Roosa Kosonen

EFFECTS OF ALCOHOL CONSUMPTION ON SERUM LIPIDOME - THE CARDIOVASCULAR RISK IN YOUNG FINNS STUDY

Syventävien opintojen kirjallinen työ

Kevätlukukausi 2024

Roosa Kosonen

EFFECTS OF ALCOHOL CONSUMPTION ON SERUM LIPIDOME – THE CARDIOVASCULAR RISK IN YOUNG FINNS STUDY

Sydäntutkimuskeskus

Kevätlukukausi 2024

Vastuuhenkilö: Juha Mykkänen

TURUN YLIOPISTO
Lääketieteellinen tiedekunta

KOSONEN, ROOSA: Effects of alcohol consumption on serum lipidome – The Cardiovascular Risk in Young Finns Study

Syventävien opintojen kirjallinen työ, 31 s.,7 liites.
Sydäntutkimuskeskus
Tammikuu 2024

There is an association between alcohol consumption and clinical lipid markers but lipidomidome-wide connections are currently unclear. Aim of this study was to study cross-sectional association of alcohol consumption with serum lipidome in a population cohort of 1969 men and women aged 30-45 years. The study subjects were participants of the Cardiovascular Risk in Young Finns Study.

The Cardiovascular Risk in Young Finns Study is an on-going follow-up study of atherosclerosis precursors of Finnish children and adolescents. The first cross-sectional survey was conducted in 1980, when 3596 participants, were randomly chosen in each university hospital area in Finland from the national population register. 2204 of these individuals were re-examined in 2007, then aged 30-45 years. Individuals with type 2 diabetes, subjects with missing risk factor data and outliers of glucose and daily amount of drinks were excluded from the present analyses. Therefore, the present study cohort included 1969 subjects. Serum lipidome analysed by using liquid chromatography-tandem mass spectrometry. The statistical tests were performed with Statistical Analysis System version 9.4. Multiple testing corrections were performed using the Bonferroni method.

After Bonferroni correction alcohol intake was found to be significantly associated with concentrations of 195 out of 437 metabolites, after adjusting age and sex. Acylcarnitines, ceramides, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols and triacylglycerols were positively correlating with alcohol consumption. Previous studies have reported similar results but nothing in this scale. More research taking into account additional confounding factors are needed to clarify whether some of these lipids could be used as potential biomarkers for alcohol risk use.

Avainsanat: Alkoholi, lipidomiikka

TABLE OF CONTENTS

1. INTRODUCTION

Coronary heart disease (CHD) is a slowly developing chronic disease, which is mainly caused by atherosclerosis. Atherosclerosis is a progressive inflammatory process which narrows the blood vessels that supply of the myocardium with oxygenated blood. Older age, male gender, smoking, hypertension, insulin resistance and diabetes, hyperlipidemia, physical inactivity and obesity have been identified as independent risk factors of CHD. (Wirtz & von Känel, 2017.) Also, alcohol consumption is associated with elevated plasma lipids and cardiovascular disease due to increased secretion of very low-density lipoprotein (LDL) from liver, impaired lipolysis and increased hepatic delivery of free fatty acids from adipose tissue (Rosales et al. 2020).

Lipids are group of organic compounds which have many biological functions. Lipids can act as structural components of cell membrane, participate in signalling pathways, and serve as energy storage sources. Lipids have been broadly divided in to simple and complex groups. Simple group includes lipids that yield at most two types of distinct entities upon hydrolysis. Acylglycerols are an example of this group, it degrades to fatty acids and glycerol. Complex group includes lipids that yield three or more types of distinct entities upon hydrolysis. Glycerophospholipids are an example of this group, it degrades to fatty acids, glycerol, and head group. (Fahy et al., 2011.)

Circulating blood lipoproteins are consisted of various amounts of triglycerides (TAGs), cholesterol (C), phospholipids and apolipoproteins (Apos). LDL-C is associated with higher risk of CHD and ApoB is the major structural protein of it. High-density lipoprotein cholesterol (HDL-C) have many antiatherogenic functions and it is strongly and inversely associated with the risk of CHD. The major structural protein of HDL particle is ApoA1. ApoB:ApoA1-ratio is then used as a surrogate marker of the risk of CHD related to lipoproteins. (Muscella et al., 2020.)

LIPID MAPS (LIPID Metabolites and Pathways Strategy, www.lipidmaps.org/) was created in 2003 to provide access example to lipid nomenclature and serving the international lipid research community. They have been focused on depositing data in a database for the global research community. The result was a database of classified structures which lead to a lipid classification and structural representation system. (O'Donnell et al., 2019.) In this system lipids are divided into eight categories which are fatty acyls, glycerolipids,

glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides. Each of these categories have subclasses. (Fahy et al., 2005.)

1.1 Definition of lipidomics

Terminology ”-omics” is derived from the Latin suffix “ome” meaning many or mass. Primary omics sciences contain the measurement of parameter related to mRNA, genes, proteins, or metabolites. Lipidomics is a major subdiscipline of metabolomics because the units it studies are metabolites. These metabolites are a special type and that is why it deserves to be studied as a special subdiscipline. Lipidomics is the discipline for complete characterization of all lipids in a given system. More complex and exact way to define lipidomics is that “lipidomics is the discipline to obtain integral information of all lipids in a biological system concerning cellular signals, membrane architecture, transcriptional and translational modulation, cell–cell and cell–protein interactions and response to environmental changes with time”. (Luque de Castro & Quiles-Zafra, 2020.)

1.2 Definition of lipidome and human plasma lipidome

Lipid molecules found in a given system are called lipidome. Therefore, lipid molecules in human blood plasma is called plasma lipidome (Burla et al., 2018). In 2010, LIPID MAPS formed a consortium to define mammalian lipidome using the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1950–Metabolites in Frozen Human Plasma. SRM 1950 were prepared by plasma samples that were collected from both male and female donors, and donor ethnicity was representative of the US population. SRM consisted samples from 100 individuals between the age of 40 to 50 years. The resulting lipidome consisted of 588 different lipid molecular species covering six mammalian lipid categories (total amount): fatty acyls (107), glycerolipids (73), glycerophospholipids (160), sphingolipids (204), sterol lipids (36), and prenol lipids (8). Sphingolipid category contained the greatest number of lipid molecular species. In a molar basis (nmol/ml) sterol category was the most abundant lipid species and the glycerophospholipid category was the most abundant in a weight basis (mg/dl). (Quehenberger et al., 2010.) The following lipid classification paragraphs are mostly based on Quehenberger et al., (2010).

