The metabolomic signatures of alcohol consumption in young adults

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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 metabolites, (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 and different types of alcohol consumption. 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: Alcohol consumption in young adults is related to a range of metabolites associated with benefits and harms to health. Associations with metabolites were similar for total and types of alcohol. There were some differences between our results and the only previous study.

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

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Introduction

Recent studies have contradicted the previous evidence of the benefits of low-to-moderate consumption of alcohol for reducing cardiovascular disease (CVD) risk [1]. The emerging technology of metabolomic analysis provides a snapshot of systemic metabolism that 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 a host 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, revealing that higher alcohol consumption was associated with higher HDL cholesterol, monounsaturated fatty acids, but lower omega-6 fatty acids, glutamine, citrate and lipoprotein particle size in three cohorts using an NMR platform [2]. Most of these biomarkers have been shown to be associated with inreased rik of CVD events [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 potentially 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 different types of alcoholic beverages (beer and wine) with metabolites; and (3) consider covariates not previously examined (e.g. diet, cardiorespiratory fitness and mental health) in associations between metabolic profiles and alcohol consumption.

Methods

Participants

Participants were from the 2004-2006 Childhood Determinants of Adult Health (CDAH) study, a twenty year follow-up of the Australian Schools Health and Fitness Survey (ASHFS) conducted in 1985 [4]. In total, 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 original 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 from 2004 to 2006 when aged between 26 and 36 years (herein refer to this study) [4]. Of these participants, 2,863 (74%) had data available for alcohol consumption completed by questionnaires, gave a fasting blood sample, and had metabolomic profile measured by a serum NMR platform. A detailed description of the cohort has been published elsewhere [5]. The flow of participants from baseline to follow-up is described in the Supplemtary material 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

Each participant reported his or her 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 or full strength beer; red, white and 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 drinking occasion. We estimated alcohol consumed per day for each beverage type by multiplying the

frequency of drinking by the estimated grams of alcohol for each beverage type: beer (sum of light beer, medium beer and full strength beer) and wine (sum of 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 [6].

Metabolomics

Serum fasting blood samples were stored at -80ºC for 11-13 years. The Computational Medicine metabolomic platform used high-throughput serum nuclear magnetic resonance (NMR) spectroscopy to quantify 223 key metabolic markers [7]. As per previous studies, we focused on 73 metabolic measures covering major biological pathways [7].

Covariates

Full details of the measurement of covariates are provided in the supplement. In brief, covariates were: age, sex, socio-economic status (SES) as quartile based 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), smoking status (never, former, or current), total physical activity (minutes per week), cardiorespiratory fitness (CRF) (PWC170 uncorrelated with lean body mass) [8], diet quality (Dietary Guideline Index, DGI) [9] and depression and anxiety diagnosis in the previous 12 months (Composite International Diagnostic Interview, CIDI) [10].

Statistical analysis

We used multivariable linear regression models to examine associations between alcohol consumption as the explanatory variable and each metabolic measure as the outcome (β coefficients and 95% CIs). Alcohol consumption was examined as 1) total alcohol

consumption (grams per week) and 2) by beverage type (beer or wine). All metabolic variables were scaled to standard deviation units and those with skewed distributions were log transformed before analysis. Results are presented graphically with numerical results also provided in the supplement.

The continuous shape of the significant linear metabolic pathways associated with alcohol consumption were examined graphically using local quadratic regression fitting, with each smoothing function segment evaluated at 25 points through the range of alcohol intake. More complex shapes were further examined using polynomial regression models to obtain the standard deviation changes of metabolite measures with log transformation when needed.

Potential covariates were included in the models in accordance with purposeful model building procedures [11]. 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). These covariates has been shown to be associated with alcohol consumption [12-14], jointly contribute mortality and morbidity from a range of diseases [15, 16].

The following sensitivity analyses were performed: (1) using total alcohol consumption in grams per day instead of grams per week, and (2) to test whether drinking alcohol the day before the blood test influenced the results.

We adjusted for multiple stastical testing using the number of principal components that explained over 99% variance of the metabolomic data to determine the independent number of tests [2]. As 36 principal components were identified the corrected significance threshold was $P \leq 0.002$ (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

The study population consisted of 1,785 participants in the cohort who had complete data on alcohol consumption and metabolomic measures (Table 1). Participants with and without metabolomics data had similar characteristics (Table 1).

