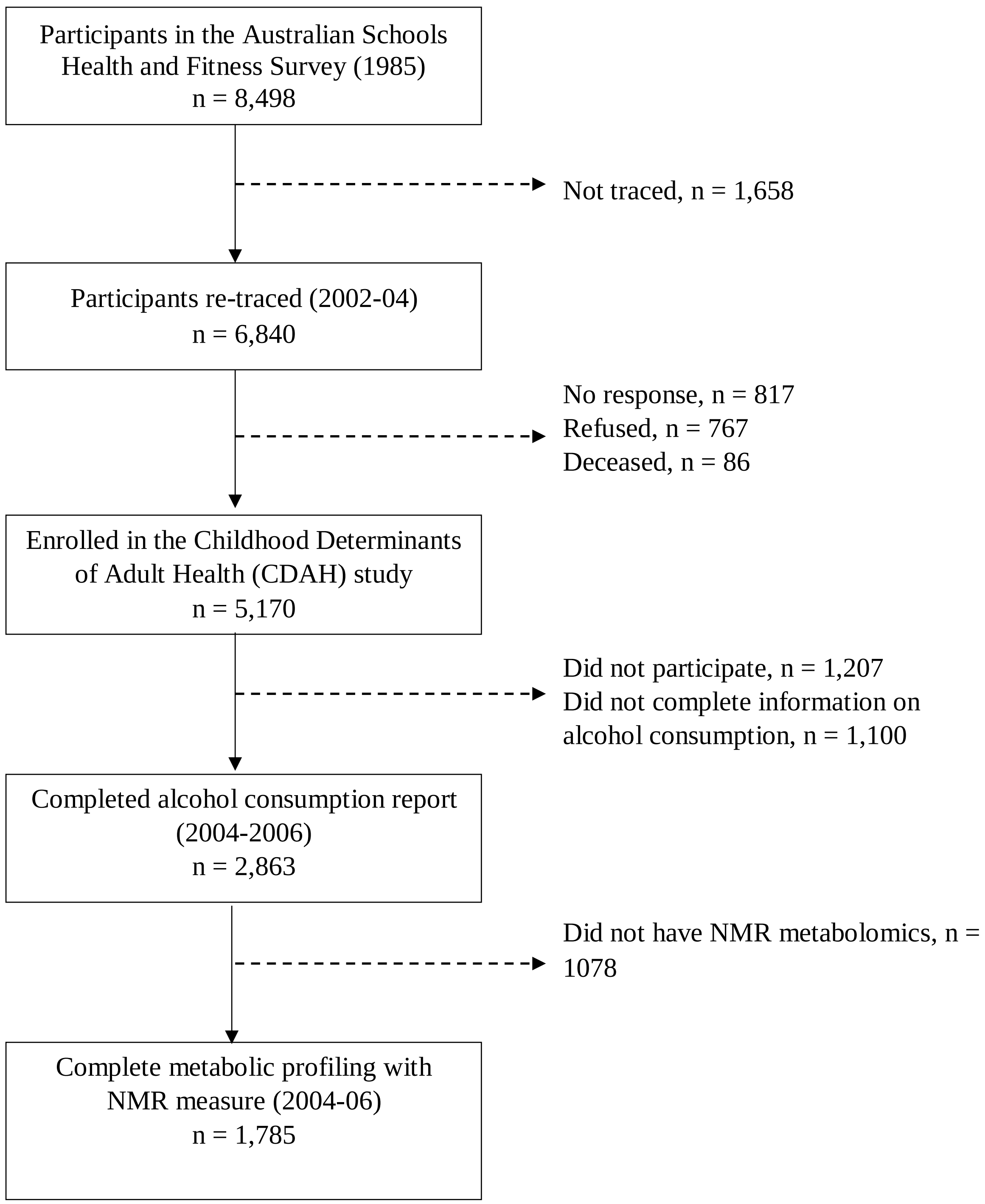


Figure 1. Flow chart of alcohol consumption status and metabolite outcomes during follow-up periods

Supplementary results

Table 1. Multivariable linear regression on association between total alcohol consumption by 100 grams per week and metabolite measures

	Model 1			Model 2		
	β†	**95% CI**	**p-value**	**β†**	**95% CI**	**p-value**
Lipoprotein lipid concentration						
Extremely large VLDL	0.011	(-0.027, 0.050)	0.568	0.019	(-0.019, 0.057)	0.320
Very large VLDL	-0.004	(-0.048, 0.040)	0.863	0.007	(-0.034, 0.047)	0.749
Large VLDL	-0.011	(-0.058, 0.036)	0.642	-0.017	(-0.062, 0.029)	0.477
Medium VLDL	-0.042	(-0.091, 0.007)	0.091	-0.063	(-0.116, -0.010)	0.020
Small VLDL	-0.056	(-0.107, -0.005)	0.033	-0.099	(-0.158, -0.040)	0.001
Very small VLDL	-0.061	(-0.114, -0.008)	0.024	-0.083	(-0.145, -0.021)	0.01
IDL	-0.079	(-0.131, -0.026)	0.004	-0.076	(-0.139, -0.014)	0.017
Large IDL	-0.046	(-0.098, 0.006)	0.084	-0.048	(-0.109, 0.014)	0.127
Medium LDL	-0.019	(-0.070, 0.032)	0.472	-0.020	(-0.080, 0.040)	0.516
Small LDL	0.006	(-0.045, 0.057)	0.810	0.009	(-0.052, 0.069)	0.778
Very large HDL	0.064	(0.015, 0.114)	0.011	0.107	(0.053, 0.161)	0.0001
Large HDL	0.170	(0.123, 0.217)	3.03e-12	0.250	(0.194, 0.300)	<2e-16
Medium HDL	0.228	(0.180, 0.276)	<2e-16	0.270	(0.211, 0.330)	<2e-16
Small HDL	0.145	(0.097, 0.192)	3.1e-09	0.150	(0.088, 0.204)	1.0e-06
Lipoprotein particle size						
VLDL particle size	-0.042	(-0.088, 0.005)	0.082	-0.059	(-0.112, -0.006)	0.028
LDL particle size	-0.123	(-0.174, -0.072)	2.6e-06	-0.130	(-0.193, -0.067)	5.8e-05
HDL particle size	0.103	(0.057, 0.148)	9.8e-06	0.153	(0.100, 0.206)	1.9e-08

Apolipoprotein						
Apolipoprotein B	-0.083	(-0.135, -0.032)	0.001	-0.093	(-0.154, -0.032)	0.003
Apolipoprotein A-1	0.188	(0.140, 0.237)	6.0e-14	0.234	(0.176, 0.291)	3.6e-15
Cholesterol						
Total C	0.027	(-0.025, 0.079)	0.312	0.046	(-0.016, 0.107)	0.145
Remnant C	-0.085	(-0.135, -0.035)	0.001	-0.092	(-0.160, -0.032)	0.003
VLDL C	-0.067	(-0.117, -0.018)	0.008	-0.085	(-0.143, -0.027)	0.004
IDL C	-0.091	(-0.143, -0.039)	0.001	-0.088	(-0.150, -0.026)	0.005
LDL C	-0.037	(-0.088, 0.015)	0.163	-0.037	(-0.098, 0.024)	0.229
HDL C	0.200	(0.152, 0.248)	4.8e-16	0.250	(0.194, 0.307)	<2e-16
HDL_2 C	0.209	(0.161, 0.257)	<2e-16	0.259	(0.202, 0.316)	<2e-16
HDL_3 C	-0.021	(-0.070, 0.028)	0.400	-0.016	(-0.074, 0.042)	0.589
Esterified C	0.035	(-0.018, 0.087)	0.193	0.052	(-0.010, 0.114)	0.097
Free C	0.006	(-0.046, 0.058)	0.816	0.024	(-0.039, 0.086)	0.435
Triglycerides						
Total TG	-0.029	(-0.080, 0.022)	0.268	-0.038	(-0.095, 0.019)	0.191
VLDL TG	-0.034	(-0.084, 0.016)	0.187	-0.049	(-0.104, 0.006)	0.083
IDL TG	-0.032	(-0.083, 0.019)	0.222	-0.033	(-0.092, 0.026)	0.271
LDL TG	-0.004	(-0.054, 0.045)	0.860	0.001	(-0.056, 0.057)	0.983
HDL TG	0.001	(-0.050, 0.052)	0.978	0.002	(-0.055, 0.060)	0.939
Phosphatidylcholine	0.179	(0.129, 0.229)	2.1e-12	0.212	(0.154, 0.270)	1.5e-12
Sphingomyein	0.035	(-0.016, 0.085)	0.178	0.057	(-0.002, 0.117)	0.060
Phosphoglycerides	0.178	(0.128, 0.228)	3.6e-12	0.209	(0.149, 0.268)	7.9e-12
Fatty acids						
Total FA	0.075	(0.023, 0.126)	0.005	0.881	(0.028, 0.148)	0.004
Saturated FA	0.083	(0.032, 0.135)	0.002	0.111	(0.051, 0.170)	0.001
MUFA	0.082	(0.031, 0.133)	0.002	0.070	(0.010, 0.127)	0.021

PUFA	0.038	(-0.014, 0.090)	0.148	0.064	(0.003, 0.125)	0.041
Omega-6 FA	0.025	(-0.027, 0.077)	0.342	0.047	(-0.014, 0.109)	0.133
Linoleic acid	0.011	(-0.041, 0.063)	0.676	0.029	(-0.033, 0.091)	0.358
Omega-3 FA	0.113	(0.061, 0.165)	2.1e-05	0.156	(0.096, 0.217)	4.5e-07
DHA	0.093	(0.043, 0.144)	0.001	0.125	(0.071, 0.180)	7.7e-06
Fatty acid ratios						
Saturated FA (%)	0.060	(0.007, 0.112)	0.027	0.126	(0.062, 0.188)	9.8e-05
MUFA (%)	0.078	(0.027, 0.129)	0.003	0.019	(-0.040, 0.079)	0.524
PUFA (%)	-0.115	(-0.166, -0.064)	9.8e-06	-0.087	(-0.146, -0.028)	0.004
Omega-6 FA (%)	-0.134	(-0.185, -0.083)	2.6e-07	-0.114	(-0.173, -0.055)	0.001
Linoleic acid (%)	-0.128	(-0.179, -0.077)	1.1e-06	-0.117	(-0.178, -0.057)	0.001
Omega-3 FA (%)	0.054	(0.002, 0.106)	0.042	0.095	(0.032, 0.158)	0.003
DHA (%)	0.059	(0.009, 0.108)	0.020	0.089	(0.033, 0.145)	0.002
Unsaturation Degree	-0.049	(-0.101, 0.002)	0.061	-0.010	(-0.070, 0.050)	0.739
Amino acids						
Alanine	0.018	(-0.035, 0.072)	0.502	0.046	(-0.018, 0.110)	0.157
Glutamine	-0.021	(-0.068, 0.027)	0.390	-0.011	(-0.067, 0.045)	0.709
Glycine	-0.081	(-0.133, -0.030)	0.002	-0.080	(-0.140, -0.020)	0.01
Branched-chain amino acids						
Isoleucine	-0.080	(-0.128, -0.032)	0.001	-0.094	(-0.150, -0.038)	0.01
Leucine	-0.015	(-0.065, 0.035)	0.562	-0.038	(-0.096, 0.021)	0.210
Valine	-0.116	(-0.165, -0.068)	2.4e-06	-0.139	(-0.197, -0.081)	2.7e-06
Aromatic amino acids						
Phenylalaine	-0.079	(-0.135, -0.024)	0.005	-0.097	(-0.167, -0.027)	0.01
Tyrosine	0.046	(-0.006, 0.098)	0.083	0.060	(-0.003, 0.122)	0.060
Histidine	-0.023	(-0.074, 0.028)	0.374	-0.032	(-0.093, 0.029)	0.306

Glycolysis and Gluconeogenesis						
Glucose	0.020	(-0.033, 0.073)	0.455	0.010	(-0.044, 0.064)	0.725
Lactate	-0.045	(-0.096, 0.007)	0.090	-0.063	(-0.125, -0.002)	0.043
Pyruvate	-0.022	(-0.075, 0.030)	0.402	-0.030	(-0.089, 0.029)	0.317
Citrate	-0.098	(-0.135, -0.061)	2.1e-07	-0.105	(-0.161, -0.049)	0.001
Glycerol	0.043	(-0.008, 0.094)	0.095	0.016	(-0.043, 0.076)	0.590
Ketone bodies						
Acetoacetate	0.019	(-0.033, 0.070)	0.473	0.019	(-0.045, 0.082)	0.564
Beta-hydroxybutyrate	0.019	(-0.032, 0.070)	0.458	0.022	(-0.040, 0.085)	0.488
Miscellaneous						
Creatinine	0.009	(-0.033, 0.051)	0.667	0.014	(-0.035, 0.063)	0.572
Albumin	0.053	(0.002, 0.105)	0.041	0.074	(0.015, 0.133)	0.015
Acetate	0.057	(0.003, 0.111)	0.038	0.049	(-0.014, 0.112)	0.129
Inflammation						
Glycoprotein acetyls	-0.038	(-0.085, 0.009)	0.109	-0.020	(-0.074, 0.034)	0.474

CI, confidence interval; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; C, cholesterol; TG, triglycerides; FA, fatty acid; DHA, docosahexaenoic acid.

Model 1 adjusted for sex, age. Model 2 adjusted for Model 1 + region, SES status, educational level, occupation, marital status, smoking, diet, physical activity, cardiorespiratory fitness, depression and anxiety.

† β=beta coefficients expressed in standard deviation unit change per 100 grams of alcohol consumption per week

Table 2. Multivariable polynomial regression on the non-linear associations between total alcohol consumption by 100 grams per week and metabolite measures

		Model 1			Model 2		
	N	β †	95% CI	p-value	β †	95% CI	p-value
Lipoprotein lipid concentration							
Medium VLDL	1,740	-0.036	(-0.056, -0.016)	<0.001	-0.017	(-0.033, -0.001)	0.034
Small VLDL	1,758	-0.122	(-0.178, -0.067)	<0.001	-0.119	(-0.183, -0.056)	<0.001
Large HDL	1,758	0.296	(0.224, 0.369)	<0.001	0.373	(0.292, 0.453)	<0.001
Medium HDL	1,758	0.378	(0.311, 0.446)	<0.001			
Small HDL	1,758	0.202	(0.133, 0.271)	<0.001	0.199	(0.115, 0.283)	<0.001
Lipoprotein particle size							
HDL particle size	1,759	0.001	(-0.073, 0.073)	0.400	0.271	(0.194, 0.348)	<0.001
Apolipoprotein							
Apolipoprotein A-1	1,783	0.321	(0.245, 0.397)	<0.001	0.379	(0.290, 0.469)	<0.001
Cholesterol							
HDL C	1,767	0.354	(0.276, 0.431)	<0.001	0.415	(0.324, 0.506)	<0.001
HDL_2 C	1,783	0.373	(0.295, 0.450)	<0.001	0.432	(0.340, 0.523)	<0.001
Triglycerides							
Phosphatidylcholine	1,765	0.290	(0.213, 0.368)	<0.001	0.325	(0.235, 0.414)	<0.001
Phosphoglycerides	1,766	0.277	(0.200, 0.354)	<0.001	0.316	(0.226, 0.406)	<0.001
Fatty acids							
Saturated FA	1,766	0.118	(0.041, 0.182)	0.001	0.147	(0.066, 0.228)	<0.001
Omega-3 FA	1,766	0.179	(0.103, 0.254)	<0.001	0.211	(0.125, 0.297)	<0.001
DHA	1,766	0.190	(0.111, 0.269)	<0.001	0.213	(0.129, 0.297)	<0.001
Fatty acid ratios							
Saturated FA (%)	1,766	0.088	(0.014, 0.162)	0.020	0.162	(0.075, 0.249)	<0.001

Omega-6 FA (%)	1,765	-0.103	(-0.172, -0.033)	0.001	-0.118	(-0.198, -0.038)	0.004
Linoleic acid (%)	1,766	-0.128	(-0.200, -0.055)	<0.001			
DHA (%)	1,766	0.165	(0.088, 0.243)	<0.001	0.182	(0.098, 0.267)	<0.001
Amino acids							
Branched-chain amino acids							
Valine	1,739	-0.114	(-0.173, -0.055)	<0.001	-0.154	(-0.224, -0.084)	<0.001
Glycolysis and Gluconeogenesis							
Citrate	1,772	-0.134	(-0.185, -0.083)	<0.001	-0.129	(-0.188, -0.069)	<0.001

CI, confidence interval; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; C, cholesterol; TG, triglycerides; FA, fatty acid; DHA, docosahexaenoic acid.

Model 1 adjusted for sex, age. Model 2 adjusted for Model 1 + region, SES status, educational level, occupation, marital status, smoking, diet, physical activity, cardiorespiratory fitness, depression and anxiety.

† β=beta coefficients expressed in standard deviation unit change per 100 grams of alcohol consumption per week

Table 3. Multivariable linear regression models examining potential confounding factors on the associations between total alcohol consumption by 100 grams per week and metabolite measures

	Unadjusted				Adjusted								
					Model 1			Model 2			Model 3		
	N	β†	95% CI	p-value	β†	95% CI	p-value	β†	95% CI	p-value	β†	95% CI	p-value
Lipoprotein lipid concentration													
Log(Large HDL)	1,758	0.083	(0.000, 0.001)	0.0006	0.180	(0.134, 0.225)	1.7e-14	0.250	(0.196, 0.301)	<2e-16	0.250	(0.194, 0.300)	<2e-16
Medium HDL	1,758	0.150	(0.100, 0.199)	4.4e-09	0.223	(0.175, 0.272)	<2e-16	0.270	(0.211, 0.330)	<2e-16	0.270	(0.211, 0.330)	<2e-16
Small HDL	1,758	0.167	(0.008, 0.015)	2.84e-11	0.141	(0.093, 0.190)	1.1e-08	0.142	(0.084, 0.200)	1.8e-06	0.150	(0.088, 0.204)	1.0e-06
Lipoprotein particle size													
LDL particle size	1,758	-0.128	(-0.178, -0.078)	5.66e-07	-0.115	(-0.167, -0.064)	1.1e-05	-0.125	(-0.187, -0.062)	0.0001	-0.130	(-0.193, -0.067)	5.8e-05
HDL particle size	1,759	-0.008	(-0.058, 0.042)	0.7539	0.100	(0.053, 0.145)	2.3e-05	0.157	(0.104, 0.209)	7.5e-09	0.153	(0.100, 0.206)	1.9e-08
Apolipoprotein													
Apolipoprotein A-1	1,783	0.136	(0.087, 0.186)	8.56e-08	0.185	(0.136, 0.234)	2.6e-13	0.231	(0.174, 0.289)	4.9e-15	0.234	(0.176, 0.291)	3.6e-15
Cholesterol													
Remnant C	1,761	-0.063	(-0.113, -0.014)	0.0125	-0.077	(-0.128, -0.026)	0.0030	-0.091	(-0.151, -0.031)	0.0029	-0.092	(-0.160, -0.032)	0.0028
IDL C	1,758	-0.074	(-0.124, -0.024)	0.0037	-0.083	(-0.136, -0.031)	0.0019	-0.088	(-0.150, -0.027)	0.0051	-0.088	(-0.150, -0.026)	0.0054