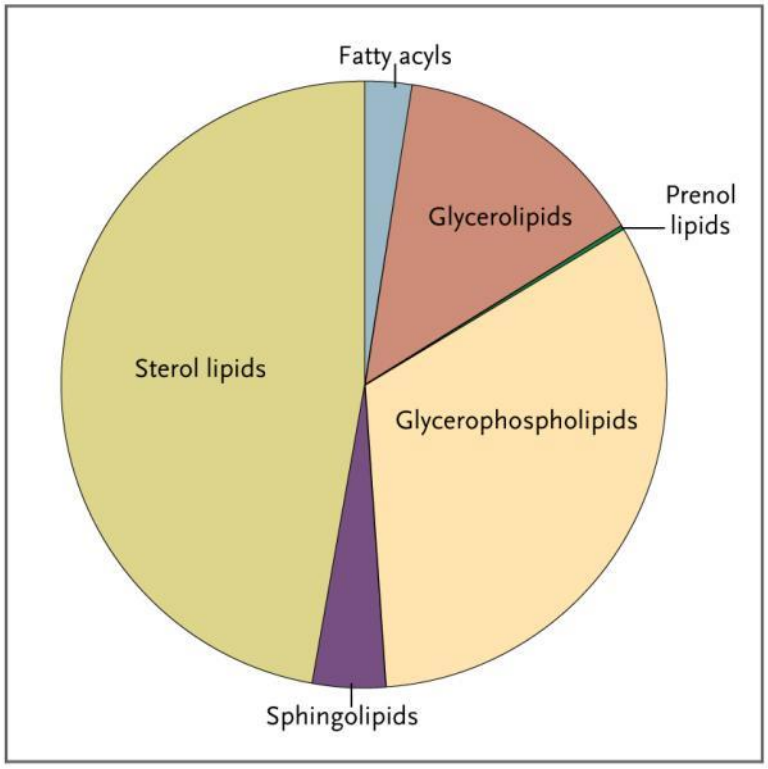

Figure 1. Relative distribution of lipids in a molar basis in human plasma (Quehenberger & Dennis, 2011).

1.2.1 Fatty acyls

Fatty acyls consist of subclasses and every subclass has separate function in a human body. Free fatty acids are taking care of tissues energy demands under normal condition and the three major constituents are oleic acid, palmitic acid and stearic acid. These three species comprise almost 80% of all free fatty acids in the circulation (Quehenberger et al., 2010.) Increasing level of free fatty acid molecules act as signalling molecules and long-term increase of plasma free fatty acid levels leads to insulin resistance and type 2 diabetes (Quehenberger & Dennis, 2011). Linolenic acid and arachidonic acid are the main polyunsaturated fatty acids (PUFA), and eicosanoids are derived from the metabolism of PUFAs. Eicosanoids such as leukotrienes and prostaglandins are tissue hormones, and they are known to regulate inflammatory responses (Whelan & Fritsche, 2013.)

1.2.2 Glycerolipids

Triacylglycerols (TAG) are the most abundant class of glycerolipid category by concentration. Diacylglycerols (DAG) are also present but significantly lower levels

(Quehenberger et al., 2010). TAGs are storage form for fatty acids and TAGs are stored by the liver into very low-density lipoproteins and secreted in plasma (Burdge & Calder, 2015). DAGs act as membrane second messenger and activates protein kinases e.g. PKC, PKD, Ca(2+)/calmodulin-dependent protein kinase (Gómez-Fernández et al., 2004; Wood et al., 2015). PKCs are involved in a multitude of physiological processes, for example, regulating lymphocytes migrating ability (Spitaler & Cantrell, 2004). DAGs are also precursors for structural glycerophospholipids and precursors for TAGs and monoacylglycerols. Immune function is also modulated by DAGs which are involved in immunological synapse function and respiratory burst like microglia. (Wood et al., 2015.)

1.2.3 Glycerophospholipids

SRM contained over 200 species of glycerophospholipids but only 158 out of all were identified due to isobaric species with the same m/z (mass/charge number of ions) (Quehenberger et al. 2010). Glycerophosphocholines (PC) and glycerophosphoethanolamines (PE) were the two major species of glycerophospholipids by concentration. Other species identified were glycerophosphate (PA), glycerophosphoglycerol (PG), glycerophosphoinositol (PI), and glycerophosphoserine (PS). (Quehenberger et al., 2010.) Glycerophospholipids are structural components of the cell, and some are secondary messengers or their precursors (Fahy et al., 2005). For example, inositol 1,4,5-trisphosphates are working as secondary messengers by inducing calcium release from the intracellular stores and in this way initiates aldosterone production in adrenal glomerulosa cells (Spaulding and Bollag 2022). Small part of the lysophosphatidylcholines (LPC) are made intracellular from lipoproteins or cell-membrane-derived PCs via phospholipase A2 or A1. A major part of LPCs is made extracellularly in plasma by endothelial lipase or by lecithin-cholesterol acyl-transferase which is secreted from the liver. LPCs are working as secondary messengers and they activate many cell types in the vascular system. LPCs are seen both antiatherogenic and atherogenic for example by increasing cellular permeability and apoptosis. (Schmitz and Ruebsaamen 2010.) Increased levels of LPC have been found in atherosclerosis, inflammatory disease, squamous cervical cancer, adrenoleukodystrophy and diabetes. Lower levels have been found in ovarian cancer, colorectal cancer, and infectious diseases (Liu et al. 2020).

1.2.4 Sphingolipids

Sphingolipids were the second largest category by total concentration and the total number of different sphingolipids in plasma SRM was 204. Sphingomyelins were the largest fraction followed by monohexosylceramides, free ceramides and free sphingoid bases. (Quehenberger et al. 2010). Sphingolipids are particularly found in cellular membranes of nervous tissues but can also be found in human plasma. Ceramides have also a role in regulation of cell growth and therefore they are potential anticancer agents. (Merrill, 2011.) Elevated plasma levels of sphingomyelins have been associated with CHD and ceramides with Alzheimer´s disease and type 2 diabetes (Quehenberger & Dennis, 2011). Especially Cer16, Cer18, Cer20 and Cer22 have been positively associated with type 2 diabetes in resent studies (Chen et al. 2020; Fretts et al. 2021). In FINRISK 2002 cohort study four ceramides were analysed and Cer(d18:1/18:0)/Cer(d18:1/16:0) ratio was the most significant predictor of type 2 diabetes (Hilvo et al. 2018). Havulinna et al. (2016) compared major adverse cardiovascular events cases with asymptomatic subjects also in FINRISK 2002 cohort study and concentrations of Cer(d18:1/16:0), Cer(d18:1/18:0), and Cer(d18:1/24:1) were significantly higher in subjects with an incident major adverse cardiovascular event compared with asymptomatic subjects.

1.2.5 Sterol lipids

Total amount of sterol lipid species was 36 in plasma SRM but it was the largest category by total concentration. Sterol lipids exist in free and esterified forms in plasma and the most abundant sterol in plasma is cholesterol and it exist primarily in the form of fatty acyl ester. Cholesterol is associated with lipoproteins in plasma. The most of plasma cholesterol is associated with LDL and HDL. LDL-C and HDL-C level in plasma are used to predict a risk of cardiovascular events and a high level of LDL-C is considered the major risk factor. (Quehenberger et al., 2010).