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 the fully adjusted model, alcohol consumption per 100 grams per week was strongly associated with higher lipid concentrations for all high-density lipoproteins (HDL) subclasses, particularly for the medium-sized and large HDL particles. Concurrently, the HDL cholesterol, apolipoprotein A-1, phosphoglycerides and phosphatidylcholine concentrations were robustly elevated in relation to 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 as 10 grams of alcohol per day instead of the weekly basis (Supplementary Figure 5).

In the fully adjusted model, beer consumption and wine consumption were positively associated with all HDL concentrations including large, medium and small-sized particle, HDL particle size, apoliporotein A-1, HDL cholestetol, phosphatidylcholine, and phosphoglycerides concentration while inversely associated with apoliporotein B, remnant

cholesterol, VLDL cholesterol. There was evidence that beer consumption was associated with larger increases in lipid concentrations in the large, medium and small HDL subclasses, HDL particle size, HDL cholesterol, apolipoprotein A-1, phosphoglycerides and phosphatidylcholine concentrations compared to consumption of wine or total alcohol consumption.

When further examining the shape of the associations, 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 with higher 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), monounsaturated fatty acids (MUFA), omega-3 FA, DHA in absolute concentrations, and higher proportion of saturated FA, 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, 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) caused most of the changes which increase the magnitudes of the associations (Supplementary Table 3).

In the fully adjusted models, similar results were observed with beer consumption and wine consumption and fatty acids measures. There was evidence that consumption of beer led to a larger increase of the magnitudes in associations with total FA, saturated FA, MUFA, PUFA, omega-6 FA, omega-3 FA, DHA and a larger decrease in associations with PUFA ratio, omega-6 FA ratio, linolenic acid ratio to total FA compared to consumption of wine or total alcohol consumption.

Similar results were observed when the standard deviation differences of metabolite concentrations were compared per 10 grams of alcohol per day instead of the weekly basis (Supplementary Figure 6).

When examining the shapes of these associations, most were linear across the range of alcohol consumption. Inverse linear associations were observed in the measures of omega-6 fatty acid ratio, linoleic acid ratio, PUFA to total fatty acids. In contrast, positive linear associations were observed in the measures of omega-3 FA, DHA or largely positive in measures of total FA, saturated FA, MUFA, PUFA, omega-6 FA concentrations, saturated FA 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 between alcohol and low-molecular-weight metabolites 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 which increase the magnitude of the associations compared to

the unadjusted model (Supplementary Table 3). While most of the small molecular metabolites were not strongly associated with alcohol consumption based on the linear models, subtle non-linear associations were evident for several measures, e.g. phenylanaline (Supplementary Table 2 and Figure S3). Similar results were observed when the standard deviation differences of metabolite concentration were compared per 10 grams of alcohol per day instead of the weekly basis (Supplementary Figure 7).

In the fully adjusted model, similar results were observed with beer consumption and wine consumption.

Discussion

The metabolomic signatures of this young adult cohort revealed diverse molecular processes 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 of 9,778 young adults 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, appeared to increase 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 the associations between alcohol consumption and lipid and lipoprotein measures were mostly consistent with the only previous study on alcohol consumption and metabolomic profiling in young adults [2]. This included that alcohol consumption was positively associated with several lipid and lipoprotein 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.

Our findings that alcohol consumption was not associated with serum total triglycerides, triglycerides in HDL contrast to the previous study [2]. A potential explanation for this disparity is the differences in the populations in determinants of trigylcerides (e.g. diet and body weight [17]), however, we adjusted for these and no associations were evident in unadjusted analyses. This suggests that further examination is needed to confirm the associations between alcohol consumption and triglycerides.

We found that beer and wine had associations with lipids and lipoproteins that were similar to total alcohol consumption. There was some evidence that consumption of beer was associated with larger increases in lipid concentrations in the 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 people that drink beer as these factors accounted for large increases in the associations, suggesting negative confounding. These findings suggest that common components of alcohol may affect lipids noting a meta-analyses showing similar J-shaped associations between beer and wine consumption with cardiovascular events [18].