HDL C	1,767	0.129	(0.080, 0.180)	3.89e-07	0.195	(0.147, 0.243)	4.1e-15	0.249	(0.193, 0.305)	<2e-16	0.250	(0.194, 0.055)	<2e-16
HDL_2 C	1,783	0.131	(0.081, 0.181)	3.03e-07	0.203	(0.154, 0.251)	4.0e-16	0.258	(0.202, 0.315)	<2e-16	0.259	(0.202, 0.316)	<2e-16
Triglycerides													
Phosphatidylcholine	1,765	0.130	(0.081, 0.180)	3.09e-07	0.179	(0.128, 0.229)	4.8e-12	0.211	(0.153, 0.269)	1.4e-12	0.212	(0.154, 0.270)	1.5e-12
Phosphoglycerides	1,766	0.134	(0.084, 0.183)	1.46e-07	0.178	(0.128, 0.229)	7e-12	0.207	(0.148, 0.266)	8.7e-12	0.209	(0.149, 0.268)	7.9e-12
Fatty acids													
Total FA	1,766	0.078	(0.028, 0.128)	0.0021	0.079	(0.027, 0.132)	0.003	0.086	(0.026, 0.145)	0.005	0.081	(0.028, 0.148)	0.004
Log(Saturated FA)	1,766	0.090	(0.043, 0.137)	0.0002	0.097	(0.046, 0.147)	0.002	0.118	(0.060, 0.175)	7.1e-05	0.121	(0.063, 0.179)	5.0e-05
Log(MUFA)	1,766	0.093	(0.046, 0.139)	0.0001	0.086	(0.036, 0.136)	0.001	0.069	(0.012, 0.127)	0.018	0.070	(0.012, 0.129)	0.018
Omega-3 FA	1,766	0.104	(0.054, 0.154)	5.1e-05	0.111	(0.059, 0.164)	3.2e-05	0.154	(0.094, 0.214)	6.0e-07	0.156	(0.096, 0.217)	4.5e-07
DHA	1,766	0.038	(-0.012, 0.088)	0.135	0.086	(0.036, 0.137)	0.0001	0.123	(0.069, 0.178)	9.6e-06	0.125	(0.071, 0.180)	7.7e-06
Fatty acid ratios													
Saturated FA (%)	1,766	0.054	(0.004, 0.104)	0.0349	0.059	(0.006, 0.112)	0.030	0.123	(0.060, 0.186)	0.0001	0.126	(0.062, 0.188)	9.8e-05
MUFA (%)	1,766	0.118	(0.068, 0.168)	3.58e-06	0.089	(0.038, 0.139)	0.001	0.020	(-0.039, 0.079)	0.511	0.019	(-0.040, 0.079)	0.524
PUFA (%)	1,766	-0.154	(-0.204, -0.105)	1.17e-09	-0.126	(-0.176, -0.075)	1.3e-06	-0.087	(-0.145, -0.028)	0.004	-0.087	(-0.146, -0.028)	0.004
Omega-6 FA (%)	1,765	-0.172	(-0.221, -0.122)	1.16e-11	-0.143	(-0.194, -0.092)	3.9e-08	-0.113	(-0.171, -0.054)	0.002	-0.114	(-0.173, -0.055)	0.001
Linoleic acid (%)	1,766	-0.159	(-0.209, -0.109)	3.89e-10	-0.137	(-0.189, -0.086)	1.8e-07	-0.118	(-0.178, -0.058)	0.001	-0.117	(-0.178, -0.057)	0.001
Log(DHA (%))	1,766	0.016	(-0.031, 0.063)	0.509	0.046	(-0.003, 0.095)	0.064	0.088	(0.032, 0.144)	0.002	0.089	(0.033, 0.145)	0.002
Amino acids													

Glycine	1,728	-0.109	(-0.160, -0.058)	3.21e-05	-0.079	(-0.131, -0.027)	0.003	-0.077	(-0.137, -0.017)	0.012	-0.080	(-0.140, -0.020)	0.01
Branched-chain amino acids													
Isoleucine	1,778	-0.002	(-0.052, 0.049)	0.9510	-0.081	(-0.130, -0.032)	0.01	-0.092	(-0.149, -0.036)	0.001	-0.094	(-0.150, -0.038)	0.01
Valine	1,739	-0.027	(-0.078, 0.024)	0.3030	-0.114	(-0.163, -0.066)	4.6e-06	-0.139	(-1.964, -0.081)	2.7e-06	-0.139	(-0.197, -0.081)	2.7e-06
Aromatic amino acids													
Phenylalaine	1,766	-0.092	(-0.140, -0.045)	0.0002	-0.075	(-0.131, -0.019)	0.01	-0.094	(-0.164, -0.025)	0.01	-0.097	(-0.167, -0.027)	0.01
Glycolysis and Gluconeogenesis													
Citrate	1,772	-0.109	(-0.155, -0.062)	4.96e-06	-0.122	(-0.171, -0.074)	9.5e-07	-0.099	(-0.155, -0.043)	0.001	-0.105	(-0.161, -0.049)	0.001

CI, confidence interval; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; C, cholesterol; TG, triglycerides; FA, fatty acid; DHA, docosahexaenoic acid.

Model 1 adjusted for sex, age, region, SES status, educational level, occupation, marital status. Model 2 adjusted for Model 1 + smoking, diet, physical activity, cardiorespiratory fitness. Model 3 adjusted for Model 2 + depression and anxiety.

† β=beta coefficients expressed in standard deviation unit change per 100 grams of alcohol consumption per week

Table 4. Multivariable linear regression models examining the potential interaction between total alcohol consumption by 100 grams per week and covariates on metabolite measures

		Unadjusted			Adjusted		
	N	**β†**	**95% CI**	**p-value**	**β†**	**95% CI**	**p-value**
Lipoprotein lipid concentration							
Log(Large HDL)	1,756						
Alc (100g/week)		0.218	(0.145, 0.291)	6.0e-09	0.285	(0.202, 0.368)	2.3e-11
Sex (male)		-0.818	(-0.924, -0.713)	<2e-16	-0.769	(-0.926, -0.611)	<2e-16
Alc x Sex		-0.105	(-0.199, -0.011)	0.028	-0.135	(-0.243, -0.026)	0.015
Log(Large HDL)	1,407						
Alc (100g/week)		0.322	(0.162, 0.482)	8.3e-05	0.393	(0.234, 0.551)	1.4e-06
Pwc170		-0.004	(-0.005, -0.003)	2.3e-13	0.001	(-4e-04, 0.003)	0.154
Alc x Pwc170		-0.001	(-0.002, -3e-05)	0.043	-0.001	(-0.002, -0.001)	0.016
Log(Large HDL)	1,556						
Alc (100g/week)		0.062	(0.004, 0.119)	0.035	0.192	(0.131, 0.253)	1.0e-09
Depression/anxiety		0.116	(-0.047, 0.278)	0.163	0.019	(-0.148, 0.186)	0.826
Alc x Dep/Anx		0.191	(0.047, 0.335)	0.009	0.085	(-0.052, 0.223)	0.224
Medium HDL	1,556						
Alc (100g/week)		0.134	(0.076, 0.192)	5.8e-06	0.265	(0.201, 0.329)	9.6e-16
Depression/anxiety		0.010	(-0.153, 0.174)	0.900	-0.109	(-0.283, 0.065)	0.220
Alc x Dep/Anx		0.159	(0.014, 0.304)	0.032	0.021	(-0.123, 0.164)	0.777
Lipoprotein particle size							
LDL particle size	1,756						
Alc (100g/week)		-0.194	(-0.275, -0.113)	2.7e-06	-0.177	(-0.272, -0.082)	0.001
Sex (male)		-0.063	(-0.179, 0.053)	0.288	0.097	(-0.084, 0.277)	0.293
Alc x Sex		0.108	(0.004, 0.211)	0.042	0.083	(-0.041, 0.208)	0.190
LDL particle size	1,639						
Alc (100g/week)		-0.082	(-0.148, -0.016)	0.014	-0.066	(-0.145, 0.013)	0.104

Smoking		-0.037	(-0.183, 0.109)	0.620	-0.041	(-0.218, 0.136)	0.648
Alc x Smoking		-0.114	(-0.222, -0.006)	0.038	-0.169	(-0.297, -0.041)	0.010
LDL particle size	1,407						
Alc (100g/week)		-0.339	(-0.509, -0.171)	8e-05	-0.309	(-0.491, -0.127)	0.001
Pwc170		-0.001	(-0.002, 2.6e-05)	0.055	-0.002	(-0.003, 0.001)	0.078
Alc x Pwc170		0.001	(0.001, 2.2e-03)	0.005	0.001	(5.1e-05, 0.002)	0.040
HDL particle size	1,557						
Alc (100g/week)		-0.008	(-0.065, -0.008)	0.792	0.139	(0.081, 0.198)	3.6e-06
Depression/anxiety		0.133	(-0.030, 0.295)	0.109	-0.019	(-0.180, 0.141)	0.813
Alc x Dep/Anx		0.147	(0.004, 0.291)	0.045	0.070	(-0.062, 0.202)	0.301
Cholesterol							
HDL C	1,765						
Alc (100g/week)		0.258	(0.182, 0.242)	4.0e-11	0.302	(0.216, 0.387)	5.8e-12
Sex(male)		-0.630	(-0.740, -0.521)	<2e-16	-0.689	(-0.851, -0.528)	<2e-16
Alc x Sex		-0.103	(-0.201, -0005)	0.039	-0.090	(-0.201, 0.022)	0.116
HDL C	1,565						
Alc (100g/week)		0.100	(-0.060, 0.071)	0.001	0.228	(0.165, 0.290)	1.6e-12
Depression/anxiety		0.006	(-0.154, 0.166)	0.944	-0.083	(-0.253, 0.087)	0.339
Alc x Dep/Anx		0.246	(0.104, 0.388)	0.001	0.119	(-0.022, 0.259)	0.098
HDL_2 C	1,783						
Alc (100g/week)		0.266	(0.190, 0.341)	2.2e-07	0.313	(0.227, 0.398)	1.2e-12
Sex(male)		-0.675	(-0.783, -0.567)	<2e-16	-0.735	(-0.897, -0.573)	<2e-16
Alc x Sex		-0.104	(-0.201, -0.007)	0.036	-0.094	(-0.206, 0.018)	0.100
HDL_2 C	1,431						
Alc (100g/week)		0.356	(0.193, 0.519)	1.9e-05	0.417	(0.253, 0.581)	6.7e-07
Pwc170		-0.004	(-0.005, -0.002)	1.4e-09	0.001	(-0.001, 0.003)	0.165
Alc x Pwc170		-0.001	(-0.002, -0.001)	0.047	-0.001	(-0.002, -0.001)	0.044
HDL_2 C	1,581						
Alc (100g/week)		0.104	(0.046, 0.161)	0.001	0.238	(0.175, 0.040)	2e-13
Depression/anxiety		0.012	(-0.149, 0.173)	0.885	-0.082	(-0.252, 0.087)	0.341

Alc x Dep/Anx		0.240	(0.096, 0.384)	0.001	0.109	(-0.033, 0.250)	0.131
Triglycerides							
Phosphatidylcholine	1,563						
Alc (100g/week)		0.107	(0.050, 0.164)	0.001	0.192	(0.128, 0.257)	6.8e-09
Depression/anxiety		-0.082	(-0.243, 0.080)	0.321	-0.154	(-0.329, 0.022)	0.086
Alc x Dep/Anx		0.199	(0.056, 0.342)	0.006	0.104	(-0.041, 0.250)	0.159
Phosphoglycerides	1,564						
Alc (100g/week)		0.110	(0.053, 0.168)	0.001	0.190	(0.124, 0.256)	1.7e-08
Depression/anxiety		-0.080	(-0.242, 0.081)	0.330	-0.141	(-0.320, 0.037)	0.120
Alc x Dep/Anx		0.191	(0.048, 0.334)	0.009	0.096	(-0.052, 0.244)	0.203
Fatty acids							
Omega-3 FA	1,647						
Alc (100g/week)		0.169	(-0.162, -0.030)	3.5e-07	0.221	(0.145, 0.296)	1.5e-08
Smoking		-0.014	(-0.157, 0.130)	0.852	0.160	(-0.010, 0.329)	0.065
Alc x Smoking		-0.123	(-0.229, -0.017)	0.023	-0.171	(-0.293, -0.048)	0.006
Fatty acid ratios							
Saturated FA (%)	1,414						
Alc (100g/week)		0.330	(0.158, 0.501)	0.001	0.298	(0.116, 0.479)	0.001
Pwc170		0.002	(0.001, 0.003)	0.006	0.002	(-0.001, 0.003)	0.089
Alc x Pwc170		-0.001	(-0.002, -0.001)	0.005	-0.001	(-0.002, -0.000)	0.048
PUFA (%)	1,740						
Alc (100g/week)		-0.062	(-0.166, 0.043)	0.250	-0.056	(-0.179, 0.067)	0.375
Occupation		-0.048	(-0.100, 0.004)	0.707	-0.015	(-0.080, 0.050)	0.654
Alc x Occupation		-0.045	(-0.089, -0.001)	0.047	-0.015	(-0.066, 0.036)	0.565
Amino acids							
Glycine	1,726						
Alc (100g/week)		-0.156	(-0.236, -0.076)	0.001	-0.151	(-0.241, -0.061)	0.001
Sex		-0.468	(-0.583, -0.352)	4.6e-15	-0.426	(-0.589, -0.253)	1.5e-06
Alc x Sex		0.138	(0.034, 0.241)	0.009	0.127	(0.007, 0.245)	0.037
Glycine	1,726						

Alc (100g/week)		0.544	(-0.106, 1.194)	0.101	1.188	(0.435, 1.942)	0.002
Age		0.039	(0.016, 0.063)	0.001	0.042	(0.015, 0.069)	0.002
Alc x Age		-0.021	(-0.041, -0.001)	0.047	-0.040	(-0.064, -0.016)	0.001
Glycine	1,722						
Alc (100g/week)		0.015	(-0.111, 0.141)	0.819	0.044	(-0.098, 0.185)	0.547
Education		0.120	(0.049, 0.191)	0.001	0.143	(0.053, 0.233)	0.002
Alc x Education		-0.066	(-0.127, -0.004)	0.037	-0.070	(-0.142, 0.003)	0.059
Glycine	1,702						
Alc (100g/week)		0.008	(-0.101, 0.117)	0.887	0.064	(-0.061, 0.188)	0.315
Occupation		0.118	(0.065, 0.171)	1.5e-05	0.107	(0.040, 0.173)	0.002
Alc x Occupation		-0.056	(-0.102, -0.011)	0.017	-0.068	(-0.120, -0.016)	0.010
Glycine	1,387						
Alc (100g/week)		-0.307	(-0.474, -0.140)	0.001	-0.270	(-0.442, -0.097)	0.002
Pwc170		-0.003	(-0.004, -0.002)	2.2e-06	-0.001	(-0.002, 0.001)	0.467
Alc x Pwc170		0.001	(0.001, 0.002)	0.012	0.001	(0.001, 0.002)	0.022
Branched-chain amino acids							
Valine	1,737						
Alc (100g/week)		0.054	(-0.051, 0.039)	0.314	-0.109	(-0.225, 0.008)	0.069
Drink before test		0.164	(0.034, 0.294)	0.014	0.011	(-0.133, 0.155)	0.878
Alc x Drink before test		-0.134	(-0.258, -0.011)	0.033	-0.039	(-0.175, 0.097)	0.574
Valine	1,688						
Alc (100g/week)		-0.335	(-0.599, -0.071)	0.013	-0.315	(-0.618, -0.011)	0.042
Diet		-0.009	(-0.012, -0.006)	2.1e-08	-0.003	(-0.007, 0.001)	0.082
Alc x Diet		0.003	(0.001, 0.006)	0.027	0.002	(-0.001, 0.006)	0.248
Glycolysis and Gluconeogenesis							
Citrate	1,770						
Alc (100g/week)		-0.165	(-0.271, -0.059)	0.002	-0.209	(-0.296, -0.122)	2.9e-06
Drink before test		-0.165	(-0.296, -0.034)	0.013	-0.174	(-0.282, -0.066)	0.002
Alc x Drink before test		0.129	(0.005, 0.253)	0.041	0.186	(0.084, 0.288)	0.001

CI=confidence interval; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; C, cholesterol; TG, triglycerides; FA, fatty acid; DHA, docosahexaenoic acid.

Model adjusted for sex, age, region, SES status, educational level, occupation, marital status, smoking, diet, physical activity, cardiorespiratory fitness, depression and anxiety.

† β=beta coefficients expressed in standard deviation unit change per 100 grams of alcohol consumption per week

Table 5. Sensitivity analysis of the association between total alcohol consumption by 100 grams per week and metabolite measures after excluding participants with alcohol use disorders (AUDs)

	All participants			Excluding those with an AUD		
	β†	**95% CI**	**p-value**	**β†**	**95% CI**	**p-value**
Lipoprotein lipid concentration						
Extremely large VLDL	0.019	(-0.019, 0.057)	0.320	0.015	(-0.028, 0.058)	0.494
Very large VLDL	0.007	(-0.034, 0.047)	0.749	0.006	(-0.039, 0.050)	0.800
Large VLDL	-0.017	(-0.062, 0.029)	0.477	-0.010	(-0.060, 0.041)	0.717
Medium VLDL	-0.063	(-0.116, -0.010)	0.020	-0.056	(-0.114, -0.033)	0.040
Small VLDL	-0.099	(-0.158, -0.040)	0.001	-0.102	(-0.169, -0.037)	0.001
Very small VLDL	-0.083	(-0.145, -0.021)	0.01	-0.090	(-0.162, -0.020)	0.012
IDL	-0.076	(-0.139, -0.014)	0.017	-0.072	(-0.153, -0.009)	0.024
Large IDL	-0.048	(-0.109, 0.014)	0.127	-0.035	(-0.115, 0.044)	0.387
Medium LDL	-0.020	(-0.080, 0.040)	0.516	-0.010	(-0.086, 0.072)	0.860
Small LDL	0.009	(-0.052, 0.069)	0.778	0.024	(-0.054, 0.104)	0.538
Very large HDL	0.107	(0.053, 0.161)	0.0001	0.123	(0.053, 0.194)	0.0006
Large HDL	0.250	(0.194, 0.300)	<2e-16	0.276	(0.215, 0.336)	<2e-16
Medium HDL	0.270	(0.211, 0.330)	<2e-16	0.303	(0.228, 0.379)	<2e-16
Small HDL	0.150	(0.088, 0.204)	1.0e-06	0.134	(0.057, 0.210)	0.0006
Lipoprotein particle size						
VLDL particle size	-0.059	(-0.112, -0.006)	0.028	-0.066	(-0.136, -0.001)	0.048
LDL particle size	-0.130	(-0.193, -0.067)	5.8e-05	-0.139	(-0.221, -0.056)	0.0009
HDL particle size	0.153	(0.100, 0.206)	1.9e-08	0.202	(0.133, 0.271)	1.9e-08
Apolipoprotein						
Apolipoprotein B	-0.093	(-0.154, -0.032)	0.003	-0.089	(-0.168, -0.011)	0.026

Apolipoprotein A-1	0.234	(0.176, 0.291)	3.6e-15	0.261	(0.186, 0.337)	1.7e-11
Cholesterol						
Total C	0.046	(-0.016, 0.107)	0.145	0.069	(-0.011, 0.149)	0.090
Remnant C	-0.092	(-0.160, -0.032)	0.003	-0.091	(-0.168, -0.013)	0.022
VLDL C	-0.085	(-0.143, -0.027)	0.004	-0.090	(-0.166, -0.015)	0.018
IDL C	-0.088	(-0.150, -0.026)	0.005	-0.078	(-0.158, 0.002)	0.057
LDL C	-0.037	(-0.098, 0.024)	0.229	-0.022	(-0.101, 0.057)	0.590
HDL C	0.250	(0.194, 0.307)	<2e-16	0.283	(0.209, 0.356)	1.4e-13
HDL_2 C	0.259	(0.202, 0.316)	<2e-16	0.297	(0.223, 0.371)	7.6e-15
HDL_3 C	-0.016	(-0.074, 0.042)	0.589	-0.054	(-0.130, 0.021)	0.159
Esterified C	0.052	(-0.010, 0.114)	0.097	0.077	(-0.003, 0.157)	0.060
Free C	0.024	(-0.039, 0.086)	0.435	0.042	(-0.037, 0.123)	0.293
Triglycerides						
Total TG	-0.038	(-0.095, 0.019)	0.191	-0.047	(-0.120, 0.027)	0.212
VLDL TG	-0.049	(-0.104, 0.006)	0.083	-0.055	(-0.125, 0.016)	0.128
IDL TG	-0.033	(-0.092, 0.026)	0.271	-0.050	(-0.126, 0.027)	0.202
LDL TG	0.001	(-0.056, 0.057)	0.983	-0.004	(-0.077, 0.069)	0.916
HDL TG	0.002	(-0.055, 0.060)	0.939	-0.009	(-0.085, 0.066)	0.810
Phosphatidylcholine	0.212	(0.154, 0.270)	1.5e-12	0.235	(0.159, 0.311)	2.0e-09
Sphingomyein	0.057	(-0.002, 0.117)	0.060	0.068	(-0.011, 0.146)	0.091
Phosphoglycerides	0.209	(0.149, 0.268)	7.9e-12	0.233	(0.156, 0.311)	4.9e-09
Fatty acids						
Total FA	0.881	(0.028, 0.148)	0.004	0.901	(0.013, 0.167)	0.022
Saturated FA	0.111	(0.051, 0.170)	0.001	0.113	(0.036, 0.190)	0.002
MUFA	0.070	(0.010, 0.127)	0.021	0.067	(0.008, 0.141)	0.041
PUFA	0.064	(0.003, 0.125)	0.041	0.070	(0.001, 0.142)	0.050
Omega-6 FA	0.047	(-0.014, 0.109)	0.133	0.054	(-0.026, 0.134)	0.186