1.3 Definition of alcohol consumption by amount and gender

Alcohol consumption is defined as mean ethanol consumption in grams of ethanol per day by World Health Organization (WHO). Alcohol consumption is classified into categories of low, moderate, high, or very high-risk drinking. These categories differ by gender as follows: In men low risk drinkers consumes between 1-40g, moderate risk 41-60g, high risk 61-100g and very high risk >100g alcohol per day. In women low drinkers consumes between 1-20g, moderate risk 21-40g, high risk 41-60g and very high risk >60g alcohol per day. The ethanol content varies between different beverages and portion sizes as follows:

Regular beer 12g (0,33l), strong beer 15,5g (0,33l), long drink 15,5g (0,33l), cider 12g (0,33l), spirit 12g (4cl) and wine 12g (12cl). (Niemelä et al., 2019).

1.4 Alcohol consumption and conventional serum lipid markers

A dose-response relationship between alcohol intake and blood lipids has been shown in several studies. Especially HDL-C, LDL-C, ApoA1 and TAG levels have relationship with alcohol intake. TAG and HDL-C levels rises with alcohol intake increase. (Park and Kim 2012, Waśkiewicz and Sygnowska 2013.) It has also been seen that changes in HDL-C concentrations and total alcohol intake have an umbrella-shaped association. Slower HDL-C decrease was associated with moderate alcohol consumption (<15g alcohol per day). Consistently, the slowest increase in the TAG, HDL and ApoA1 levels rises with alcohol intake increase but lipoprotein(a), ApoB/ApoA1 and LDL/HDL ratio decreased in males. In this study there were no significant difference between alcohol consumption and ApoB and LDL levels in males. (Hao et al., 2015)

Piano et al. (2018) observed associations between binge-drinking (4/5 drinks of any alcohol beverage 1-12 or >12 times in the past year) and total cholesterol, LDL-C, HDL-C, ApoB, TAG between sexes. Among men, binge drinking was associated with higher total cholesterol and these levels were significantly different compared with women. LDL-C and ApoB levels were also higher in a binge-drinking group of men, but in women there were no effects in these lipid parameters between drinking groups. HDL-C levels were higher in both binge-drinking groups of men and women. In this study there were no significant different in TAG levels in women or men between drinking groups.

Linear increase of serum myristic acid, monosaturated fatty acids such as palmitoleic acid and oleic acid, and n-6-PUFA such as linoleic acid and arachidonic acid are associated with increasing alcohol consumption in a group of 60 years old men. Increasing alcohol consumption is also associated to a linear decrease of serum unsaturated linoleic acid and saturated pentadecanoic acid. Increasing alcohol consumption had also non-linear association to increased serum n-6 PUFA gamma-linolenic acid, saturated palmitic acid, and n-3 polyunsaturated eicosapentaenoic acid. It also had non-linear association to decreased serum n-6 PUFA dihomo-gamma-linolenic acid, n-3 PUFA decosahexaenoic acid and saturated stearic acid. (Laguzzi et al., 2018).

1.5 Alcohol consumption and single lipids or lipid patterns

There are studies showing that alcohol consumption is associated with single lipids and some studies describe associations of alcohol consumption and metabolite patterns. Langenau et al. (2019) studied association of alcohol consumption on metabolite patterns. They used data-driven approach called Treelet transform. It was used to capture interrelationships among metabolites and the metabolite patterns were diacyl-glycerophosphocholines and acyl-alkyl-phosphatidylcholine I (diacyl PCs and acyl-alkyl PCs I), sphingomyelins (SMs), lysophosphatidylcholines (LPCs), diacyl-phosphatidylcholines (diacyl PCs), diacyl-phosphatidylcholines and acyl-alkyl-phosphatidylcholine II (diacyl PCs and acyl-alkyl PCs II) and acyl-alkyl-phosphatidylcholine (acyl-alkyl PCs). Intake of 12g alcohol per day has significant difference in decreased levels of lipid patterns of SMs in a group of men. There was also significant difference in an increase of diacyl PCs and acyl-alkyl PCs I, LPCs, diacyl PCs and diacyl PCs and acyl-alkyl PCs II. Van Roekel et al. (2018) discovered the same in their study. Alcohol consumption was associated with four LPCs, 13 diacyl PCs, seven acyl-alkyl PCs, and six SMs concentration.

Alcohol seems to affect mostly on the glycerophospholipid, sphingolipid and ether lipid metabolism. LPCs have been found to have cytotoxic effects and alcohol consumption accumulates them. (Weltzien, 1979).

When comparing moderate to heavy drinkers (men >40g/day, women >20g/day) to light drinkers (men <40g/day, women <20g/day) there has been shown to be association with increase in LPC acyl and diasyl PCs but a decrease in several acyl-alkyl PCs and SM. There were also decrease in LPC acyl 17:0. Gender also seems to have an impact on lipid metabolite levels. In alcohol consuming men there are more lipid metabolites than in women. (Jaremek et al., 2013; Lacruz et al., 2016).

Paapstel et al., (2018) investigated serum PC and LPC species in relation to hemodynamic, endothelial dysfunction and arterial stiffness in atherosclerotic patients who has also symptoms. In comparison to healthy subjects there was decreased serum levels of several individual PC and LPC species among atherosclerotic patients. These species were e.g., diacyl-PCs, acyl-alkyl-PCs and LPC-acyls. This study also revealed that coronary atherosclerosis and peripheral atherosclerosis showed distinct PC and LPC profiles.

1.6 Impact of different types of alcoholic beverages on lipids

Intake of wine has a more pronounced positive relation between alcohol consumption and serum n-3 PUFA eicosapentaenoic acid, n-3 PUFA decosahexaenoic acid and n-6 arachidonic acid as compared to a moderate intake of other types of beverages (Laguzzi et al., 2018).

Differences in HDL concentration rates and TAG:HDL-C ratio rates in a longitudinal study shows that difference of the alcohol type that is consumed modifies the change in HDL-C. Increased consumption of beer increased the mean differences in HDL-C and decreased the TAG:HDL-C ratio. Umbrella-shaped association was observed in the changes of HDL-C concentrations and TAG:HDL-C ratios in hard-liquor consumption. (Huang et al., 2017).

30g of alcohol per day in the forms of red wine or gin were given to healthy volunteers during 4 weeks in a clinical trial. The aim was to compare the effects of a polyphenol-rich (red wine) and polyphenol-free (gin) alcoholic beverages on lipid profile. (Estruch et al., 2011).