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 [19, 20]. Alcohol may influence HDL cholesterol through cholesteryl ester transfer protein (CETP) activity [21], in combination with increased transport rate of apolipoproteins [22], reduced hepatic lipase activity [23]. In turn, HDL cholesterol moves excess cholesterol molecules from peripheral cells to the liver [24]. Alcohol was associated with higher apolipoprotein A-1 concentration mainly due to the increase of A-1 lipoprotein particle, which has been suggested to represent the antiatherogenic fraction of HDL [25]. 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, such as phosphoglycerides and phosphatidylcholine [26], to progression of coronary heart disease [27]. These conflicting effects of alcohol consumption on lipids and lipoproteins coupled with recent findings on metabolic markers differentially predicting myocardial infarction and stroke [28] may explain some of the conflicting effects of alcohol on risk of 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, 29], with mostly adverse effects in relation to cardiovascular

risk. While total FA, saturated FA, MUFA, omega-3 FA concentrations, saturated FA 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 these fatty acids measures support higher risks of some cardiovascular disease apparently associated with alcohol consumption in recent studies [1] when considered alongside studies of metabolites and risk of cardiovascular events [28].

Beer and wine showed similar associations to total alcohol consumption with fatty acids. There was, again, evidence that consumption of beer led to a larger increase of the magnitudes of the associations with a range of measures compared to consumption of wine or total alcohol consumption, which might be due to residual confounding despite efforts to adjust for covariates.

We found that demographic factors including sex, age, SES status and other health behaviours including smoking, diet, physical activity and cardiorespiratory fitness, but not mental health accounted for significant 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 [30]. Saturated FA increase low-density lipoprotein (LDL) cholesterol, potentially increasing risk for CVD [31]. While dietary MUFAs protective against CVD [32-35], MUFA ratio to total fatty acids is a biomarker of higher cardiovascular and diabetes risk [3, 36]. Likewise, the robust association of alcohol intake with lower proportion of omega-6 fatty acids has been related to higher cardiometabolic risk [3, 29, 36],

noting recent findings with different effects on myocardial infarction and stroke by the influence of circulating triglycerides levels [28]. On the other hand, omega-3 FA concentrations have been associated with lower cardiovascular risk [37-39]. Within the omega-3 series, the long-chain docosahexaenoic acid (DHA) are also associated with decreased coronary events, whereas the role of linolenic acid is less clear [40, 41]. 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 later in life.

Associations of alcohol with low-molecular-weight metabolites

In line with the previous finding, citrate and phenylalanine were strongly inversely associated with alcohol consumption, while it was not associated with glutamine. A strong linear association was observed for citrate, while phenylalanine showed a non-linear shape where the slope of the association initially declined 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 its effects on enzymes in oxidative pathways such as the citric acid or glyoxylate cycle that bypasses part of the citric acid cycle, including succinate dehydrogenase [42]. The effects of alcohol on phynylalanine might be related to the production of the 2-phenylethyl alcohol which is found in fresh beer or other volatiles such as ethyl alcohol [43]. In turn, higher citrate levels have been linked with modestly lower risk for cardiovascular diseases [3, 44]. 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 and metabolic risk related to higher alcohol consumption.

Strengths and limitations

The strength of our study is that we comprehensively examined potential linear and non-linear relationships of individual metabolite measures and alcohol consumption including different types of alcohol (noting the limited power for spirit consumption due to its infrequent consumption). Furthermore, several sensitivity analyses were performed showing the robustness of our findings. In addition, we took multiple confounding factors into account in the analysis, including diet, cardiorespiratory fitness, and mental health that were not examined in previous studies.

Our study has several limitations. The cross-sectional analyses mean we cannot confirm casual associations between alcohol consumption and metabolites. Our young and relatively 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 [45]. We had substantial loss to follow-up since childhood, which may affect the generalisability of our findings to other populations. However, a comparison of the CDAH sample without the metabolomics data showed similar characteristics. Furthermore, the proportion of current drinkers in this study was very similar to that in the general Australian population [46].

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.

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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)

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.