Linoleic acid	0.029	(-0.033, 0.091)	0.358	0.036	(-0.045, 0.116)	0.387
Omega-3 FA	0.156	(0.096, 0.217)	4.5e-07	0.154	(0.077, 0.232)	0.0001
DHA	0.125	(0.071, 0.180)	7.7e-06	0.125	(0.058, 0.201)	0.0004
Fatty acid ratios						
Saturated FA (%)	0.126	(0.062, 0.188)	9.8e-05	0.136	(0.054, 0.217)	0.001
MUFA (%)	0.019	(-0.040, 0.079)	0.524	0.009	(-0.068, 0.086)	0.820
PUFA (%)	-0.087	(-0.146, -0.028)	0.004	-0.082	(-0.158, -0.006)	0.030
Omega-6 FA (%)	-0.114	(-0.173, -0.055)	0.001	-0.107	(-0.182, -0.032)	0.002
Linoleic acid (%)	-0.117	(-0.178, -0.057)	0.001	-0.115	(-0.192, -0.037)	0.002
Omega-3 FA (%)	0.095	(0.032, 0.158)	0.003	0.091	(0.010, 0.173)	0.003
DHA (%)	0.089	(0.033, 0.145)	0.002	0.097	(0.023, 0.170)	0.009
Unsaturation Degree	-0.010	(-0.070, 0.050)	0.739	-0.014	(-0.092, 0.064)	0.725
Amino acids						
Alanine	0.046	(-0.018, 0.110)	0.157	0.048	(-0.036, 0.131)	0.266
Glutamine	-0.011	(-0.067, 0.045)	0.709	-0.008	(-0.081, 0.065)	0.831
Glycine	-0.080	(-0.140, -0.020)	0.01	-0.096	(-0.176, -0.016)	0.019
Branched-chain amino acids						
Isoleucine	-0.094	(-0.150, -0.038)	0.01	-0.124	(-0.196, -0.052)	0.001
Leucine	-0.038	(-0.096, 0.021)	0.210	-0.054	(-0.130, 0.021)	0.159
Valine	-0.139	(-0.197, -0.081)	2.7e-06	-0.166	(-0.241, -0.091)	1.6e-05
Aromatic amino acids						
Phenylalaine	-0.097	(-0.167, -0.027)	0.01	-0.118	(-0.214, -0.022)	0.016
Tyrosine	0.060	(-0.003, 0.122)	0.060	0.075	(-0.008, 0.158)	0.076
Histidine	-0.032	(-0.093, 0.029)	0.306	-0.056	(-0.137, 0.024)	0.168
Glycolysis and Gluconeogenesis						

Glucose	0.010	(-0.044, 0.064)	0.725	0.043	(-0.030, 0.115)	0.251
Lactate	-0.063	(-0.125, -0.002)	0.043	-0.067	(-0.148, 0.015)	0.108
Pyruvate	-0.030	(-0.089, 0.029)	0.317	-0.019	(-0.097, 0.059)	0.631
Citrate	-0.105	(-0.161, -0.049)	0.001	-0.113	(-0.177, -0.048)	0.001
Glycerol	0.016	(-0.043, 0.076)	0.590	0.007	(-0.070, 0.085)	0.852
Ketone bodies						
Acetoacetate	0.019	(-0.045, 0.082)	0.564	0.002	(-0.079, 0.082)	0.969
Beta-hydroxybutyrate	0.022	(-0.040, 0.085)	0.488	0.022	(-0.102, 0.058)	0.594
Miscellaneous						
Creatinine	0.014	(-0.035, 0.063)	0.572	0.016	(-0.081, 0.049)	0.629
Albumin	0.074	(0.015, 0.133)	0.015	0.148	(0.070, 0.226)	0.001
Acetate	0.049	(-0.014, 0.112)	0.129	0.064	(-0.017, 0.145)	0.123
Inflammation						
Glycoprotein acetyls	-0.020	(-0.074, 0.034)	0.474	-0.021	(-0.091, 0.049)	0.560

CI=confidence interval; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; C, cholesterol; TG, triglycerides; FA, fatty acid; DHA, docosahexaenoic acid.

Model adjusted for sex, age, region, SES status, educational level, occupation, marital status, smoking, diet, physical activity, cardiorespiratory fitness, depression and anxiety.

† β=beta coefficients expressed in standard deviation unit change per 100 grams of alcohol consumption per week

Table 6. Sensitivity analysis of the association between total alcohol consumption by 100 grams per week and metabolite measures after excluding participants who were non-drinkers

	All participants			Exclusion of non-drinkers		
	β†	**95% CI**	**p-value**	**β†**	**95% CI**	**p-value**
Lipoprotein lipid concentration						
Extremely large VLDL	0.019	(-0.019, 0.057)	0.320	0.025	(-0.028, 0.062)	0.496
Very large VLDL	0.007	(-0.034, 0.047)	0.749	0.008	(-0.036, 0.048)	0.812
Large VLDL	-0.017	(-0.062, 0.029)	0.477	-0.021	(-0.072, 0.029)	0.407
Medium VLDL	-0.063	(-0.116, -0.010)	0.020	-0.069	(-0.128, -0.011)	0.019
Small VLDL	-0.099	(-0.158, -0.040)	0.001	-0.082	(-0.146, -0.019)	0.011
Very small VLDL	-0.083	(-0.145, -0.021)	0.01	-0.069	(-0.136, -0.018)	0.014
IDL	-0.076	(-0.139, -0.014)	0.017	-0.073	(-0.137, -0.009)	0.027
Large IDL	-0.048	(-0.109, 0.014)	0.127	-0.043	(-0.106, 0.020)	0.179
Medium LDL	-0.020	(-0.080, 0.040)	0.516	-0.016	(-0.077, 0.046)	0.623
Small LDL	0.009	(-0.052, 0.069)	0.778	0.012	(-0.049, 0.074)	0.695
Very large HDL	0.107	(0.053, 0.161)	0.0001	0.099	(0.042, 0.155)	0.0006
Large HDL	0.250	(0.194, 0.300)	<2e-16	0.184	(0.128, 0.239)	1.3e-10
Medium HDL	0.270	(0.211, 0.330)	<2e-16	0.250	(0.191, 0.309)	<2e-16
Small HDL	0.150	(0.088, 0.204)	1.0e-06	0.142	(0.082, 0.203)	3.8e-06
Lipoprotein particle size						
VLDL particle size	-0.059	(-0.112, -0.006)	0.028	-0.062	(-0.118, -0.006)	0.028
LDL particle size	-0.130	(-0.193, -0.067)	5.8e-05	-0.127	(-0.193, -0.061)	0.0001
HDL particle size	0.153	(0.100, 0.206)	1.9e-08	0.137	(0.082, 0.192)	1.1e-06
Apolipoprotein						
Apolipoprotein B	-0.093	(-0.154, -0.032)	0.003	-0.088	(-0.151, -0.025)	0.006
Apolipoprotein A-1	0.234	(0.176, 0.291)	3.6e-15	0.222	(0.162, 0.281)	5.7e-13

Cholesterol						
Total C	0.046	(-0.016, 0.107)	0.145	0.045	(-0.017, 0.108)	0.155
Remnant C	-0.092	(-0.160, -0.032)	0.003	-0.088	(-0.149, -0.026)	0.005
VLDL C	-0.085	(-0.143, -0.027)	0.004	-0.084	(-0.166, -0.015)	0.006
IDL C	-0.088	(-0.150, -0.026)	0.005	-0.083	(-0.146, -0.020)	0.010
LDL C	-0.037	(-0.098, 0.024)	0.229	-0.031	(-0.092, 0.032)	0.337
HDL C	0.250	(0.194, 0.307)	<2e-16	0.238	(0.180, 0.297)	3.9e-15
HDL_2 C	0.259	(0.202, 0.316)	<2e-16	0.245	(0.186, 0.304)	9.8e-16
HDL_3 C	-0.016	(-0.074, 0.042)	0.589	-0.012	(-0.072, 0.048)	0.692
Esterified C	0.052	(-0.010, 0.114)	0.097	0.054	(-0.009, 0.117)	0.091
Free C	0.024	(-0.039, 0.086)	0.435	0.042	(-0.044, 0.082)	0.293
Triglycerides						
Total TG	-0.038	(-0.095, 0.019)	0.191	-0.042	(-0.102, 0.018)	0.167
VLDL TG	-0.049	(-0.104, 0.006)	0.083	-0.051	(-0.110, 0.007)	0.085
IDL TG	-0.033	(-0.092, 0.026)	0.271	-0.035	(-0.097, 0.026)	0.260
LDL TG	0.001	(-0.056, 0.057)	0.983	-0.004	(-0.063, 0.054)	0.885
HDL TG	0.002	(-0.055, 0.060)	0.939	-0.009	(-0.069, 0.051)	0.764
Phosphatidylcholine	0.212	(0.154, 0.270)	1.5e-12	0.201	(0.141, 0.261)	7.4e-11
Sphingomyein	0.057	(-0.002, 0.117)	0.060	0.050	(-0.011, 0.110)	0.107
Phosphoglycerides	0.209	(0.149, 0.268)	7.9e-12	0.194	(0.133, 0.255)	5.8e-10
Fatty acids						
Total FA	0.081	(0.028, 0.148)	0.004	0.084	(0.022, 0.146)	0.008
Saturated FA	0.111	(0.051, 0.170)	0.001	0.105	(0.044, 0.167)	0.001
MUFA	0.070	(0.010, 0.127)	0.021	0.062	(0.001, 0.123)	0.046
PUFA	0.064	(0.003, 0.125)	0.041	0.065	(0.002, 0.127)	0.043
Omega-6 FA	0.047	(-0.014, 0.109)	0.133	0.048	(-0.014, 0.111)	0.131
Linoleic acid	0.029	(-0.033, 0.091)	0.358	0.031	(-0.032, 0.094)	0.336

Omega-3 FA	0.156	(0.096, 0.217)	4.5e-07	0.140	(0.091, 0.216)	1.6e-06
DHA	0.125	(0.071, 0.180)	7.7e-06	0.112	(0.056, 0.168)	8.8e-05
Fatty acid ratios						
Saturated FA (%)	0.126	(0.062, 0.188)	9.8e-05	0.121	(0.056, 0.186)	0.001
MUFA (%)	0.019	(-0.040, 0.079)	0.524	0.009	(-0.052, 0.071)	0.775
PUFA (%)	-0.087	(-0.146, -0.028)	0.004	-0.074	(-0.134, -0.014)	0.016
Omega-6 FA (%)	-0.114	(-0.173, -0.055)	0.001	-0.101	(-0.160, -0.040)	0.001
Linoleic acid (%)	-0.117	(-0.178, -0.057)	0.001	-0.104	(-0.165, -0.043)	0.001
Omega-3 FA (%)	0.095	(0.032, 0.158)	0.003	0.096	(0.031, 0.161)	0.004
DHA (%)	0.089	(0.033, 0.145)	0.002	0.074	(0.016, 0.132)	0.012
Unsaturation Degree	-0.010	(-0.070, 0.050)	0.739	-0.008	(-0.071, 0.054)	0.792
Amino acids						
Alanine	0.046	(-0.018, 0.110)	0.157	0.057	(-0.009, 0.123)	0.091
Glutamine	-0.011	(-0.067, 0.045)	0.709	-0.002	(-0.060, 0.056)	0.948
Glycine	-0.080	(-0.140, -0.020)	0.01	-0.067	(-0.129, -0.006)	0.033
Branched-chain amino acids						
Isoleucine	-0.094	(-0.150, -0.038)	0.01	-0.077	(-0.135, -0.019)	0.01
Leucine	-0.038	(-0.096, 0.021)	0.210	-0.015	(-0.075, 0.045)	0.617
Valine	-0.139	(-0.197, -0.081)	2.7e-06	-0.117	(-0.177, -0.057)	0.0001
Aromatic amino acids						
Phenylalaine	-0.097	(-0.167, -0.027)	0.01	-0.086	(-0.160, -0.011)	0.024
Tyrosine	0.060	(-0.003, 0.122)	0.060	0.074	(-0.009, 0.139)	0.024
Histidine	-0.032	(-0.093, 0.029)	0.306	-0.032	(-0.096, 0.031)	0.320
Glycolysis and Gluconeogenesis						
Glucose	0.010	(-0.044, 0.064)	0.725	0.013	(-0.057, 0.052)	0.932

Lactate	-0.063	(-0.125, -0.002)	0.043	-0.061	(-0.126, 0.003)	0.060
Pyruvate	-0.030	(-0.089, 0.029)	0.317	-0.028	(-0.089, 0.034)	0.376
Citrate	-0.105	(-0.161, -0.049)	0.001	-0.075	(-0.121, -0.030)	0.001
Glycerol	0.016	(-0.043, 0.076)	0.590	0.031	(-0.030, 0.091)	0.319
Ketone bodies						
Acetoacetate	0.019	(-0.045, 0.082)	0.564	0.012	(-0.055, 0.079)	0.726
Beta-hydroxybutyrate	0.022	(-0.040, 0.085)	0.488	0.024	(-0.042, 0.090)	0.472
Miscellaneous						
Creatinine	0.014	(-0.035, 0.063)	0.572	0.011	(-0.040, 0.062)	0.680
Albumin	0.074	(0.015, 0.133)	0.015	0.081	(0.020, 0.143)	0.009
Acetate	0.049	(-0.014, 0.112)	0.129	0.048	(-0.018, 0.114)	0.155
Inflammation						
Glycoprotein acetyls	-0.020	(-0.074, 0.034)	0.474	-0.024	(-0.081, 0.032)	0.402

CI=confidence interval; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; C, cholesterol; TG, triglycerides; FA, fatty acid; DHA, docosahexaenoic acid.

Model adjusted for sex, age, region, SES status, educational level, occupation, marital status, smoking, diet, physical activity, cardiorespiratory fitness, depression and anxiety.

† β=beta coefficients expressed in standard deviation unit change per 100 grams of alcohol consumption per week

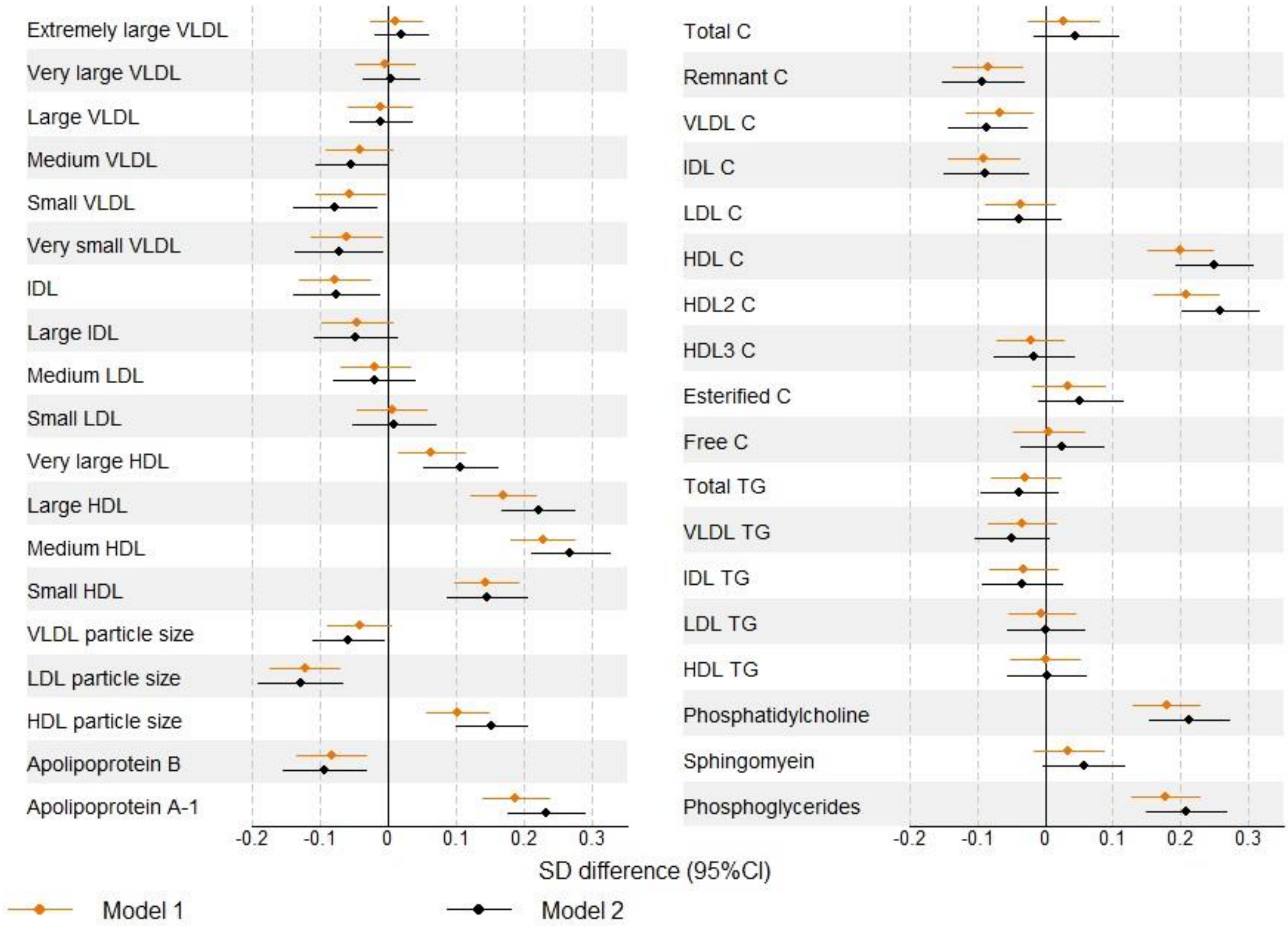


Figure 2. Cross-sectional associations between alcohol consumption (100 grams of pure alcohol per week) and lipoprotein lipid measures. All association were adjusted for age and sex in Model 1; and Model 1 plus region of residence, SES status, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety in Model 2. Error bars denote 95% confidence intervals. Differences in metabolite measures are expressed as standard deviation difference (95% CIs) in metabolite concentration per 100 grams of alcohol per week. Association magnitudes in absolute concentration units are listed in Supplementary Table 1 and continuous shapes of the metabolic associations with alcohol intake are shown in Supplementary Figure S1.