1.7 Relation between alcohol and serum lipids in animal model

Li et al., (2018) analysed the serum lipid species of rats administrated by alcohol for one year and showed that alcohol affects glycerolipids, sphingolipid and glycerophospholipid metabolism in both female and male rats. Also, the lipid fatty-acyl chain and the total degree of fatty acid unsaturation was modified significantly. In glycerophospholipid metabolism phosphatidic acid and phosphatidylethanolamine were significantly increased and lysophosphatidylcholine and phosphatidylcholine were decreased. In sphingolipid metabolism 3-O-sulfogalactosylceramide and trihexosylceramide were down-regulated and glucosylceramide was up-regulated. In glycerolipid metabolism diacylglycerol, triacylglycerol and cholesteryl ester were increased.

1.8 Relation between alcohol consumption and atherosclerosis

Atherosclerosis is a chronic low-grade inflammatory disease which involves lipid accumulation and inflammatory markers in the arteries. Carotid artery intima-media thickness is considered as a valid indicator of subclinical atherosclerosis. (Laguzzi et al., 2021). Binding of the cholesterol-containing lipoprotein particles to intimal proteoglycans is the central pathogenic process in atherogenesis according to the response-to-retention hypothesis (Williams & Tabas, 1995).

After retaining to the proteoglycans, lipoproteins get oxidized and accumulates in lipophages. Then they provoke a cascade of inflammatory process that drive the formation of atherosclerosis. There is a study that has identified lipid species that are specifically detected in atherosclerotic plaques. Those species were cholesterol ester, LPC, PC and SM. (Stegemann et al., 2011).

Juonala et al. (2009) observed that carotid intima-media thickness was directly related to alcohol consumption among healthy young adults and particularly in men. There was also direct correlation between the frequency of wine and strong alcohol beverages drinking and carotid intima-media thickness and this association was independent of age, sex, LDL-C, HDL-C, TAG, BMI, blood pressure, and smoking. In this study the frequencies of consuming mild alcohol beverages or beer did not correlate independently with carotid intima-media thickness. Binge drinking was also not associated with intima-media thickness.

Ogunmoroti et al. (2021) used American Heart Associations seven cardiovascular health metrics (BMI, diet, total cholesterol, blood pressure, blood glucose, smoking and physical activity) to determine alcohol consumptions effects on ideal cardiovascular health. The study showed that light to moderate consumption of wine was associated with better cardiovascular health and heavy consumption of liquor or beer was associated with worse cardiovascular health. Ogunmoroti et al. (2019) also showed that in women there were significant association in light and moderate drinking groups to have average and optimal cardiovascular health scores.

Oxidised LDL (oxLDL) is known to contribute to atherosclerosis whereas HDL-C is known to have an ability to inactivate and remove lipid hydroperoxides from oxLDL. HDL-C is then atheroprotective. (Rasmiena et al. 2016). Greater alcohol intake is observed to have dose-response relation for higher baseline HDL-C concentration so moderate alcohol consumption might have beneficial effect on cardiovascular health. This study showed that comparing any amount of alcohol intake to never drinkers there was significant difference in a decreasing rate of HDL-C concentration among drinkers and it was the lowest among moderate (men 15-30g/day, women 7,5-15g/day) drinkers. (Huang et al., 2017).

2. AIM OF THIS RESEARCH AND HYPOTHESIS

There is an association between alcohol consumption and clinical lipid markers but lipidome-wide connections are currently unclear. In the study, my aim was to study cross-sectional association of alcohol consumption with serum lipidome in a population cohort of 1969 men and women aged 30-45 years. The study subjects were participants of the Cardiovascular Risk in Young Finns Study.

3. METHODS

3.1 Subjects

The Cardiovascular Risk in Young Finns Study is an on-going follow-up study of atherosclerosis precursors of Finnish children and adolescents. The first cross-sectional survey was conducted in 1980, when 3596 participants, aged 3, 6, 9, 12, 15 and 18 years, were randomly chosen in each university hospital area in Finland from the national population register. (Raitakari et al., 2008). 2204 of these individuals were re-examined in 2007, then aged 30-45 years. Individuals with type 2 diabetes and subjects with missing risk factor data were excluded from the present analyses. Histograms were used to evaluate and to remove any potential outliers from the data (i.e., data points outside the 99-percentile curve in plasma glucose and daily amount of drinks). Therefore, the present study cohort included 1969 subjects (Table 1).

3.2 Alcohol consumption

Data on alcohol consumption was acquired using questionnaires. Participants were asked to report their consumption of 1/3l cans or bottles of beer, glasses (12cl) of wine, and 4cl shots of liquor or strong alcohol during the last week. These amounts are comparable to approximately 14g of alcohol (=1unit). Participants were also asked to report the frequency of consumption of different alcohol beverages with a scale: daily, weekly, monthly, less than monthly, never. Frequency of binge drinking were also asked on a scale: twice a week or more, once a week, 2-3 times per month, once per month, 2-6 times a year, less or never. Subjects consuming ≥6units of alcohol on a given occasion more frequently than once a month were considered binge drinkers. (Juonala et al., 2009). Drinks per day was calculated from reported consumptions of 1/3l cans or bottles of beer or cider, glasses (12cl) of wine and shots of liquor or strong alcohol during the last week and divided by seven.

3.3 Lipidome-wide analysis

Serum lipidome analysed by using liquid chromatography-tandem mass spectrometry (Mishra et al., 2020). Quantification of the lipidome for the stored serum samples was performed at Zora Biosciences Oy (Espoo, Finland). Lipid extraction was based on a previously described method (Mamtani et al., 2016). In brief, 10 µl 10 mM 2,6-di-tert-butyl-4-methylphenol in methanol was added to 10 µl of sample, followed by 20 µl of internal standards (Avanti Polar Lipids Inc., Alabaster, AL) and 300 µl chloroform-methanol (2:1; v:v) (Sigma-Aldrich GmbH, Steinheim, Germany). Samples were mixed and sonicated in a water bath for 10 min, followed by a 40 min incubation and centrifugation (15 min at 5,700 g). The upper phase was transferred and evaporated under nitrogen. Extracted lipids were resuspended in 100 µl water-saturated butanol and sonicated in a water bath for 5 min. Methanol (100 µl) was added to the samples before the extracts were centrifuged for 5 min at 3,500 g, and finally the supernatants were transferred to the analysis plate for mass spectrometric analysis. Details of mass spectrometric analyses have also been described in detail previously (Braicu et al., 2017). The analyses were performed on a hybrid triple-quadrupole/linear ion trap mass spectrometer (QTRAP 5500; AB Sciex, Concord, Canada) equipped with ultra-high-performance liquid chromatography (Nexera-X2; Shimadzu, Kyoto, Japan). Chromatographic separation of the lipidomic screening platform was performed on an Acquity BEH C18, 2.1 × 50 mm i.d. 1.7 µm column (Waters Corporation, Milford, MA). The data were collected using a scheduled multiple-reaction monitoring algorithm, and the data were processed using Analyst and MultiQuant 3.0 software (AB Sciex).