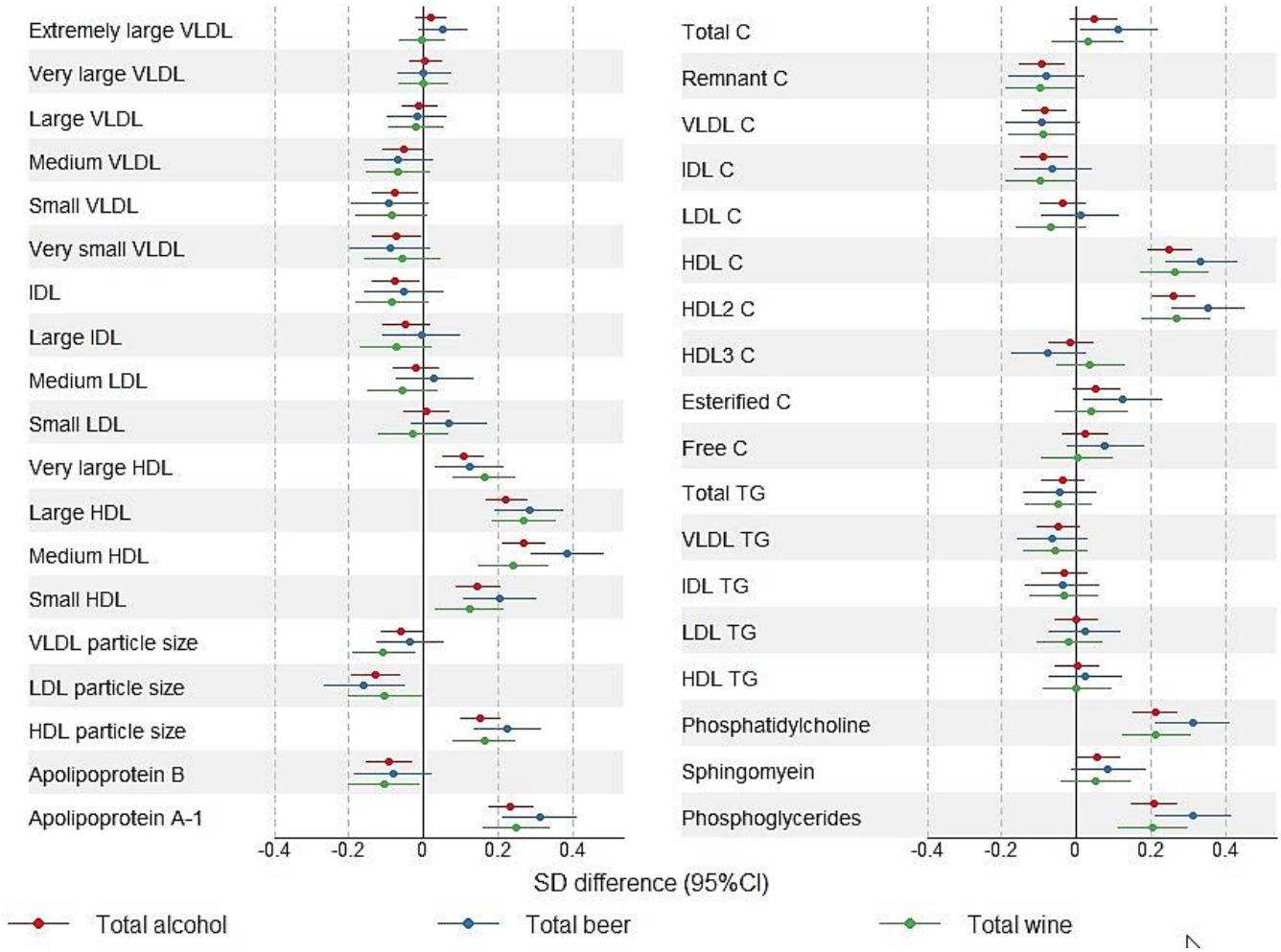

Figure 1. Cross-sectional associations between alcohol consumption as total alcohol, beer and wine consumed and lipoprotein lipid measures. All association were adjusted for age, sex, region of residence, SES, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory 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 Table 2a and continuous shapes of the metabolic associations with alcohol intake are shown in Supplementary Figure S1.