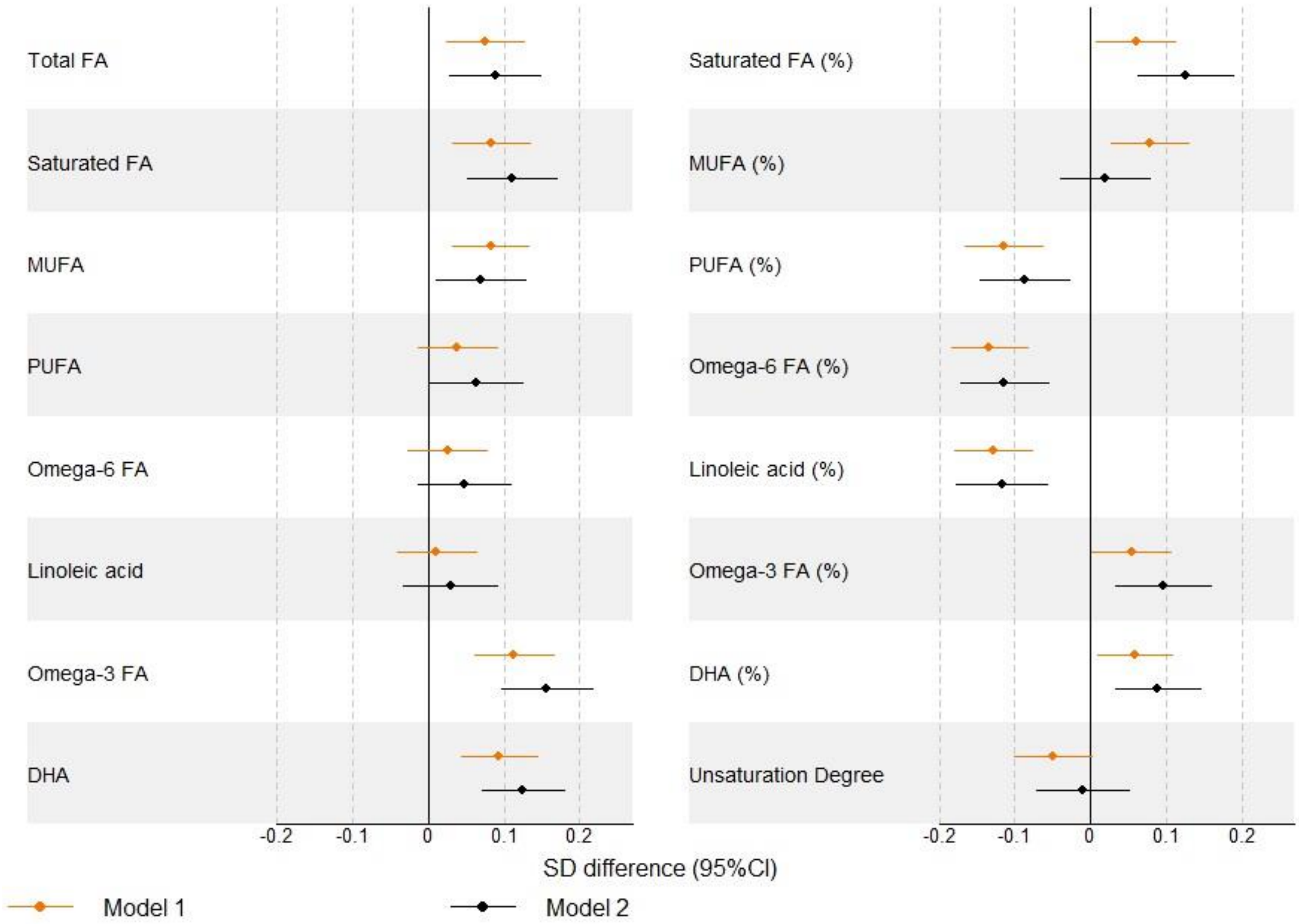


Figure 3. Cross-sectional associations between alcohol consumption and fatty acids. All association were adjusted for age and sex in Model 1; and Model 1 plus region of residence, SES status, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety in Model 2. Error bars denote 95% confidence intervals. Differences in metabolite measures are expressed as standard deviation difference (95% CIs) in metabolite concentration per 100 grams of alcohol per week. Association magnitudes in absolute concentration units are listed in Supplementary Table 1 and continuous shapes of the metabolic associations with alcohol intake are shown in Supplementary Figure S2.

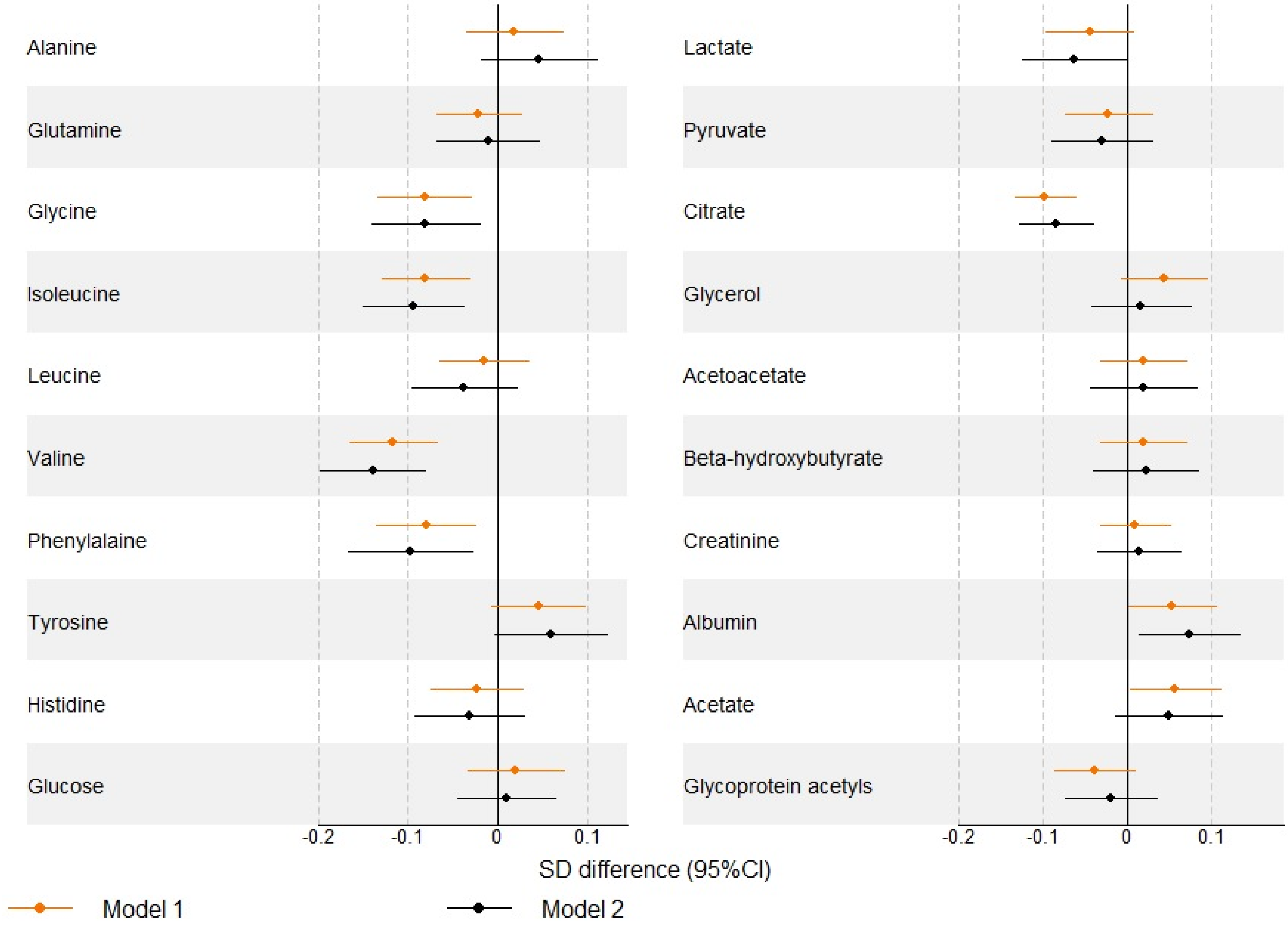


Figure 4. Cross-sectional associations between alcohol consumption and low-molecular-weight metabolites and hormonal measures. All association were adjusted for age and sex in Model 1; and Model 1 plus region of residence, SES status, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety in Model 2. Error bars denote 95% confidence intervals. Differences in metabolite measures are expressed as standard deviation difference (95% CIs) in metabolite concentration per 100 grams of alcohol per week. Association magnitudes in absolute concentration units are listed in Supplementary Table 1 and continuous shapes of the metabolic associations with alcohol intake are shown in Supplementary Figure S3.

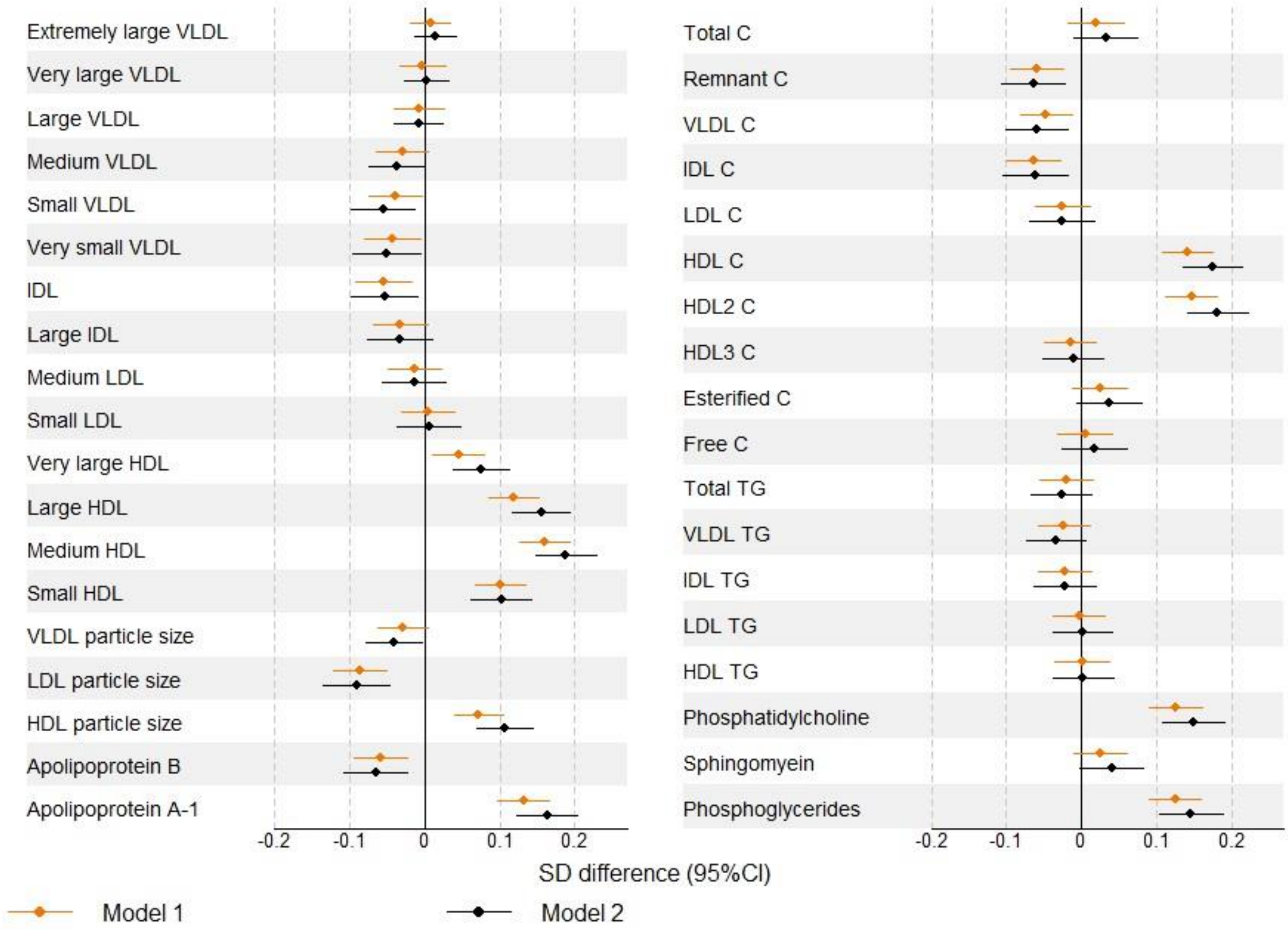


Figure 5. Cross-sectional associations between alcohol consumption and lipoprotein lipid measures. All association were adjusted for age and sex in Model 1; and Model 1 plus region of residence, SES status, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety in Model 2. Error bars denote 95% confidence intervals. Differences in metabolite measures are expressed as standard deviation difference (95% CIs) in metabolite concentration per 1 standard drink of of alcohol per day

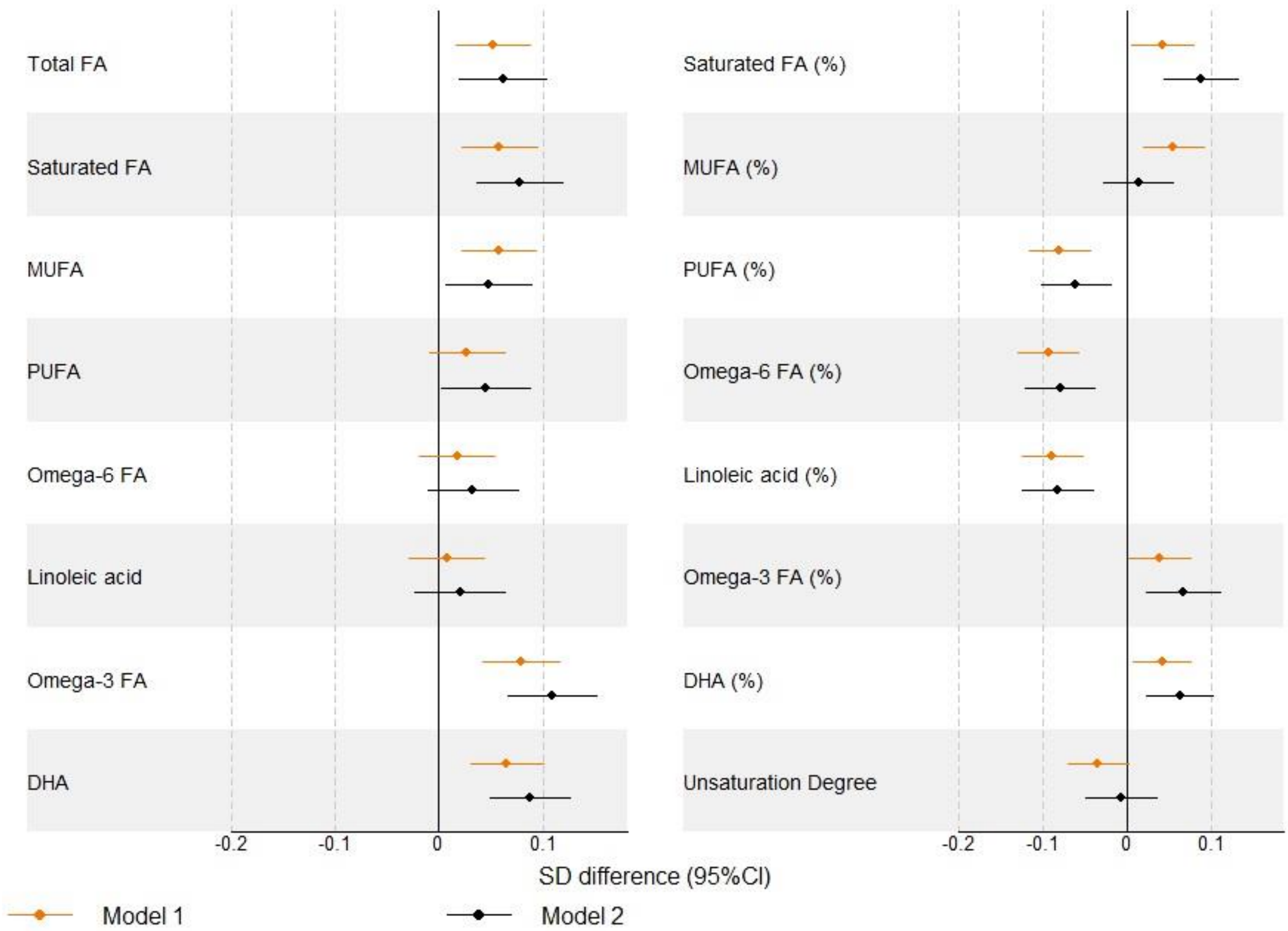


Figure 6. Cross-sectional associations between alcohol consumption and fatty acids. All association were adjusted for age and sex in Model 1; and Model 1 plus region of residence, SES status, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety in Model 2. Error bars denote 95% confidence intervals. Differences in metabolite measures are expressed as standard deviation difference (95% CIs) in metabolite concentration per 1 standard drink of alcohol per day

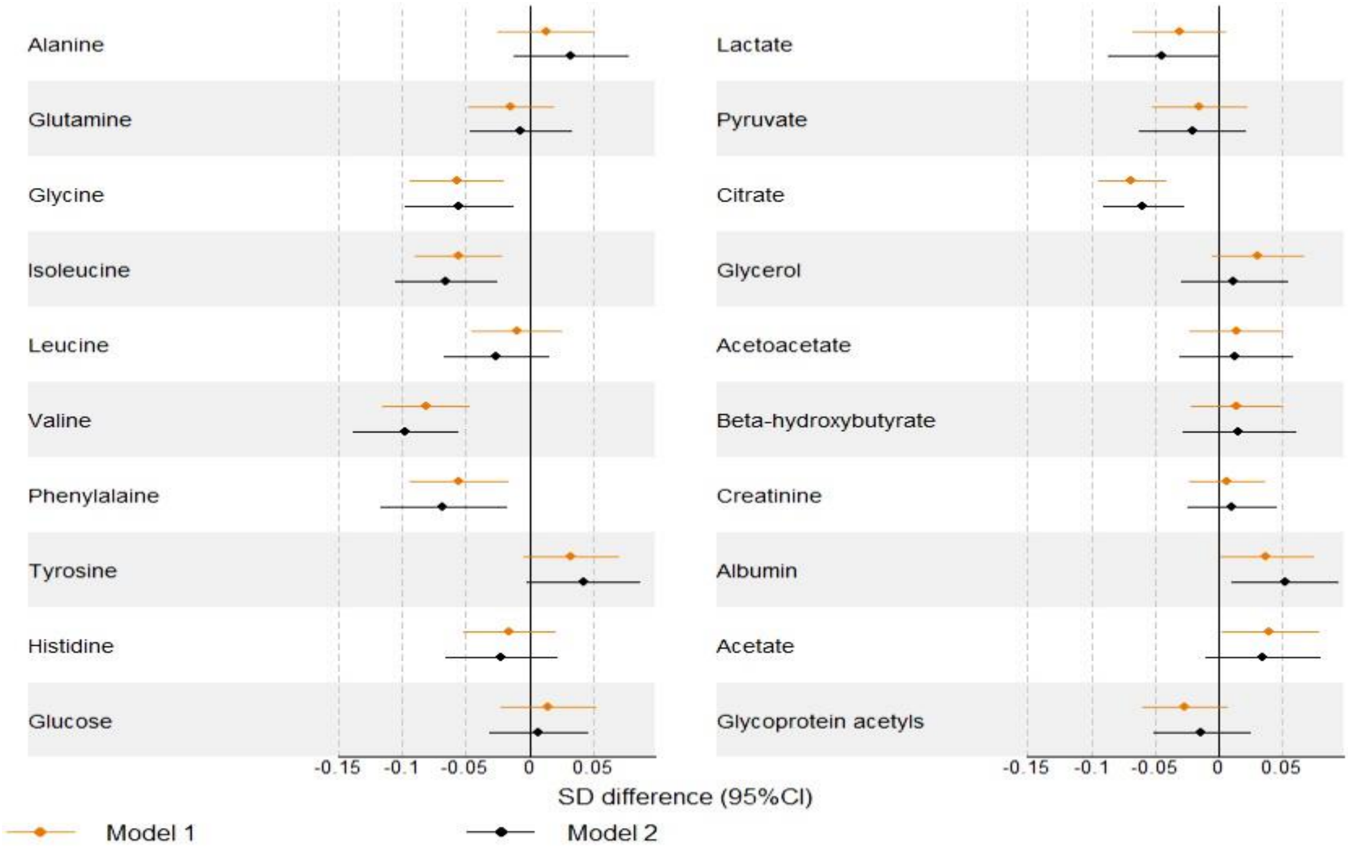


Figure 7. Cross-sectional associations between alcohol consumption and low-molecular-weight metabolites measures. All association were adjusted for age and sex in Model 1; and Model 1 plus region of residence, SES status, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety in Model 2. Error bars denote 95% confidence intervals. Differences in metabolite measures are expressed as standard deviation difference (95% CIs) in metabolite concentration per 1 standard drink of of alcohol per day