3.4 Lipidome lipid classification

The lipidomics data consisted of 437 molecular lipid species from five LIPID MAPS lipid classification categories (http://www.lipidmaps.org). Fatty Acyls (n=9), Glycerolipids (n=61), Glycerophospholipids (n=233), Sphingolipids (n=112) and Sterol Lipids (n=22). Fatty Acyls consist of ACs (n=9), glycerolipids consist of DAGs (n=19) and TAGs (n=42). Glycerophospholipids consist of PCs (n=102), LPCs (n=57), PEs (n=38), LPEs (n=19), PGs (n=2) and PIs (n=15). Sphingolipids consist of Cers (n=30), Gb3 (n=6), Glc/GalCer (n=22), LacCers (n=13) and SMs (n=41). Sterol lipids consist of CEs (n=22). The numbers in the lipid name are used to describe the fatty acid chains on the lipid. The numbers are generally presented in the format (number of carbons in fatty acid chain) : (number of

double bonds in fatty acid chain), e.g., 20:2 would be 20 carbons in the fatty acid chain with two double bonds.

3.5 Clinical characteristics and risk factors

Height and weight were measured, and body mass index (BMI) was calculated as BMI=weight/height^2 (kg/m^2). Blood pressure was measured with a random zero sphygmomanometer. For the determination of serum lipoprotein levels, venous blood samples were drawn after an overnight fast. Standard enzymatic methods were used for measuring levels of serum total cholesterol, TAGs, and HDL-C. LDL-C concentration was calculated by the Friedewald formula. Serum insulin concentrations were measured by microparticle enzyme immunoassay kits, glucose concentrations were analyzed enzymatically. ApoA1 and ApoB were analysed immunoturbidometrically (Orion Diagnostica, Espoo, Finland). Data on smoking and physical activity were acquired using questionnaires. Physical activity index calculated as metabolic equivalents (MET) was constructed by combining the information on the frequency, intensity and duration of physical activity, including leisure-time physical activity and commuting to the workplace (Kresanov et al., 2021; Raitakari et al., 2008). The participants were classified as having type 2 diabetes if they met one of the following criteria: fasting plasma glucose ≥7 mmol/l at the 2001 or 2007 follow-up visit, type 2 diabetes diagnosed by a physician and the use of glucose-lowering medication at the 2007 follow-up visit or as confirmed by the National Social Insurance Institution Drug Reimbursement Registry, which covers all Finnish citizens since 1990 (Pulkki-Råback et al. 2017).

3.6 Statistical methods

The statistical tests were performed with Statistical Analysis System version 9.4. A total of 1969 subjects of 2007 follow-up were included in the final analyses. Histograms and normal probability plots were used to evaluate the normality assumptions. BMI, TAGs, insulin, alcohol consumption, METs and all lipidomic variables were skewed distributed. Variables with skewed distributions were log-transformed. T-test was used to examine differences in normally distributed clinical characteristics between sexes. The Mann-Whitney U test was used to examine differences between not normally distributed clinical characteristics between sexes. Pearson’s chi-squared test was used to examine differences in categorical data. Analyses of covariance (ANCOVA) were used to determine differences in serum lipid concentrations between alcohol consumption, adjusting age and

sex. Multiple testing corrections were performed using the Bonferroni method, with P < 0.0001 considered statistically significant.

4. RESULTS

4.1 Participant Characteristics

Main characteristics of the study subjects in 2007 are presented in Table 1. Men had higher levels of total cholesterol, LDL-C, TAG and ApoB than in women. Accordingly, men had lower levels of HDL-C and ApoA1 than women. In men, mean alcohol consumption was 1.19 (range 0–5.57) units/day, and in women 0.56 (range 0–5.43) units/day. The rest of variable characteristics of the study subjects are presented in the Table 1.

Table 1. Main characteristics of the study subjects. Data are expressed as mean ± standard error or numbers (percentage).

Variable	Men	Women	P-value
N	868	1101	
Age (years)	37.4 ± 5.0	37.7 ± 4.9	0.169
Alcohol consumption (units/day)	1.19 ± 1.18	0.56 ± 0.72	<0.0001
Total cholesterol (mmol/l)	5.19 ± 0.94	4.91 ± 0.84	<0.0001
HDL-C (mmol/l)	1.21 ± 0.28	1.44 ± 0.33	<0.0001
LDL-C (mmol/l)	3.40 ± 0.82	2.94 ± 0.74	<0.0001
TAG (mmol/l)	1.58 ± 0.92	1.18 ± 0.64	<0.0001
ApoA1 (g/l)	1.51 ± 0.21	1.67 ± 0.27	<0.0001
ApoB (g/l)	1.11 ± 0.27	0.94 ± 0.23	<0.0001
BMI (kg/m^2)	26.5 ± 4.0	25.3 ± 5.0	<0.0001
Systolic blood pressure (mmHg)	126 ± 13	116 ± 14	<0.0001
Diastolic blood pressure (mmHg)	79 ± 11	73 ± 11	<0.0001
Insulin (IU/l)	9.54 ± 22.9	8.27 ± 6.58	0.029
Glucose (mmol/l)	5.41 ± 0.46	5.13 ± 0.46	<0.0001
Physical activity index (METs)	19.6 ± 21.9	19.3 ± 20.1	0.156

Current smokers (%)	277 (31.9%)	246 (22.3%)	<0.0001

4.2 Associations of alcohol consumption with serum lipidome

After Bonferroni correction alcohol intake was found to be significantly associated with concentrations of 195 out of 437 lipids, after adjusting for age and sex. There was seven ACs, nine DAGs, 15 TAGs, 58 PCs (in which 22 was ether-linked), 28 LPCs, 18 PEs (in which 2 was ether-linked), eight LPEs, two PGs, eight PIs, eight Cers, one Gb3, five Glc/GalCers, three LacCers, 58 PCs (in which 22 was ether-linked), 16 SMs and eight CEs. Associations with ACs, Cers, LPEs, PCs, PEs, PGs, PIs, DAGs, TAGs, and CEs were generally positive, while mostly inverse associations were observed with Gb3s, Glc/GalCer, LacCers, ether-linked PCs and SMs. LPCs were associated with both positive and negative correlation. The exact numbers of statistically significant lipids within each lipid class are presented in Table 2. The individual lipid associations of alcohol intake are presented in Supplementary Table.

Table 2. The age and sex adjusted significant associations of serum lipids with alcohol intake categorized by lipid classes and direction of the association.