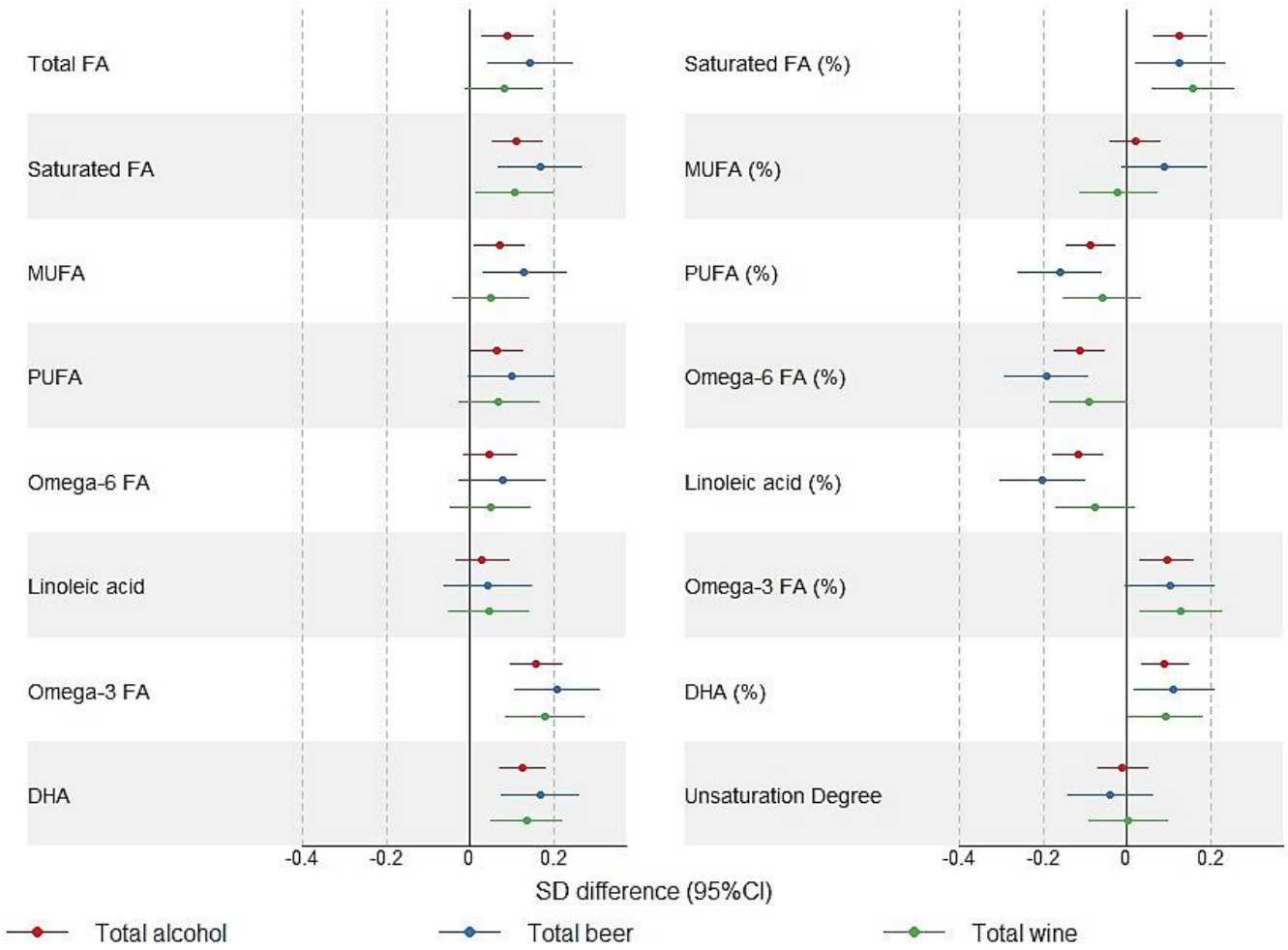

Figure 2. Cross-sectional associations between alcohol consumption as total alcohol, beer and wine consumed and fatty acids. All association were adjusted for age, sex, region of residence, SES, educational level, occupation, marital status, smoking, diet quality, physical activity, cardiorespiratory 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 2a and continuous shapes of the metabolic associations with alcohol intake are shown in Supplementary Figure S2.