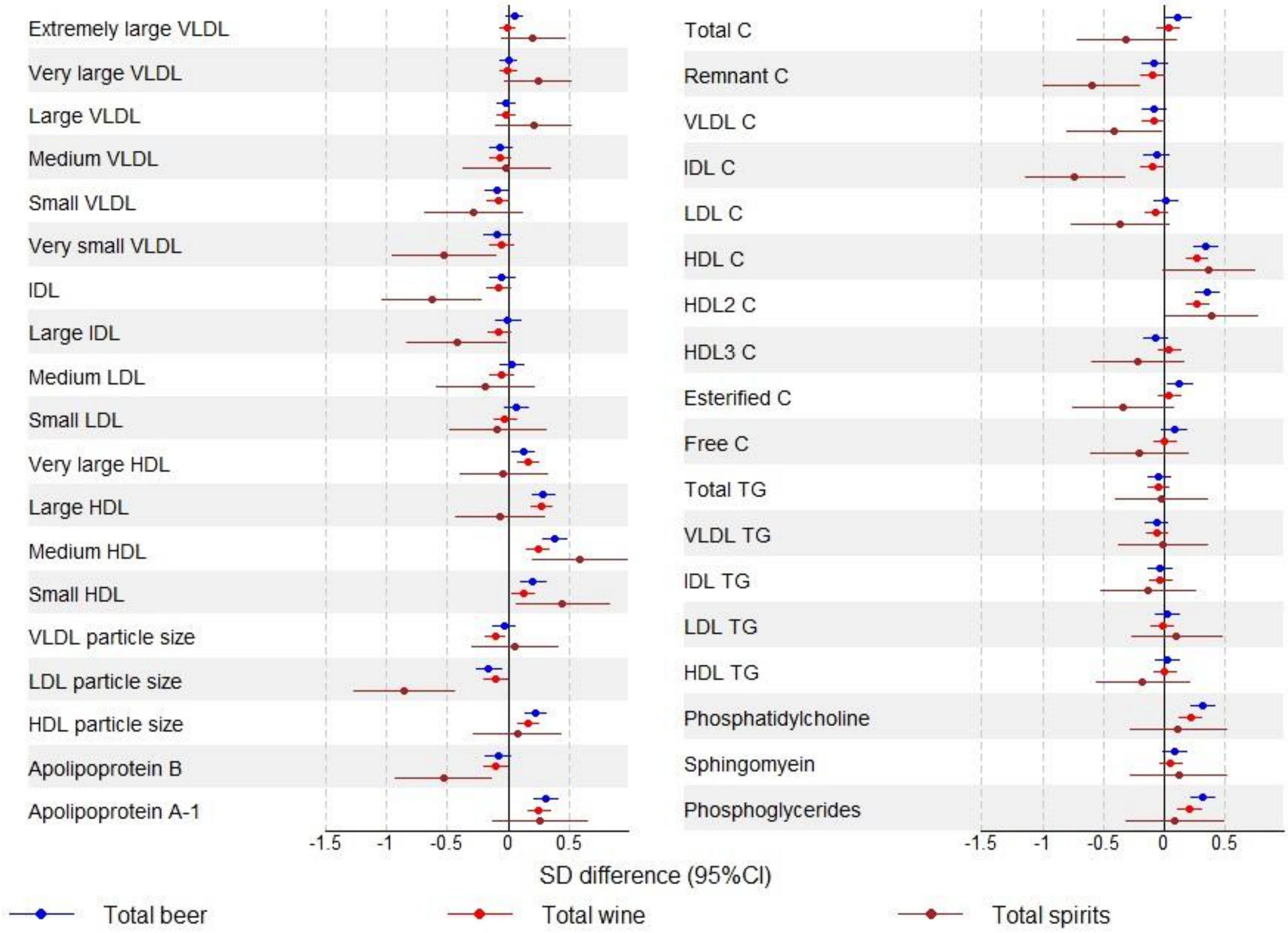


Figure 8. Cross-sectional associations between types of alcohol beverages and lipoprotein lipid measures. All association were adjusted for sex, age, region of residence, SES status, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety in Model 2. Error bars denote 95% confidence intervals. Differences in metabolite measures are expressed as standard deviation difference (95% CIs) in metabolite concentration per 100 grams of alcohol per week

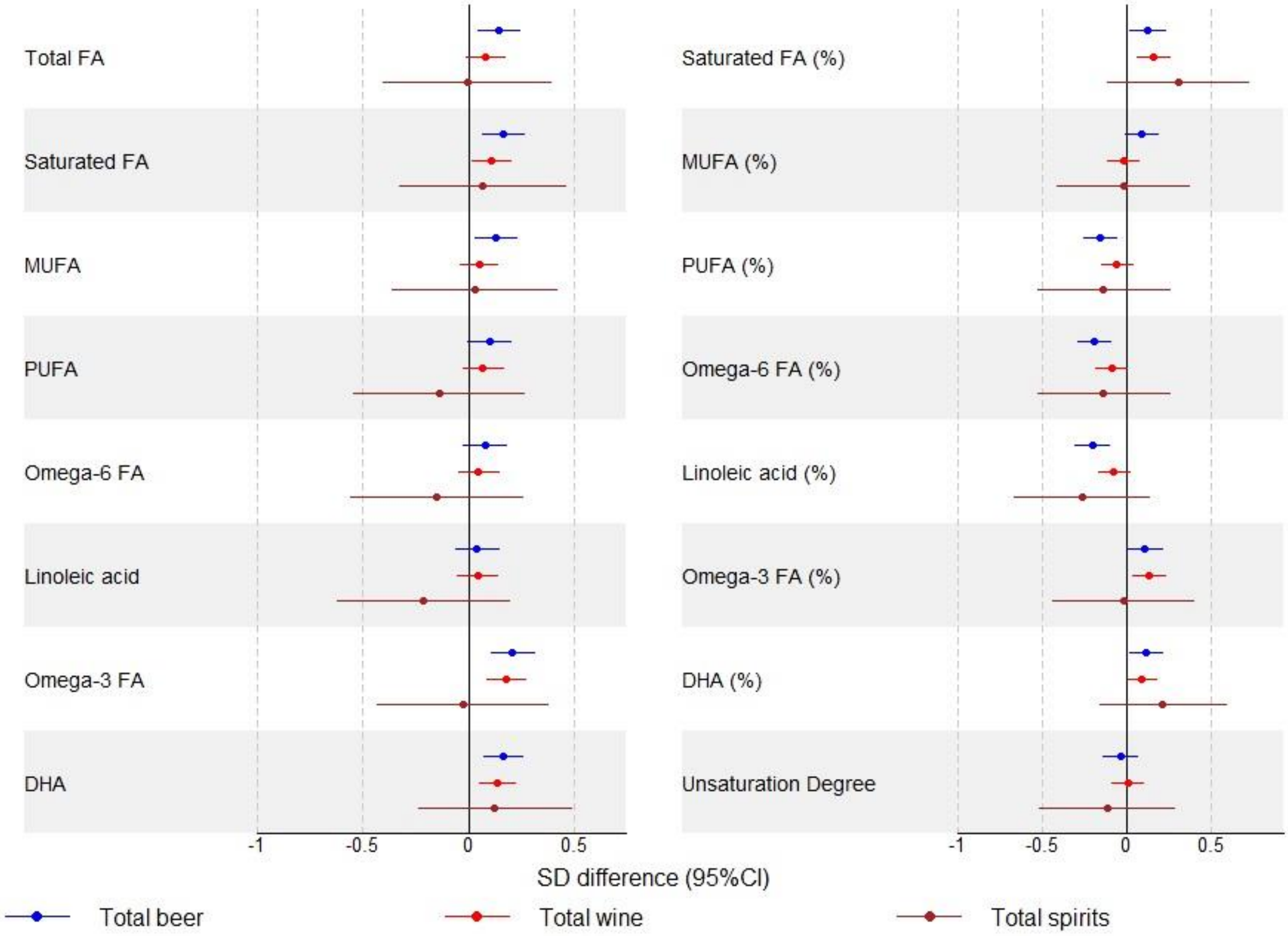


Figure 9. Cross-sectional associations between types of alcohol beverages and fatty acids. All association were adjusted for sex, age, region of residence, SES status, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety in Model 2. Error bars denote 95% confidence intervals. Differences in metabolite measures are expressed as standard deviation difference (95% CIs) in metabolite concentration per 100 grams of alcohol per week

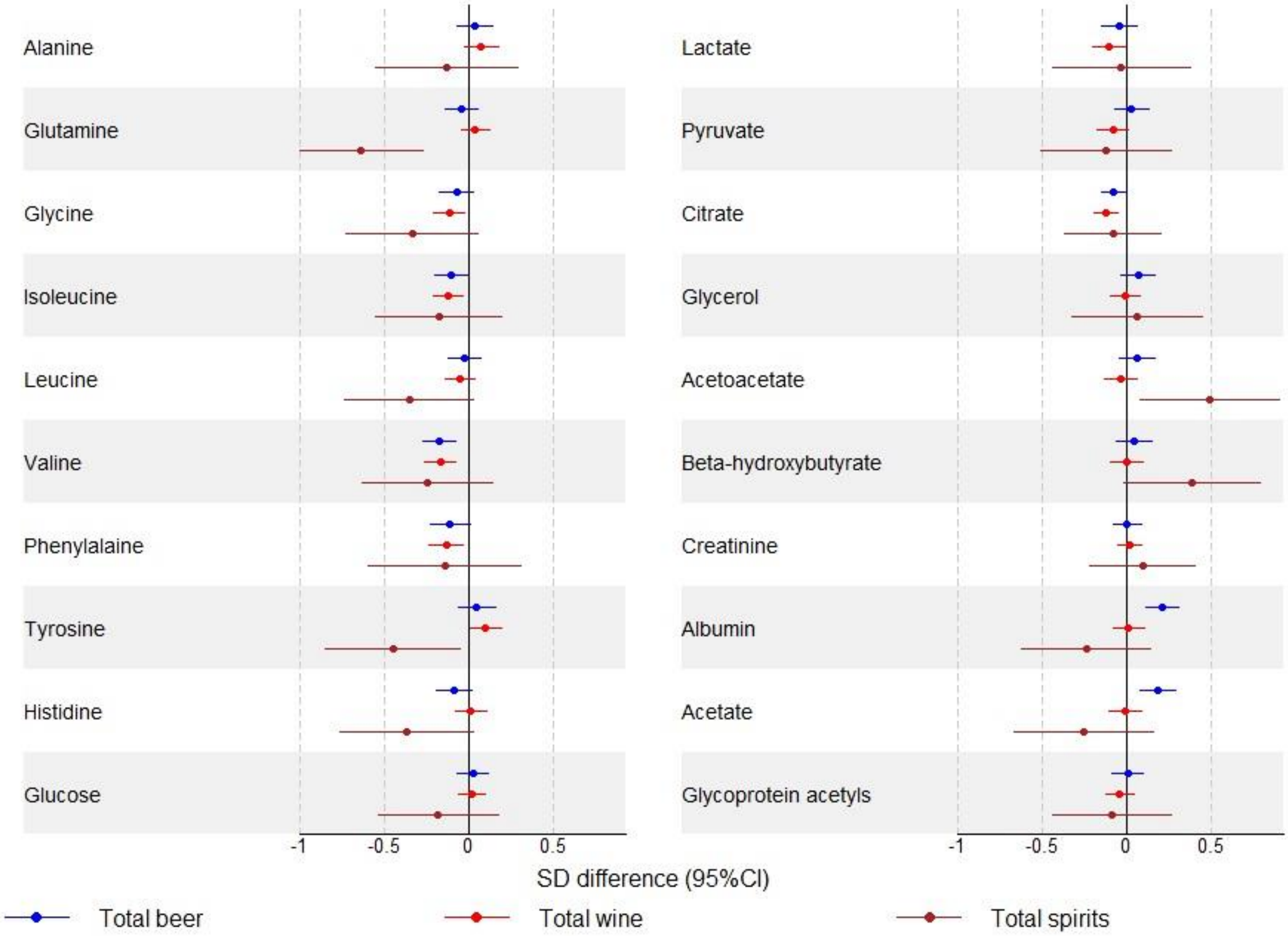
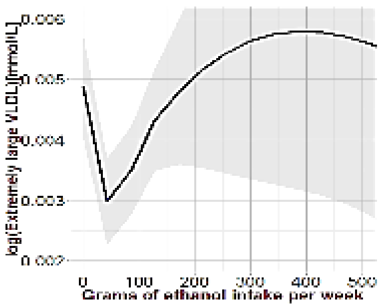


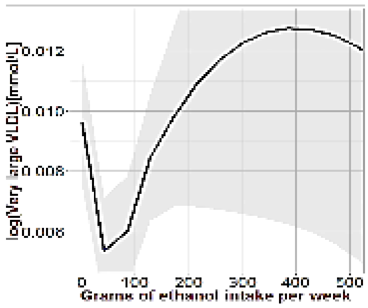
Figure 10. Cross-sectional associations between types of alcohol beverages and low-molecular-weight metabolites and measures. All association were adjusted for sex, age, region of residence, SES status, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory fitness, depression and/or anxiety in Model 2. Error bars denote 95% confidence intervals. Differences in metabolite measures are expressed as standard deviation difference (95% CIs) in metabolite concentration per 100 grams of alcohol per week

Figure S1. Continuous shape of the association between alcohol consumption and lipoprotein lipid measures. The black curves denote the shape of the association, with the grey shaded area denoting the 95% confidence interval of the fit. The association shapes were derived using local quadratic regression fitting evaluated at 25 points

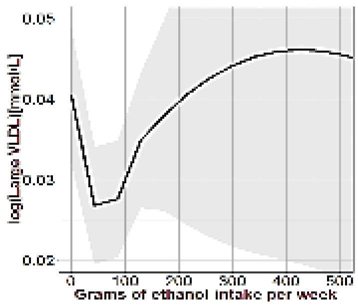
Log(Extremely large VLDL)



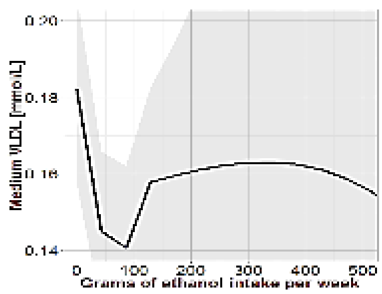
Log(Very large VLDL)



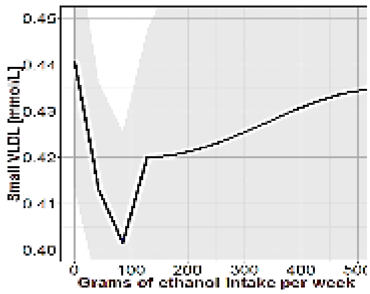
Log(Large VLDL)



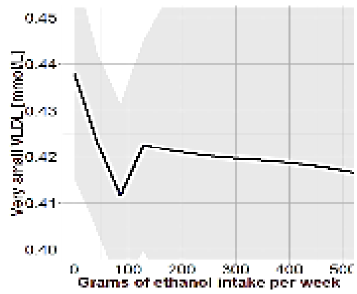
Medium VLDL



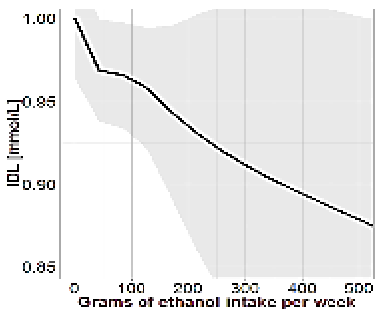
Small VLDL



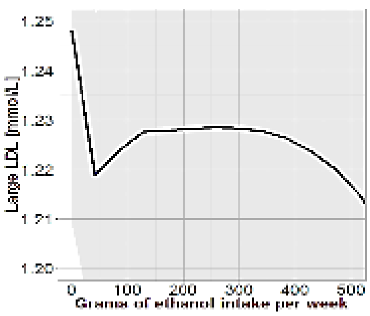
Very small VLDL



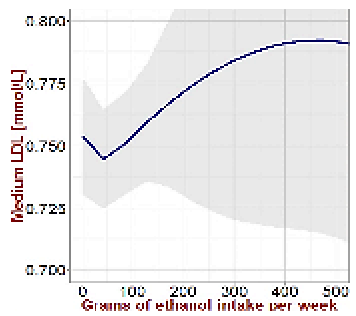
IDL



Large LDL



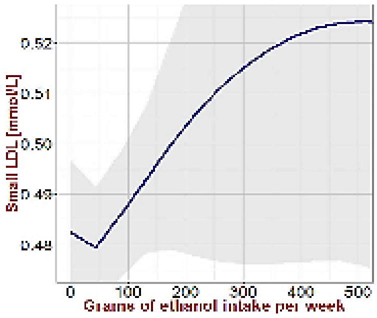
Medium LDL

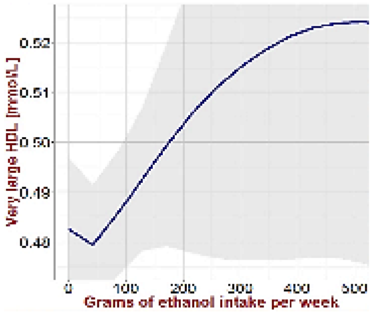


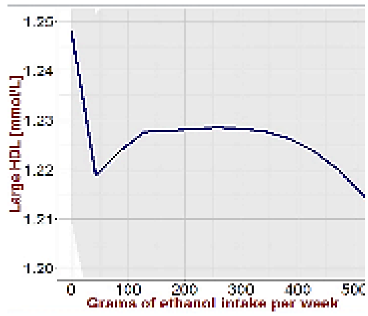
Small LDL

Very large HDL

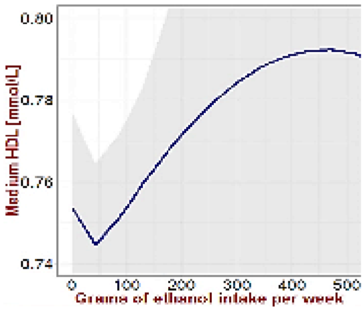
Large HDL

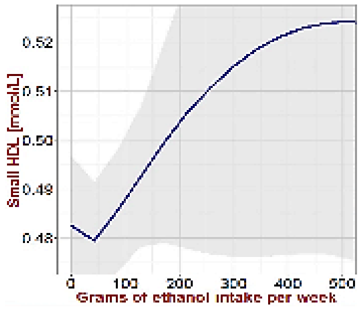


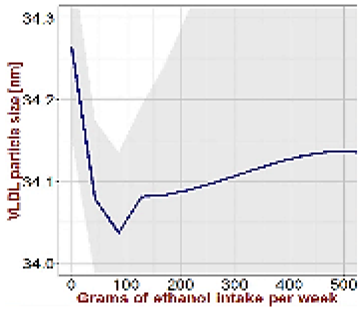




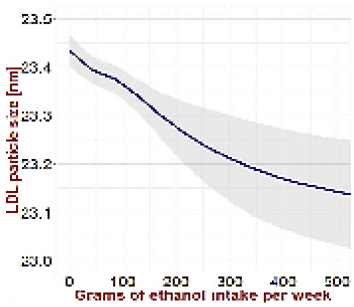
Medium HDL **Small HDL** **VLDL particle size**

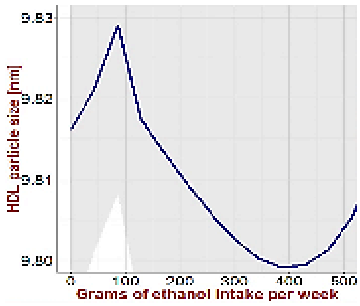


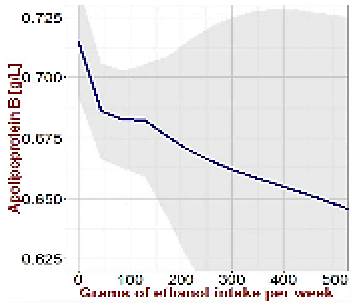




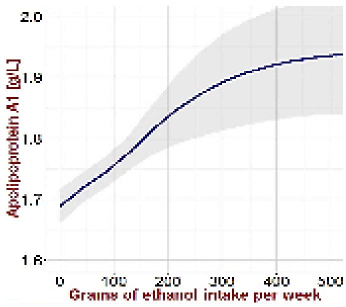
LDL particle size **HDL particle size** **Apolipoprotein B**