Lipid class	Positive association (n)	Negative association (n)
Acylcarnitines (ACs)	7	0
Cholesteryl esters (CEs)	6	2
Ceramides (Cers)	8	0
Diacylglycerols (DAGs)	8	1
Globotriasoylceramides (Gb3s)	0	1
Glucosyl/galactosylceramides (Glc/GalCers)	1	4
Lysophosphatidylcholines (LPCs)	12	16
Lysophosphatidylethanolamines (LPEs)	8	0
Lactosylceramides (LacCers)	0	4
Phosphatidylcholines (PCs)	23	13
Phosphatidylcholines (ether-linked) (PC Os) or (PC Ps)	4	18

Phosphatidylethanolamines (PEs)	16	0
Phosphatidylethanolamines (ether-linked) (PE Os) or (PE Ps)	2	0
Phosphatidylglycerols (PGs)	2	0
Phosphatidylinositols (PIs)	8	0
Sphingomyelins (SMs)	4	12
Triacylglycerols (TAGs)	15	0

5. DISCUSSION

There has been a lack of broader studies of alcohol intake and lipidomics. In this study alcohol intake was related with several fatty acyls, glycerolipids, glycerophospholipids, sphingolipids and sterol lipids.

Acylcarnitines (AC) were correlating only positively with alcohol intake. van Roekel et al. (2018) showed similar findings, including that AC 14:1, AC 16 and AC 18:1 had positive association with alcohol consumption. Langenau et al. (2019) studied effects of alcohol consumption on long- and short-chain ACs and on medium- and long-chain ACs. They discovered that the association was positive in both groups. Long-chain ACs such as AC 16, AC 18, AC 18:1 and AC 18:2 were significantly higher levels in patients with pulmonary arterial hypertension in (Brittain et al. 2016), but no significant associations were seen between short- or medium-chain ACs and pulmonary arterial hypertension. Zhao et al. (2020) studied AC concentrations among type 2 diabetes patients and risk of CVD. They discovered that higher levels of short and medium-chain ACs were significantly associated with CVD risk among type 2 diabetes patients. Alcohol consumptions positive effects on AC levels have been showed by many studies previously and ACs association with CVD risk as well. Further studies are still needed to show if there is causality between alcohol intake and CVD risk and if it´s because of increased levels of serum ACs.

Glycerophospholipids were the largest group of lipids in my study, and in this group lysophosphatidylcholines (LPC) were both positively and negatively associated with alcohol intake. Lacruz et al. (2016) and van Roekel et al. (2018) showed in their cross-sectional studies that LPC 16:0, LPC 16:1 and LPC 20:4 had positive association and LPC 17:0 had negative association with alcohol intake. I discovered similar associations in my analyses. Decreased concentrations of LPC 17:0 and LPC 18:2 have been seen to

increase risk of incident myocardial infarction (Ward-Caviness et al. 2017). A previous study has also observed independent associations of LPC 18:1 and LPC 18:2 with cardiovascular disease (Ganna et al. 2014). Israelsen et al. (2021) studied effects of alcohol intoxication on blood lipid levels. They discovered that several LPCs decreased after alcohol intake, especially LPC 18:1 and LPC 20:4. Israelsen et al. (2021) also hypothesized that alcohol increases hepatic LPC uptake which leads to decrease of LPC in circulation. However, in my study LPC 18:1 and LPC 20:4 had positive association with alcohol intake. It has been shown before that alcohol intake is inversely associated with lipoprotein-associated phospholipase A2 (lp-PLA2) (Hatoum et al. 2010) which is needed for LPC production. LPCs are also major components of ox-LDL and by that plays an important role in various pathological activities such as atherosclerosis and acute and chronic inflammation (Schmitz and Ruebsaamen 2010). On the other hand Reichel et al. (2015) discovered in their study that lysosomal acid sphingomyelinase (L-ASM) was positively correlated with LPC levels in alcohol-dependent patients. L-ASM hydrolyze among other things SMs and PCs (Breiden and Sandhoff 2021). Further research is needed to establish these interactions, but LPC levels are more likely regulated by several mechanisms.

Phosphatidylethanolamines (PE) were also correlating only positively with alcohol consumption. There is a lack of cross-sectional studies of alcohol consumptions effects of PE content in humans. In an animal model there has been seen positive relation with chronic alcohol intake with PE concentrations in serum (Li et al. 2018). Stegemann et al. (2014) showed that PE 36:5 was strongly associated with cardiovascular disease. In my study PE 36:5 was positively associated with alcohol consumption. Phosphatidylethanolamine methyltransferase (PEMT) catalyzes PE conversion to PC in the liver, and it has been seen that alcohol consumption reduces this conversion in an animal model (Kharbanda et al. 2007). Considering these findings, PE concentrations may stay in higher levels in serum because of liver dysfunction.

Phosphatidylcholines (PC) were correlating both positively and negatively with alcohol intake, but mostly positive associations were observed. Jaremek et al. (2013) and van Roekel et al. (2018) discovered similar findings, showing that PC aa C32:1, PC aa 34:1, PC aa 36:1 and PC aa 40:4 were positively associated and PC ae C30:2, PC ae 36:2, PC ae 38:3, PC ae 38:4, PC ae C40:3, PC ae 40:4 and PC ae 40:6 were negatively associated with alcohol intake. On the other hand, PC 38:3 has been seen to have strong

association with cardiovascular disease (Stegemann et al. 2014). In my study PC 38:3 had positive correlation with alcohol intake. Reichel et al. (2015) observed that plasma levels of PC were higher in alcohol-dependent patients compared with healthy controls and higher levels of PC could be result of alcohol inhibition of lp-PLA2.

Phosphatidylinositols (PI) were one of the groups that were correlating only positively with alcohol consumption. Total of 8 of all 15 PIs analyzed were positive. Similar results have been seen before and plasma concentrations of PI 40:5 and PI 36:2 have been significantly higher among alcohol using subjects (Reichel et al. 2015). PIs are known as secondary messengers, but more studies are needed to find out how alcohol is affecting their secondary messenger cascades (Hammond and Burke 2020).

Sphingomyelins (SM) levels were mostly negatively associated with alcohol intake, including low levels of SM 41:1. Lower levels of SM 41:1 have seen before to predict liver-related events (Thiele et al. 2023). Thiele et al. (2023) also showed with mendelian randomization analyses that alcohol-related liver disease causes low SM levels, but causality was not observed between alcohol dependency and SM levels in the blood. Reichel et al. (2015) showed that SM species decreased when alcohol abuse was chronic but normalize quickly when patients abstained from alcohol. Alcohol consumption may also have SM-lowering effect via alcohol-induced ASM which leads to hydrolyzation of SMs to PCs (van Roekel et al. 2018). In Pongrac Barlovic et al. (2020) prospective cohort study was discovered that serum total SM associated with incident coronary heart disease events in Type 1 diabetes patients after adjusting sex, age of diabetes onset, diabetes duration and smoking, but after further adjustment there were no significant different. However, alcohol intake was not one of the adjustments made. They discovered also that higher serum total SM was associated with a more rapid eGFR decline and proposed that SM is involved in progression of renal damage.