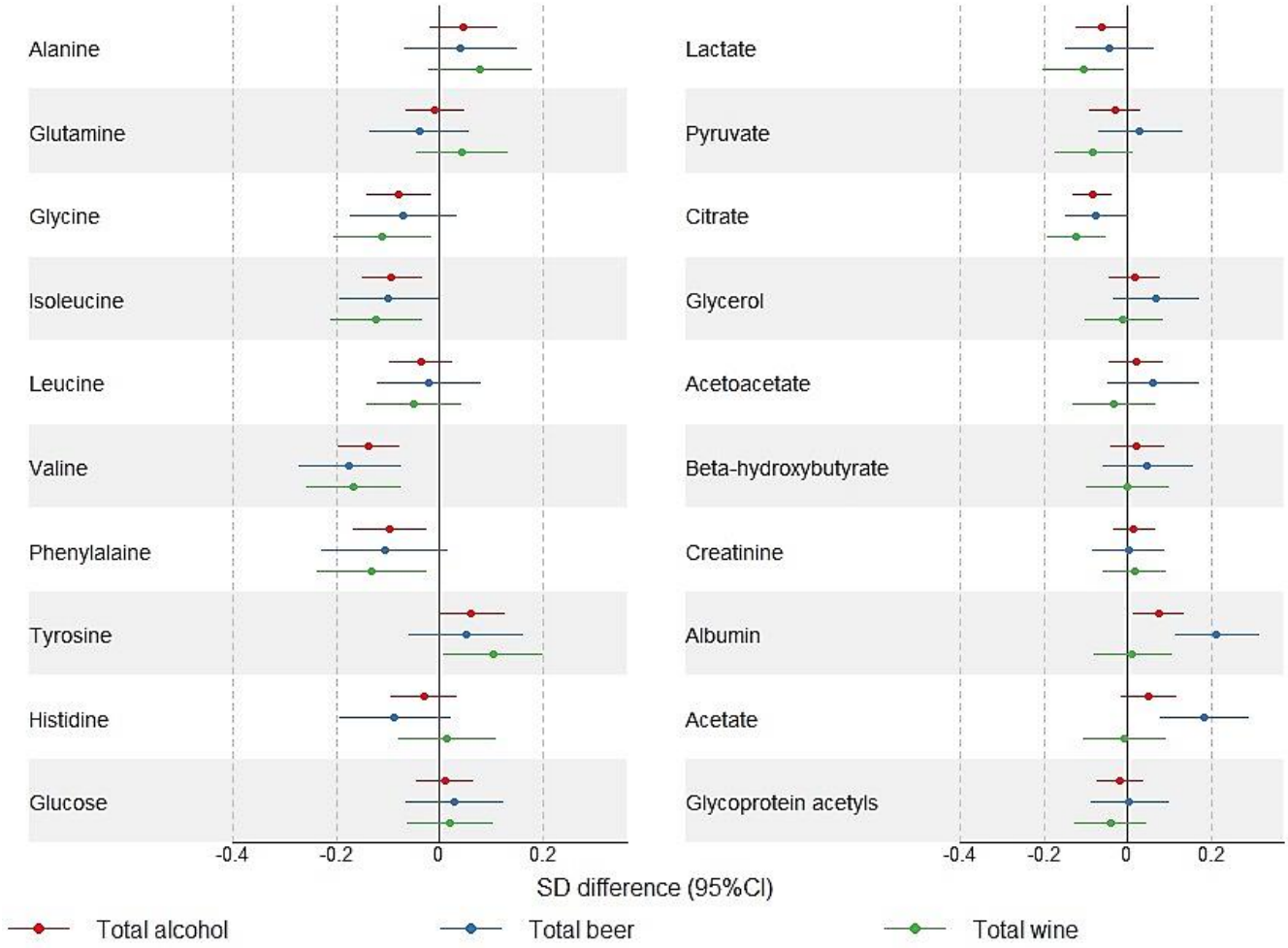

Figure 3. Cross-sectional associations between alcohol consumption as total alcohol, beer and wine consumed and low-molecular-weight metabolites. All association were adjusted for age, sex, region of residence, SES, educational level, occupation, marital status, smoking, dietary intakes, physical activity, 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 2a and continuous shapes of the metabolic associations with alcohol intake are shown in Supplementary Figure S3.