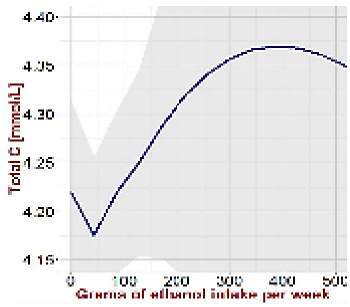


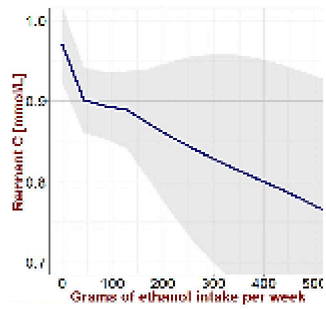




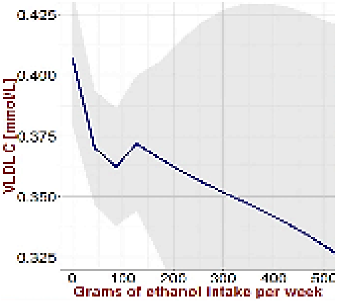
Apolipoprotein A1 **Total C** **Remnant C**

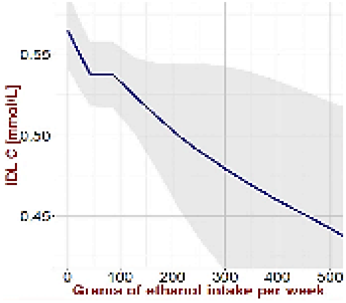


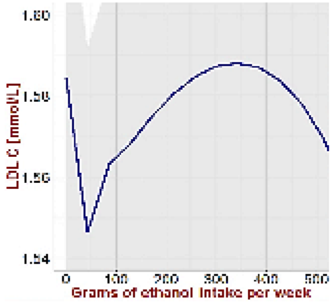




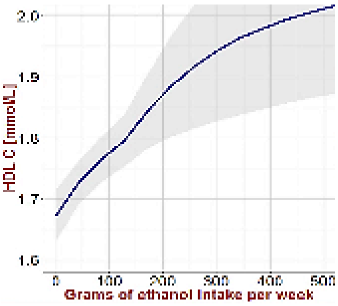
VDLD C **IDL C** **LDL C**



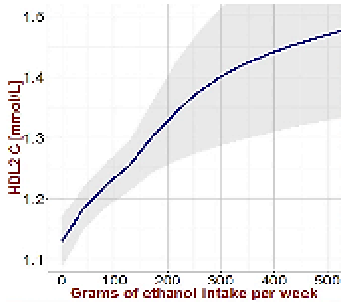




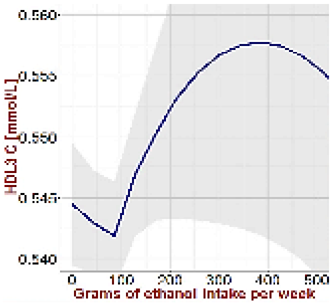
HDL C



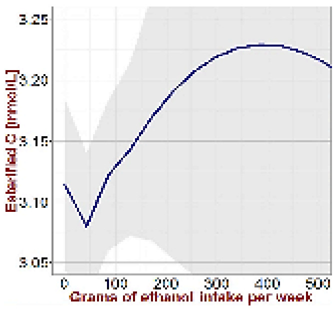
HDL2 C



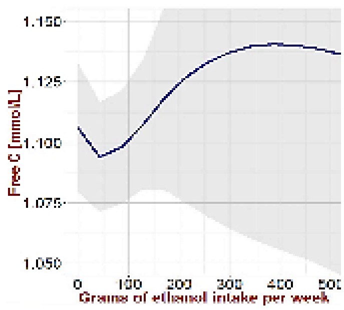
HDL 3 C



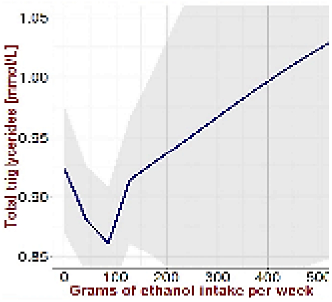
Esterified C



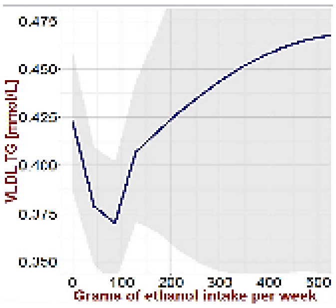
Free C



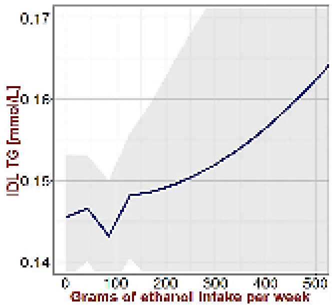
Total triglycerides



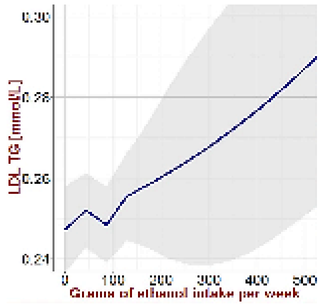
VLDL TG



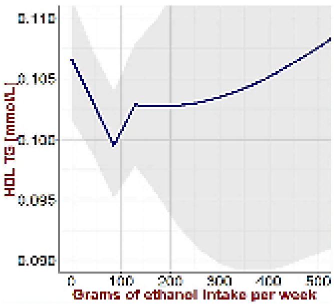
IDL TG



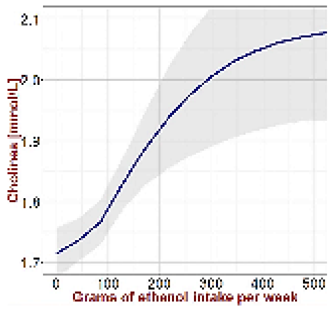
LDL TG



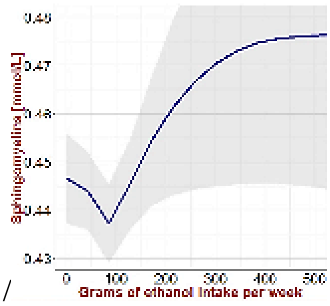
HDL TG



Cholines



Sphingomyelin



Phosphoglycerides

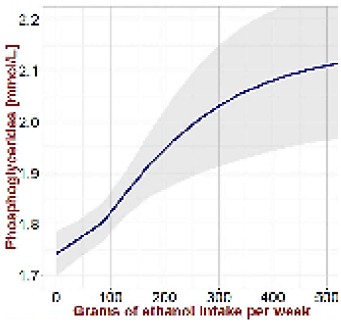
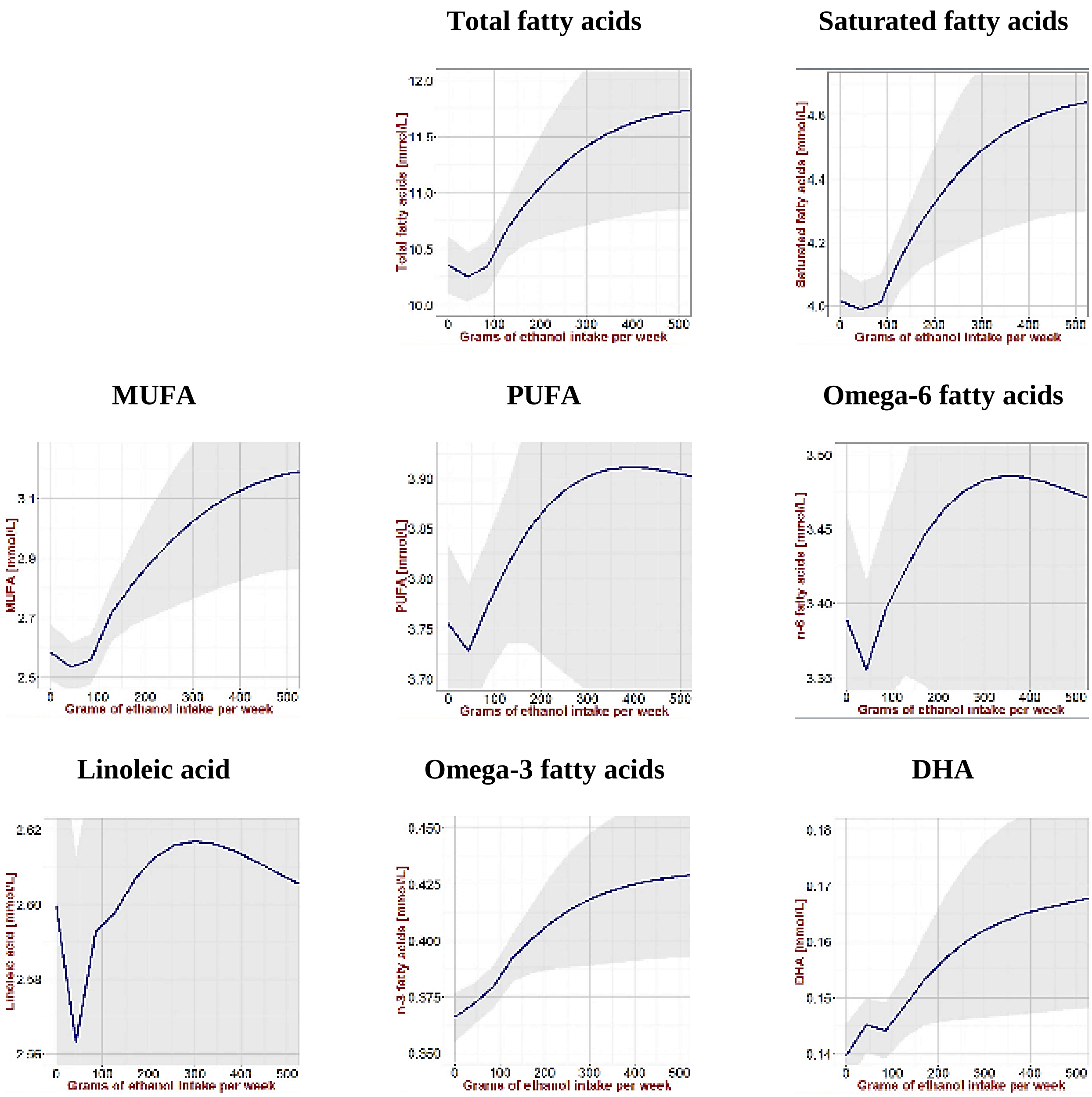
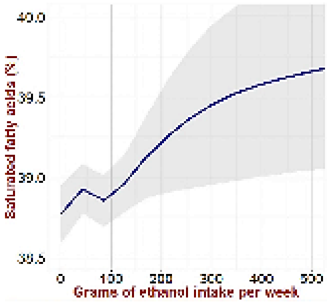


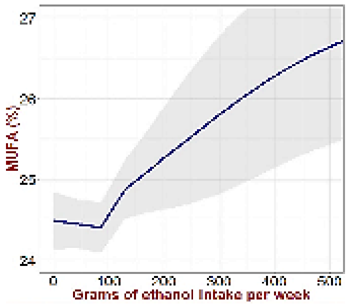
Figure S2. Continuous shape of the association between alcohol consumption and fatty acids. The black curves denote the shape of the association, with the grey shaded area denoting the 95% confidence interval of the fit. The association shapes were derived using local quadratic regression fitting evaluated at 25 points



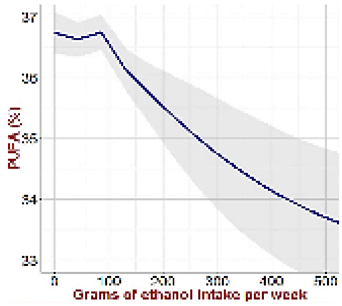
Saturated fatty acids (%)



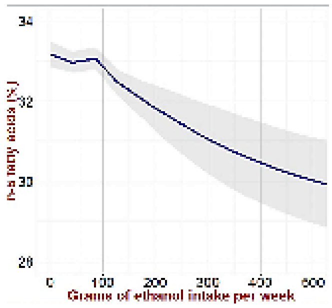
MUFA (%)



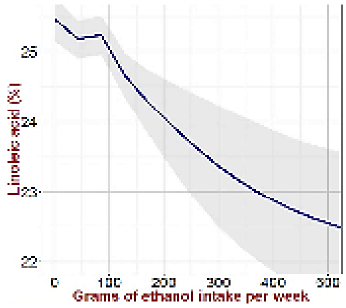
PUFA (%)



Omega-6 fatty acids (%)



Linoleic acid (%)



Omega-3 fatty acids (%)

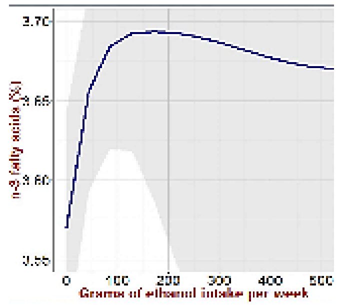
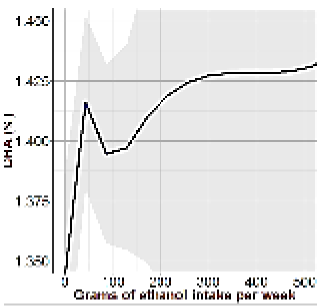
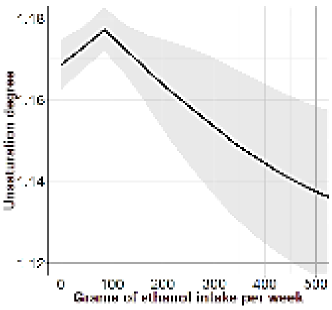


Figure S3. Continuous shape of the association between alcohol consumption and low-molecular-weight metabolites and hormonal measures. The black curves denote the shape of the association, with the grey shaded area denoting the 95% confidence interval of the fit. The association shapes were derived using local quadratic regression fitting evaluated at 25 points

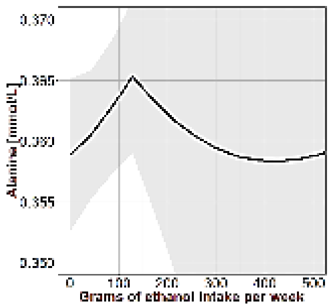
DHA (%)



Unsaturation degree



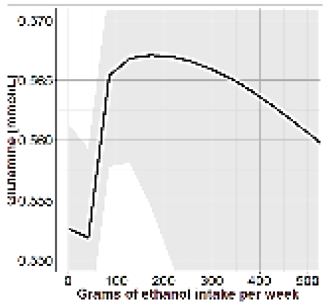
Alanine

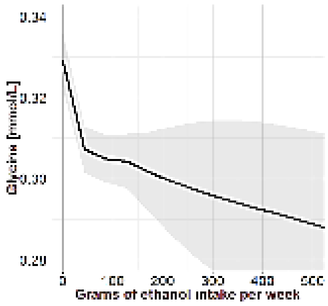


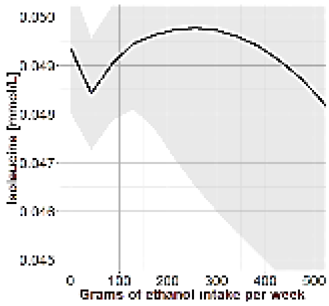
Glutamine

Glycine

Isoleucine



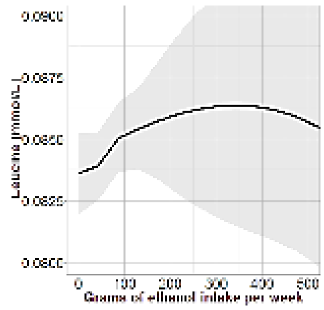


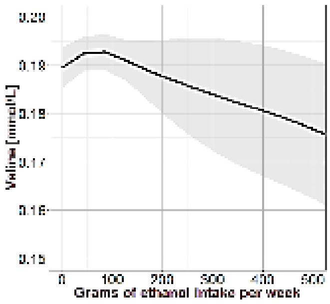


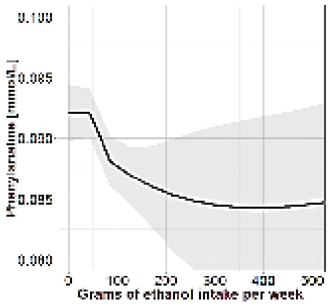
Leucine

Valine

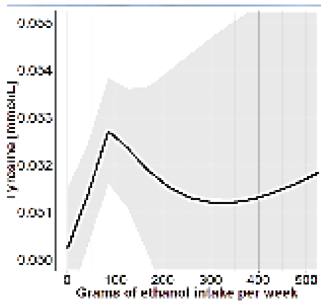
Phenylanaline





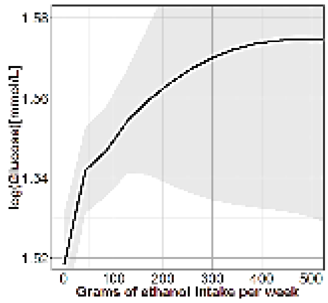


Tyrosine

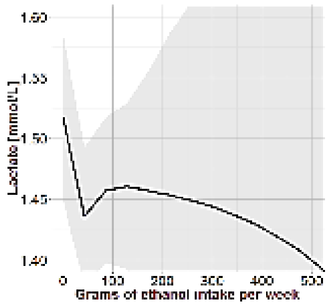


Histidine

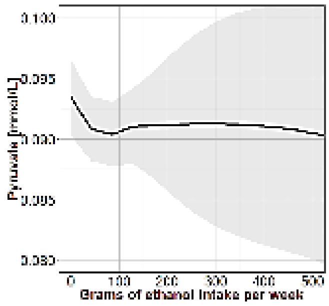
log(Glucose)



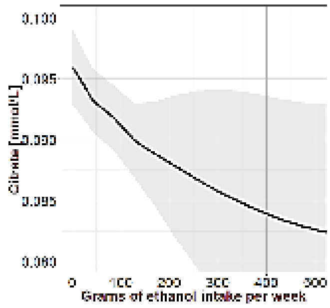
Lactate



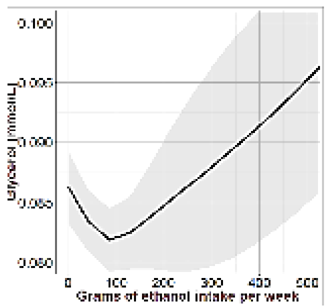
Pyruvate



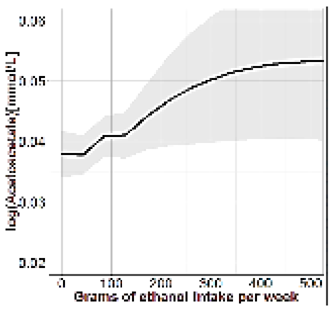
Citrate



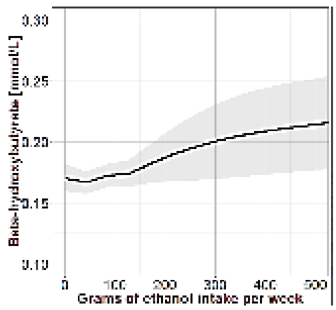
Glycerol



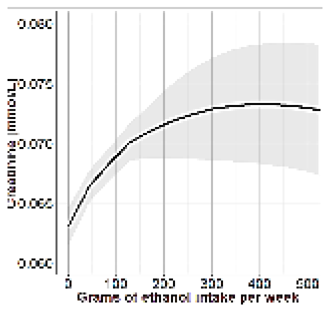
log(Acetoacetate)