Creamides (Cer) were also one of the groups that were correlating only positive with alcohol consumption. Ceramides are generated by hydrolysis of sphingomyelin and the hydrolysis is catalyzed by acid sphingomylinase (ASM) (Garcia-Ruiz et al. 2015). Alcohol consumption have been seen to lead accumulation of ceramides and that could be cause of increased activation of ASM (Saito et al. 2005). Reichel et al. (2015) showed in their study that Cer concentrations were higher among patients of alcohol abuse and after detoxification Cer concentrations remained to be on higher level. Especially Cer(d18:1/16:0) and Cer(d18:1/18:0) remained elevated. In my study Cer(d18:1/18:0) was

associated positively with alcohol consumption. Several Cer have been seen to have positive correlation with cardiovascular disease, such as Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/20:0), Cer(d18:1/22:0), Cer(d18:1/24:0) and Cer 18:1/24:1) (Mantovani et al. 2020; Poss et al. 2020). And there for alcohol consumption might add risk of CVD due accumulation of Cer. In my study alcohol consumption was associated positively with Cer(d18:1/24:0) concentrations which is seen to correlate positively with CVD (Mantovani et al. 2020).

Triacylglycerols (TAG) in general, as conventional lipid markers, have been seen to have positive association with alcohol intake in a several studies (Park and Kim 2012, Waśkiewicz and Sygnowska 2013, Piano et al. 2018). In my study, the results were the same, and a total of 15 out of 42 TAGs had positive association with alcohol consumption. Absorption of TAG and TAG hydrolysis and synthesis in human body are regulated in many ways and effects of alcohol on those cascades are under investigation. Exposure to alcohol is seen to increase hepatic uptake of fatty acyls (FA) which are needed for TAG synthesis in the liver. TAG synthesis from FAs is mediated by acetyltransferases and phosphatidate phosphatases such as Lipin-1 (You and Arteel 2019). Mishra et al. (2020) showed in their follow-up study that TAG(18:0/18:0/18:1), TAG(18:0/18:1/18:1) and TAG(16:0/18:0/18:1) were significantly associated with both atherosclerosis and osteoporosis. In my study TAG(18:0/18:0/18:1) were associated positively with alcohol intake.

5.1 Study limitations and strengths

My study has limitations. Study cohort has a long systematic follow-up which offers cross-sectional sample of subjects but because of that causality of daily alcohol consumption and the measured lipids cannot be drawn. Lipidomic analyses were adjusted only with age and sex. Analyses would need further adjustments to be more accurate. Self-reported questionnaires as measures of quantity and frequency of alcohol consumption were used in this study and that might increase margin of error by selective underreporting. Study subjects were Finns, and because of that these findings cannot be generalized to other populations.

My study cohort was relatively large. Study subjects were healthy young (30-45 years) Finns, and both men and women were represented. A strength of this study was also a large panel of investigated lipids, a total of 437, which is one of the largest in this area.

6. CONCLUSIONS

Alcohol consumption was associated with several lipids from different lipid classes. There were more positive associations than negative. Previous studies have showed similar findings, but not in this scale. Especially AC, Cer, PE, TAG were correlating positively with alcohol consumption and SM had inverse correlation. Higher levels of AC, Cer and TAG have been associated with CVD in previous studies, also SM have been implicated. Further studies are needed to evaluate whether alcohol intake related lipids are causal for the development of CVD.

REFERENCES

Braicu, E.I. et al. High-grade ovarian serous carcinoma patients exhibit profound alterations in lipid metabolism. Oncotarget, 2017;8(61):102912-102922.

Breiden, B. and Sandhoff, K. Acid sphingomyelinase, a lysosomal and secretory phospholipase c, is key for cellular phospholipid catabolism. International Journal of Molecular Sciences. 2021;22(16):9001.

Brittain, E.L. et al. Fatty acid metabolic defects and right ventricular lipotoxicity in human pulmonary arterial hypertension. Circulation 2016;133(20):1936–1944.

Burdge, G.C. and Calder, P.C. Introduction to fatty acids and lipids. World Review of Nutrition and Dietetics. 2015;112:1-16.

Burla, B. et al. MS-based lipidomics of human blood plasma: A community-initiated position paper to develop accepted guidelines. Journal of Lipid Research. 2018;59(10):2001–2017.

Chen, G.C. et al. Serum sphingolipids and incident diabetes in a US population with high diabetes burden: The Hispanic Community Health Study/Study of Latinos (HCHS/SOL). American Journal of Clinical Nutrition. 2020;112(1):57–65.

Estruch, R. et al. Moderate consumption of red wine, but not gin, decreases erythrocyte superoxide dismutase activity: A randomised cross-over trial. Nutrition, Metabolism and Cardiovascular Diseases. 2011;21(1):46–53.

Fahy, E. et al. A comprehensive classification system for lipids. Journal of Lipid Research. 2005;46(5):839–861.

Fahy, E., Cotter, D., Sud, M. and Subramaniam, S. Lipid classification, structures and tools. Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids. 2011;1811(11):637–647.

Fretts, A.M. et al. Plasma ceramides containing saturated fatty acids are associated with risk of type 2 diabetes. Journal of Lipid Research. 2021;62:100119.

Ganna, A. et al. Large-scale Metabolomic Profiling Identifies Novel Biomarkers for Incident Coronary Heart Disease. PLoS Genetics. 2014;10(12):e1004801

Garcia-Ruiz, C., Mato, J.M., Vance, D., Kaplowitz, N. and Fernández-Checa, J.C. Acid sphingomyelinase-ceramide system in steatohepatitis: A novel target regulating multiple pathways. Journal of Hepatology. 2015;62(1):219–233.

Gómez-Fernández, J.C., Torrecillas, A. and Corbalán-García, S. Diacylglycerols as activators of protein kinase C. Molecular Membrane Biology. 2004;21(6):339–349.

Hammond, G.R.V. and Burke, J.E. Novel roles of phosphoinositides in signaling, lipid transport, and disease. Current Opinion in Cell Biology. 2020;63:57–67.

Hao, G. et al. Relationship between Alcohol Consumption and Serum Lipid Profiles among Middle-Aged Population in China: A Multiple-Center Cardiovascular Epidemiological Study. Angiology. 2015:66(8):753–758.

Hatoum, I.J., Nelson, J.J., Cook, N.R., Hu, F.B. and Rimm, E.B. Dietary, lifestyle, and clinical predictors of lipoprotein-associated phospholipase A2 activity in individuals without coronary artery disease. American Journal of Clinical Nutrition. 2010;91(3):786–793.

Havulinna, A.S. et al. Circulating Ceramides Predict Cardiovascular Outcomes in the Population-Based FINRISK 2002 Cohort. Arteriosclerosis, Thrombosis, and Vascular Biology. 2016;36(12):2424–2430.