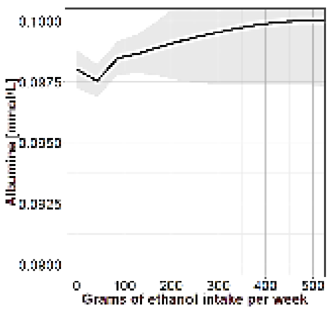
Beta-hydroxybutyrate



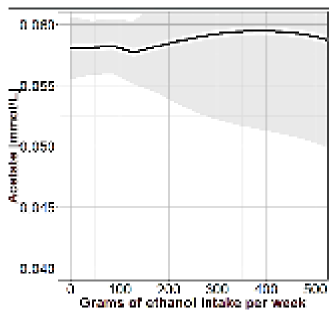
Creatinine



Albumine



Acetate



Glycoprotein acetyls

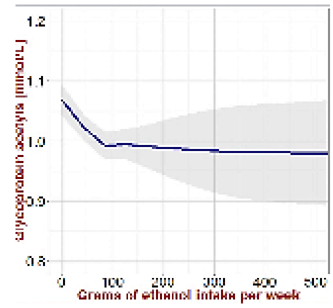
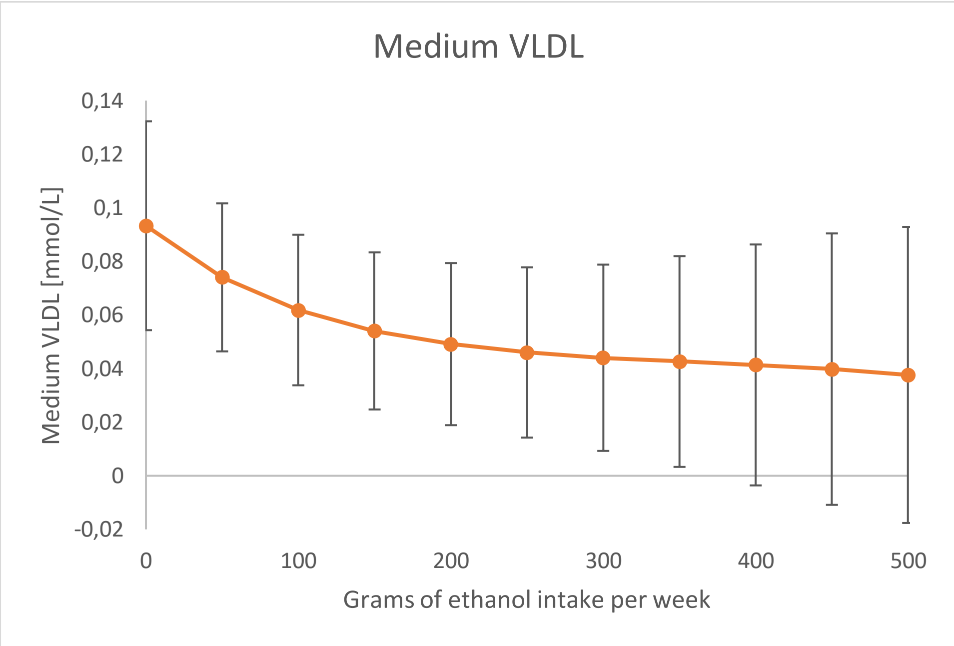
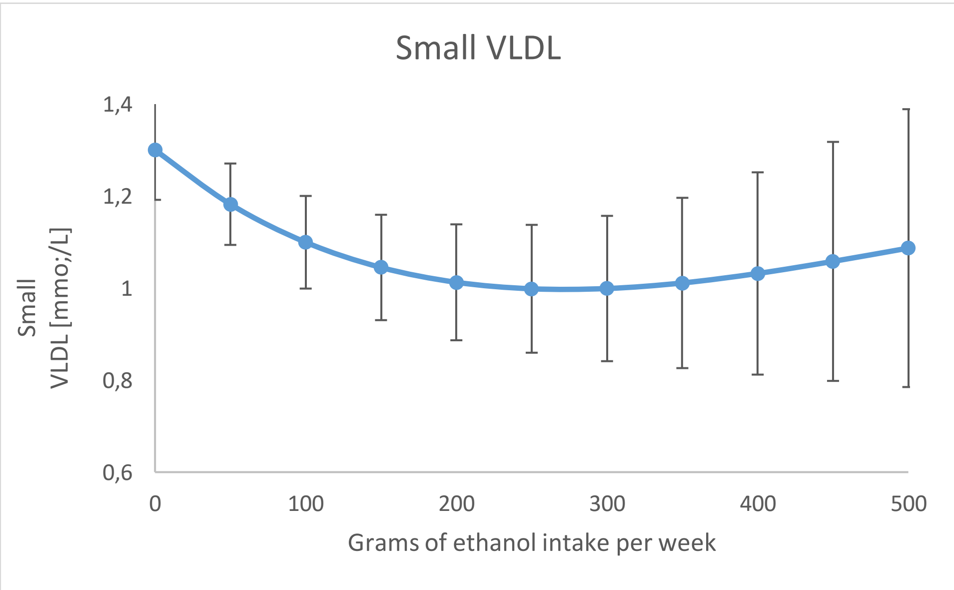
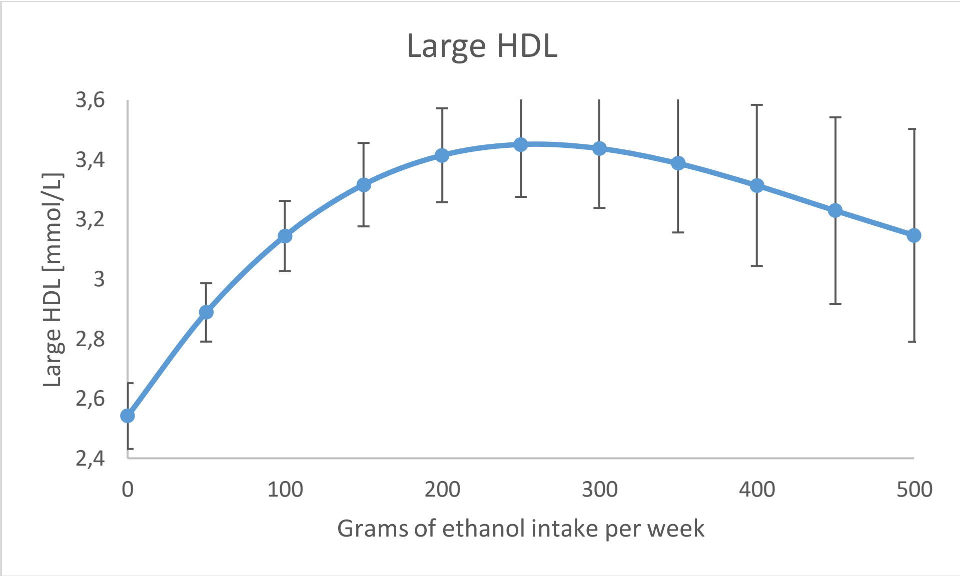
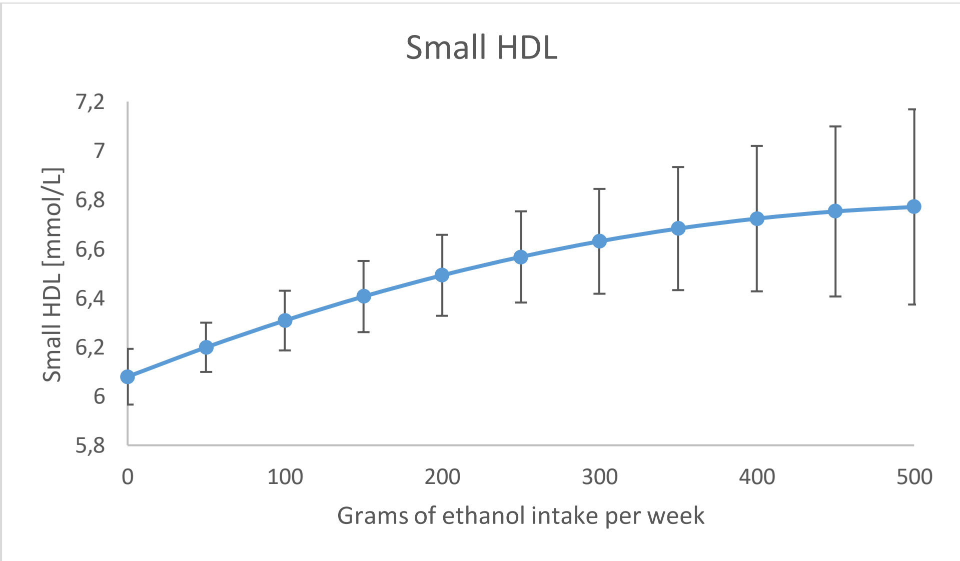

1.2
1.1
1.0
0.9
0.8
0
100
200
300
400
500
Grams of ethanol intake per week

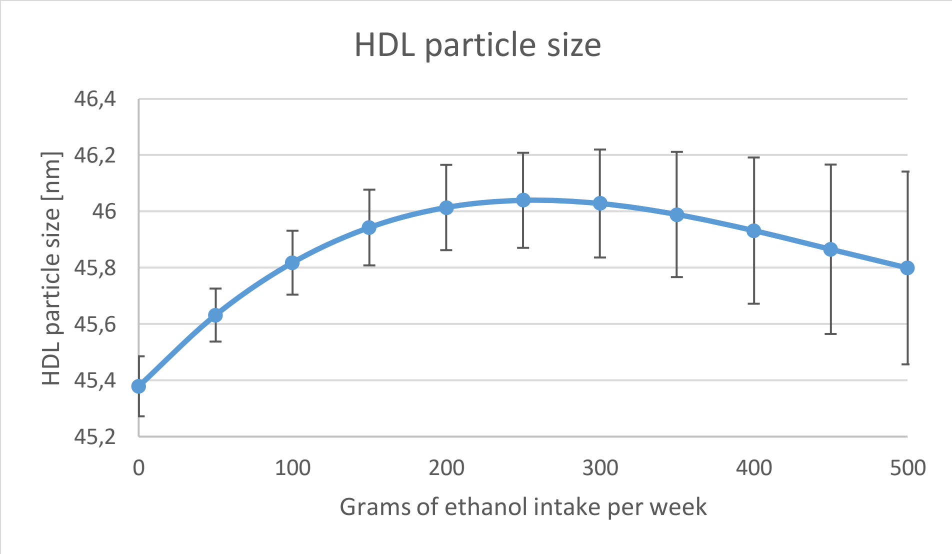
Figure S4. Continuous shape of the non-linear associations between alcohol consumption and metabolic measures. Association magnitudes in absolute concentration units are listed in Supplementary Table 2b.

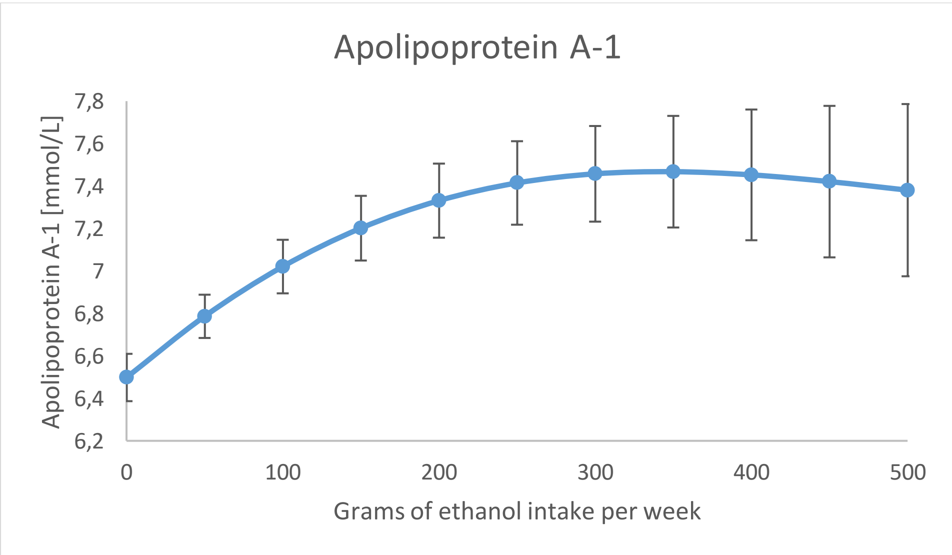


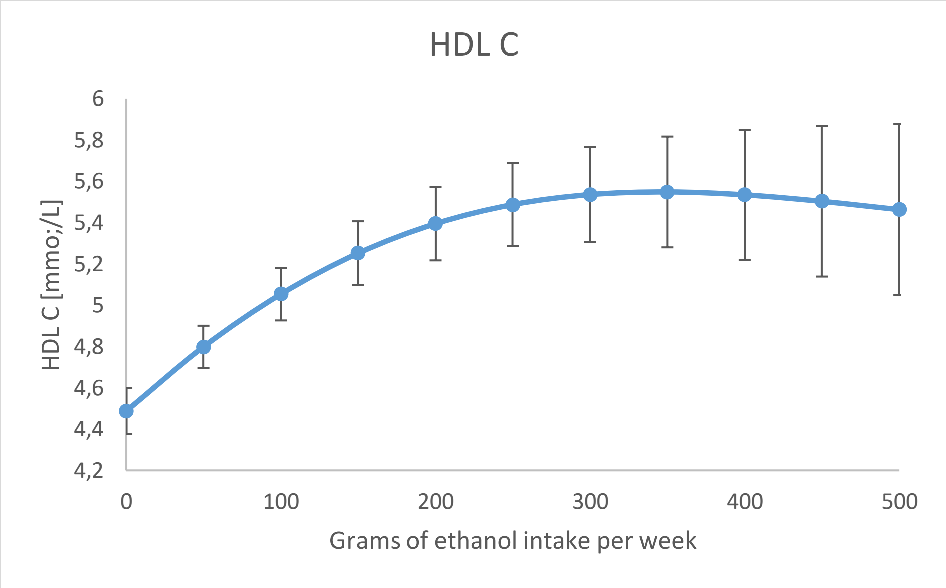


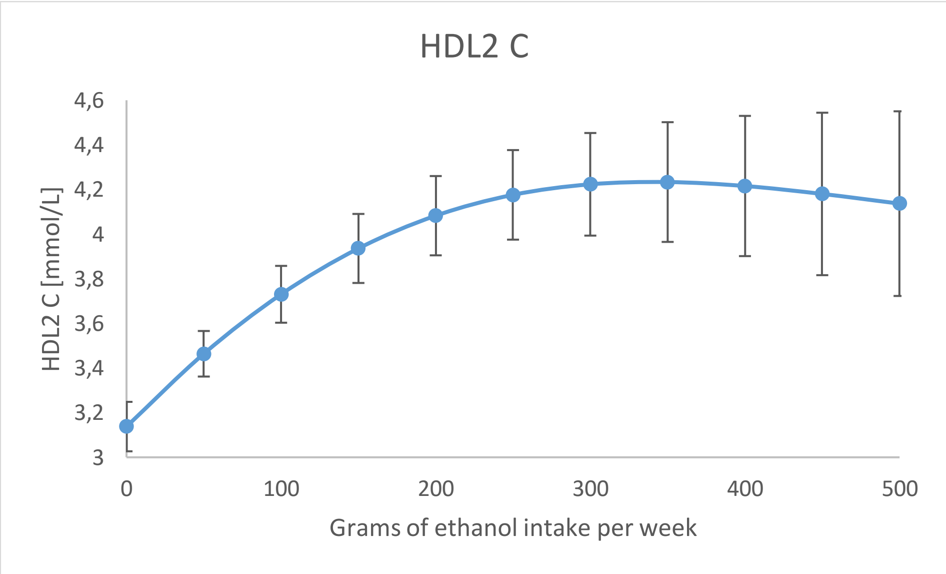

Large HDL
Large HDL [mmol/L]
3,6
3,4
3,2
3
2,8
2,6
2,4
0
100
200
300
400
500
Grams of ethanol intake per week

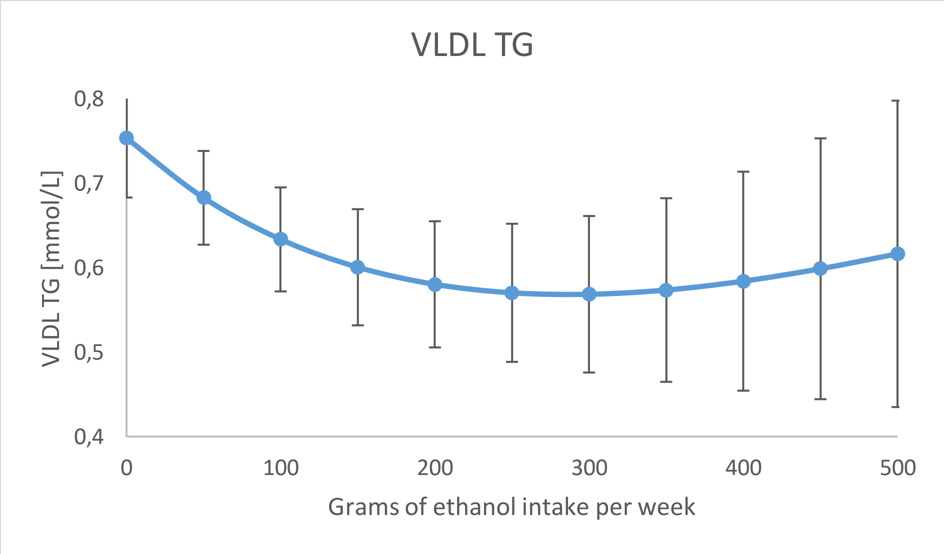

Small HDL
Small HDL [mmol/L]
7,2
7
6,8
6,6
6,4
6,2
6
5,8
0
100
200
300
400
500
Grams of ethanol intake per week

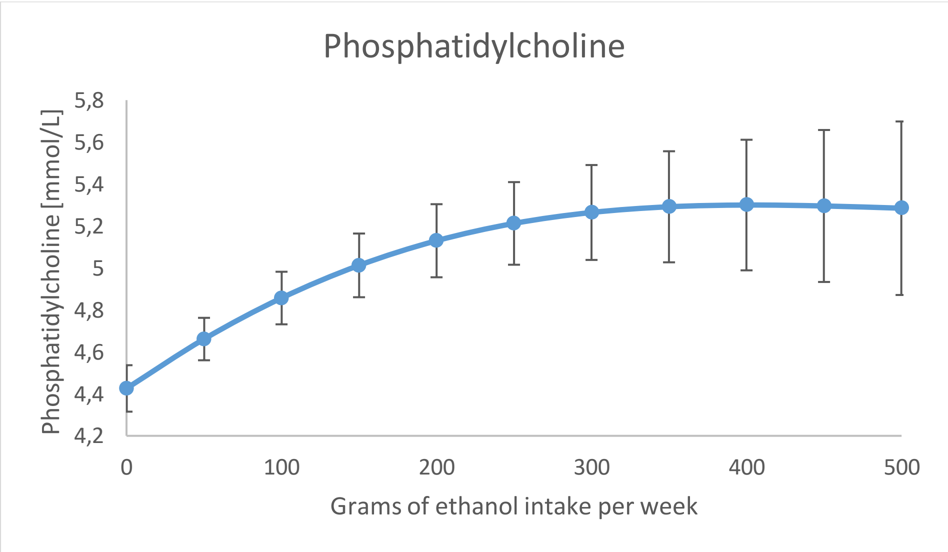

HDL particle size
46,4
46,2
46
45,8
45,6
45,4
45,2
HDL particle size [nm]
0
100
200
300
400
500
Grams of ethanol intake per week

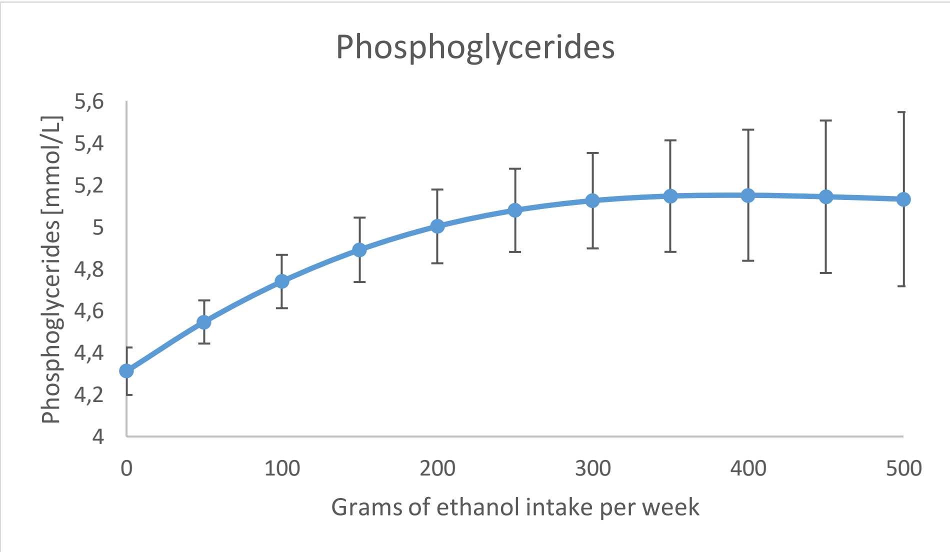

Apolipoprotein A-1
7,8
7,6
7,4
7,2
7
6,8
6,6
6,4
6,2
Apolipoprotein A-1 [mmol/L]
0
100
200
300
400
500
Grams of ethanol intake per week

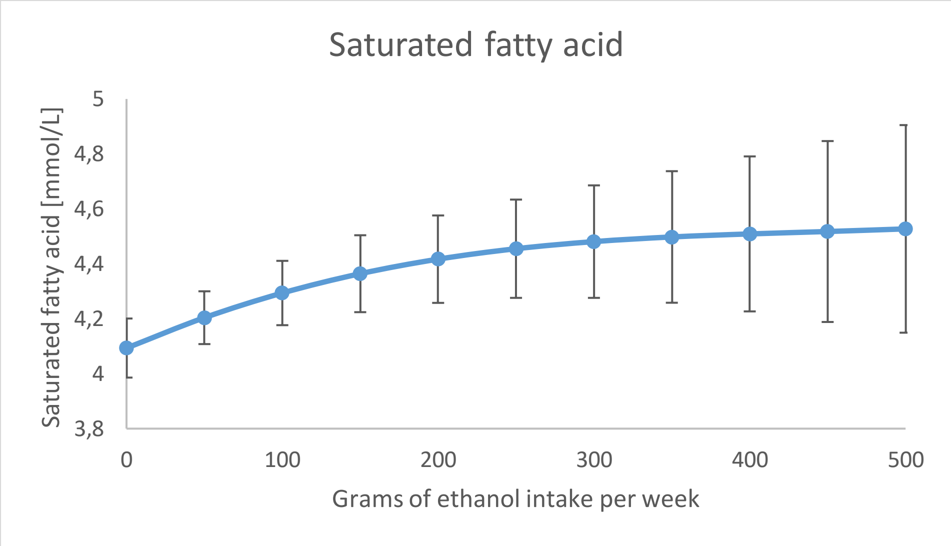

HDL C
HDL C [mmo;/L]
6
5,8
5,6
5,4
5,2
5
4,8
4,6
4,4
4,2
0
100
200
300
400
500
Grams of ethanol intake per week

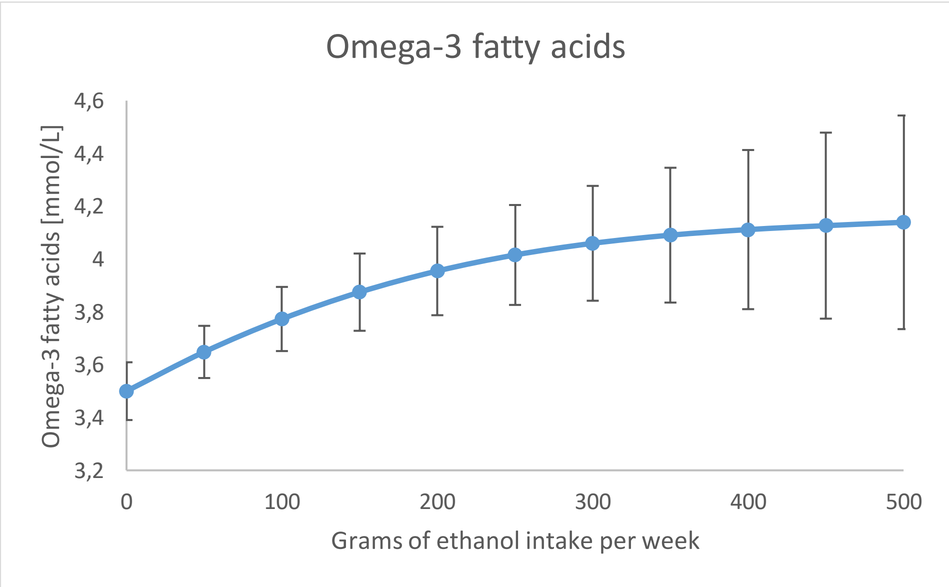

HDL2 C
HDL2 C [mmol/L]
4,6
4,4
4,2
4
3,8
3,6
3,4
3,2
3
0
100
200
300
400
500
Grams of ethanol intake per week

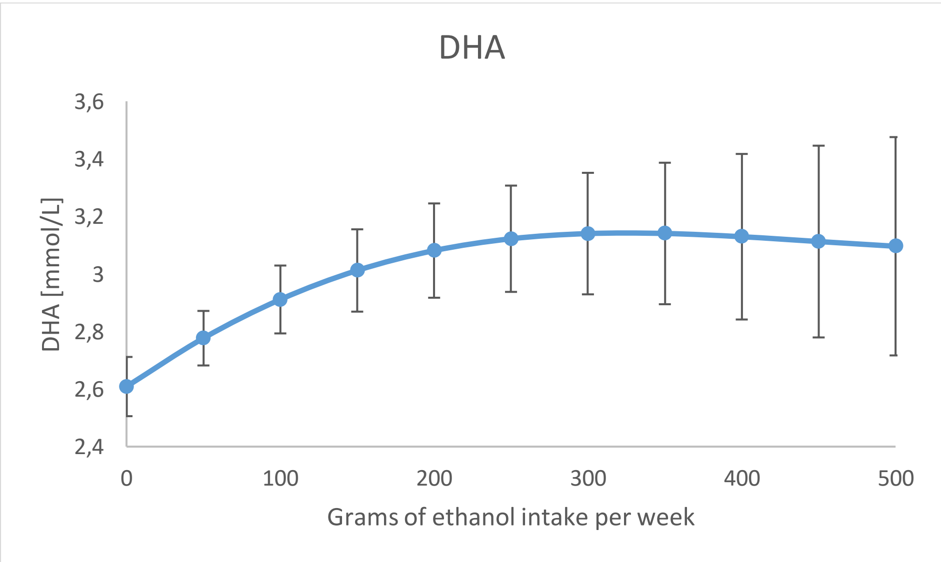

VLDL TG
VLDL TG [mmol/L]
0,8
0,7
0,6
0,5
0,4
0
100
200
300
400
500
Grams of ethanol intake per week

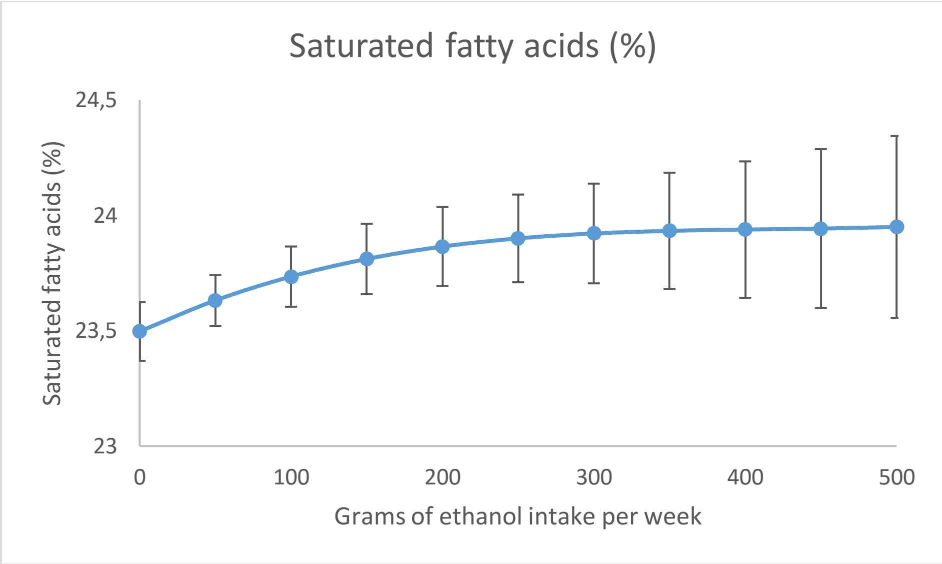

Phosphatidylcholine
Phosphatidylcholine [mmol/L]
5,8
5,6
5,4
5,2
5
4,8
4,6
4,4
4,2
0
100
200
300
400
500
Grams of ethanol intake per week

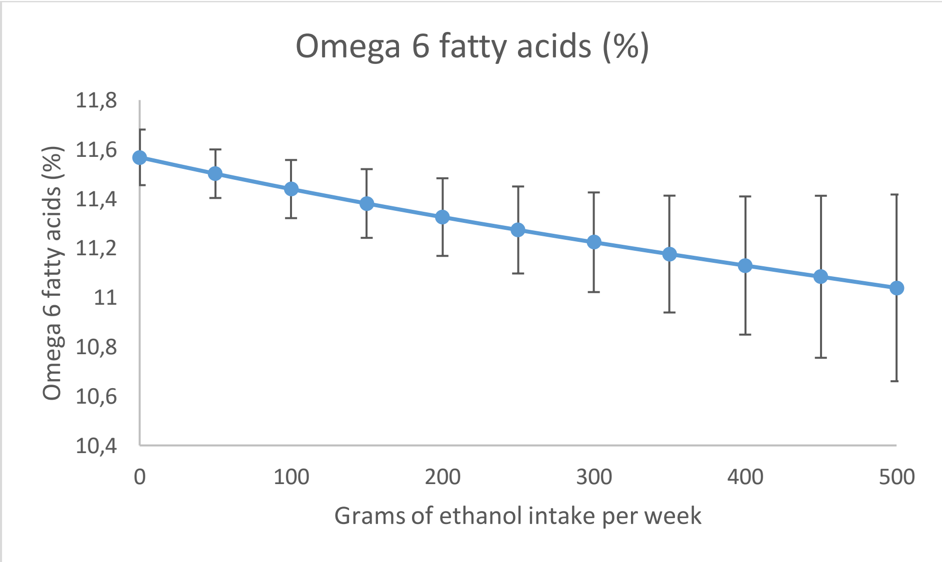

Phosphoglycerides
Phosphoglycerides [mmol/L]
5,6
5,4
5,2
5
4,8
4,6
4,4
4,2
4
0
100
200
300
400
500
Grams of ethanol intake per week

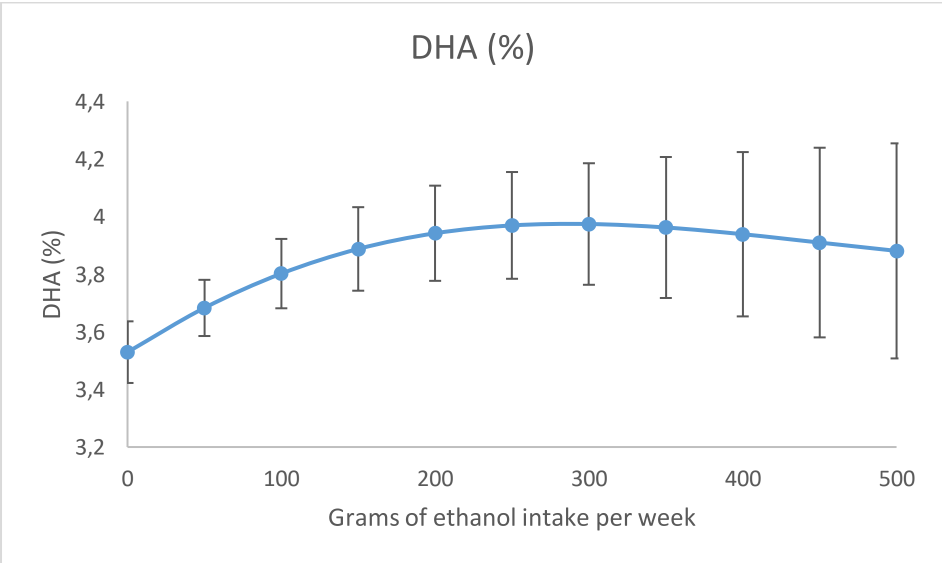

Saturated fatty acid
Saturated fatty acid [mmol/L]
5
4,8
4,6
4,4
4,2
4
3,8
0
100
200
300
400
500
Grams of ethanol intake per week

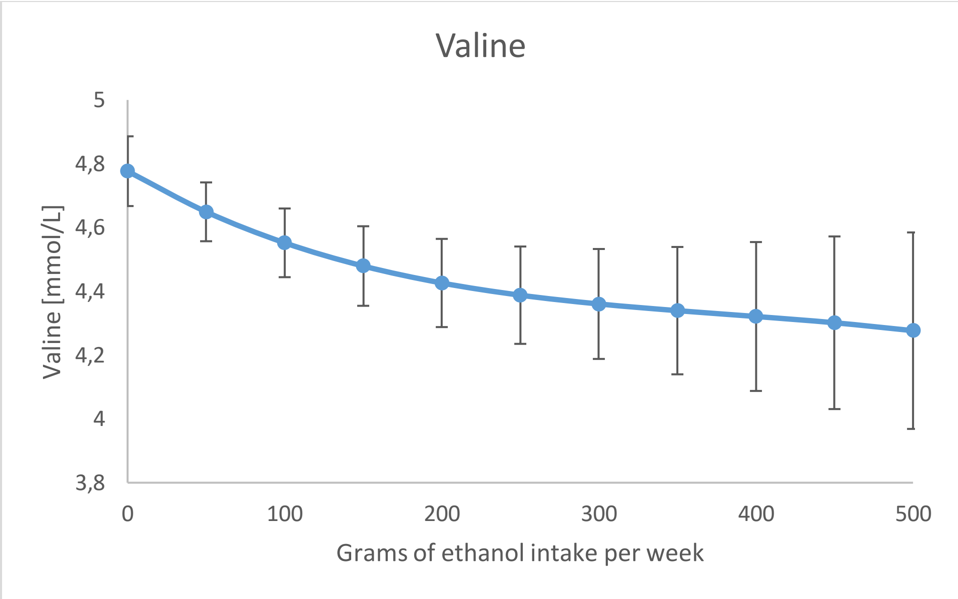

Omega-3 fatty acids
Omega-3 fatty acids [mmol/L]
4,6
4,4
4,2
4
3,8
3,6
3,4
3,2
0
100
200
300
400
500
Grams of ethanol intake per week

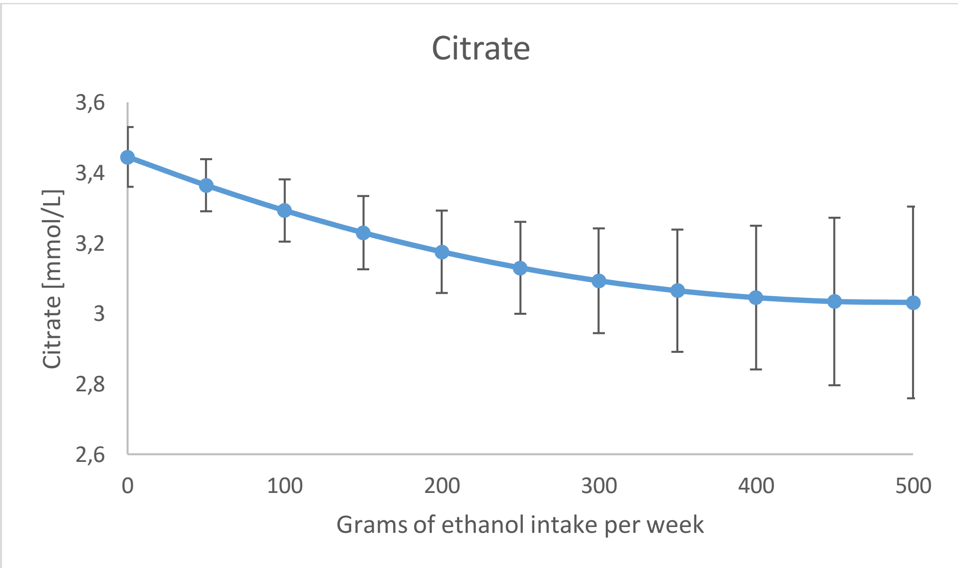

DHA
DHA [mmol/L]
3,6
3,4
3,2
3
2,8
2,6
2,4
0
100
200
300
400
500
Grams of ethanol intake per week


Saturated fatty acids (%)
Saturated fatty acids (%)
24,5
24
23,5
23
0
100
200
300
400
500
Grams of ethanol intake per week


Omega 6 fatty acids (%)
Omega 6 fatty acids (%)
11,8
11,6
11,4
11,2
11
10,8
10,6
10,4
0
100
200
300
400
500
Grams of ethanol intake per week


DHA (%)
DHA (%)
4,4
4,2
4
3,8
3,6
3,4
3,2
0
100
200
300
400
500
Grams of ethanol intake per week


Valine
5
4,8
4,6
4,4
4,2
4
3,8
Valine [mmol/L]
0
100
200
300
400
500
Grams of ethanol intake per week


Citrate
3,6
3,4
3,2
3
2,8
2,6
Citrate [mmol/L]
0
100
200
300
400
500
Grams of ethanol intake per week

References

1. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Medicine and science in sports and exercise. 2003;35(8):1381-95.

2. Withers RT, Davies GJ, Crouch RG. A comparison of three W170 protocols. European journal of applied physiology and occupational physiology. 1977;37(2):123-8.

3. Quan HL, Blizzard CL, Sharman JE, Magnussen CG, Dwyer T, Raitakari O, et al. Resting heart rate and the association of physical fitness with carotid artery stiffness. American journal of hypertension. 2014;27(1):65-71.

4. Smith KJ, McNaughton SA, Gall SL, Otahal P, Dwyer T, Venn AJ. Associations between Partnering and Parenting Transitions and Dietary Habits in Young Adults. Journal of the Academy of Nutrition and Dietetics. 2017;117(8):1210-21.

5. National Health and Medical Research Council. Dietary Guidelines for Australian Adults. Canberra2003.

6. Kellet E, Smith A, Schmerlaib Y. The Australian Guide to Healthy Eating. In: Sevices CDoHaF, editor. Canberra1998.

7. Haro JM, Arbabzadeh-Bouchez S, Brugha TS, de Girolamo G, Guyer ME, Jin R, et al. Concordance of the Composite International Diagnostic Interview Version 3.0 (CIDI 3.0) with standardized clinical assessments in the WHO World Mental Health surveys. International journal of methods in psychiatric research. 2006;15(4):167-80.

8. Burke GL, Hunter SM, Croft JB, Cresanta JL, Berenson GS. The interaction of alcohol and tobacco use in adolescents and young adults: Bogalusa Heart Study. Addictive behaviors. 1988;13(4):387-93.

9. Shuval K, Finley CE, Chartier KG, Balasubramanian BA, Gabriel KP, Barlow CE. Cardiorespiratory fitness, alcohol intake, and metabolic syndrome incidence in men. Medicine and science in sports and exercise. 2012;44(11):2125-31.

10. Baumeister SE, Finger JD, Glaser S, Dorr M, Markus MR, Ewert R, et al. Alcohol consumption and cardiorespiratory fitness in five population-based studies. European journal of preventive cardiology. 2017:2047487317738594.

11. Perreault K, Bauman A, Johnson N, Britton A, Rangul V, Stamatakis E. Does physical activity moderate the association between alcohol drinking and all-cause, cancer and cardiovascular diseases mortality? A pooled analysis of eight British population cohorts. Br J Sports Med. 2017;51(8):651-7.

12. Soedamah-Muthu SS, De Neve M, Shelton NJ, Tielemans SM, Stamatakis E. Joint associations of alcohol consumption and physical activity with all-cause and cardiovascular mortality. The American journal of cardiology. 2013;112(3):380-6.