Hilvo, M. et al. Ceramide stearic to palmitic acid ratio predicts incident diabetes. Diabetologia. 2018;61(6):1424–1434.

Huang, S. et al. Longitudinal study of alcohol consumption and HDL concentrations: A community-based study. American Journal of Clinical Nutrition. 2017;105(4):905–912.

Israelsen, M. et al. Comprehensive lipidomics reveals phenotypic differences in hepatic lipid turnover in ALD and NAFLD during alcohol intoxication. JHEP Reports. 2021;3(5):100325

Jaremek, M. et al. Alcohol-induced metabolomic differences in humans. Translational Psychiatry. 2013;3(7):e276.

Juonala, M. et al. Alcohol consumption is directly associated with carotid intima-media thickness in Finnish young adults. The Cardiovascular Risk in Young Finns Study. Atherosclerosis. 2009;204(2):e93-98.

Kharbanda, K.K., Mailliard, M.E., Baldwin, C.R., Beckenhauer, H.C., Sorrell, M.F. and Tuma, D.J. Betaine attenuates alcoholic steatosis by restoring phosphatidylcholine generation via the phosphatidylethanolamine methyltransferase pathway. Journal of Hepatology. 2007;46(2):314–321.

Kresanov, P. et al. The associations of oxidized lipoprotein lipids with lipoprotein subclass particle concentrations and their lipid compositions. The Cardiovascular Risk in Young Finns Study. Free Radical Biology and Medicine. 2021;162:225–232.

Lacruz, M.E. et al. Cardiovascular Risk Factors Associated with Blood Metabolite Concentrations and Their Alterations during a 4-Year Period in a Population-Based Cohort. Circulation: Cardiovascular Genetics. 2016;9(6):487–494.

Laguzzi, F. et al. Circulating fatty acids in relation to alcohol consumption: Cross-sectional results from a cohort of 60-year-old men and women. Clinical Nutrition. 2018;37(6):2001–2010.

Laguzzi, F. et al. Alcohol consumption in relation to carotid subclinical atherosclerosis and its progression: results from a European longitudinal multicentre study. European Journal of Nutrition. 2021;60(1):123–134.

Langenau, J., Boeing, H., Bergmann, M.M., Nöthlings, U. and Oluwagbemigun, K. The association between alcohol consumption and serum metabolites and the modifying effect of smoking. Nutrients. 2019;11(10):2331.

Li, H. et al. Lipidomic signature of serum from the rats exposed to alcohol for one year. Toxicology Letters. 2018;294:166–176.

Liu, P., Zhu, W., Chen, C., Yan, B., Zhu, L., Chen, X. and Peng, C. The mechanisms of lysophosphatidylcholine in the development of diseases. Life Sciences. 2020;247:117443.

Luque de Castro, M.D. and Quiles-Zafra, R. Lipidomics: An omics discipline with a key role in nutrition. Talanta. 2020;219:121197.

Mamtani, M. et al. Lipidomic risk score independently and cost-effectively predicts risk of future type 2 diabetes: Results from diverse cohorts. Lipids in Health and Disease 2016;15(1):65.

Mantovani, A. et al. Associations between specific plasma ceramides and severity of coronary-artery stenosis assessed by coronary angiography. Diabetes and Metabolism. 2020;46(2):150–157.

Merrill, A.H. Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. Chemical Reviews. 2011;111(10):6387–6422.

Mishra, B.H. et al. Lipidomic architecture shared by subclinical markers of osteoporosis and atherosclerosis: The Cardiovascular Risk in Young Finns Study. Bone. 2020;131:115160.

Muscella, A., Stefà, E. and Marsigliante, S. The effects of exercise training on lipid metabolism and coronary heart disease. REVIEW Energetics and Metabolism Am J Physiol Heart Circ Physiol. 2020;319(1):76–88.

Niemelä, O., Nivukoski, U., Bloigu, A., Bloigu, R., Aalto, M. and Laatikainen, T. Laboratory test based assessment of WHO alcohol risk drinking levels. Scandinavian Journal of Clinical and Laboratory Investigation. 2019;79(1–2):58–64.

O'Donnell, V.B., Dennis, E.A., Wakelam, M.J.O. and Subramaniam, S. LIPID MAPS: Serving the next generation of lipid researchers with tools, resources, data, and training. Science Signaling. 2019;12(563):eaaw2964.

Ogunmoroti, O. et al. Alcohol type and ideal cardiovascular health among adults of the Multi-Ethnic Study of Atherosclerosis. Drug and Alcohol Dependence. 2021;218:108358.

Ogunmoroti, O., Osibogun, O., McClelland, R.L., Burke, G.L., Nasir, K. and Michos, E.D. Alcohol and ideal cardiovascular health: The Multi-Ethnic Study of Atherosclerosis. Clinical Cardiology. 2019;42(1):151–158.

Paapstel, K. et al. Inverse relations of serum phosphatidylcholines and lysophosphatidylcholines with vascular damage and heart rate in patients with atherosclerosis. Nutrition, Metabolism and Cardiovascular Diseases. 2018;28(1):44–52.

Park, H. and Kim, K. Association of alcohol consumption with lipid profile in hypertensive men. Alcohol and Alcoholism. 2012;47(3):282–287.

Piano, M.R., Burke, L., Kang, M. and Phillips, S.A. Effects of Repeated Binge Drinking on Blood Pressure Levels and Other Cardiovascular Health Metrics in Young Adults: National Health and Nutrition Examination Survey, 2011-2014. J Am Heart Assoc. 2018;7(13):e008733.

Pongrac Barlovic, D., Harjutsalo, V., Sandholm, N., Forsblom, C. and Groop, P.H. Sphingomyelin and progression of renal and coronary heart disease in individuals with type 1 diabetes. Diabetologia. 2020;63(9):1847–1856.

Poss, A.M. et al. Machine learning reveals serum sphingolipids as cholesterol-independent biomarkers of coronary artery disease. Journal of Clinical Investigation. 2020;130(3):1363–1376.

Pulkki-Råback, L. et al. Positive Psychosocial Factors in Childhood Predicting Lower Risk for Adult Type 2 Diabetes: The Cardiovascular Risk in Young Finns Study, 1980–2012. American Journal of Preventive Medicine. 2017;52(6):e157–e164.

Quehenberger, O. et al. Lipidomics reveals a remarkable diversity of lipids in human plasma1. Journal of Lipid Research. 2010;51(11):3299–3305.

Quehenberger, O. and Dennis, E.A. The Human Plasma Lipidome. New England Journal of Medicine. 2011;365(19):1812–1823.

Raitakari, O.T. et al. Cohort profile: The cardiovascular risk in young Finns study. International Journal of Epidemiology. 2008;37(6):1220–1226.

Rasmiena, A.A., Barlow, C.K., Ng, T.W., Tull, D. and Meikle, P.J. High density lipoprotein efficiently accepts surface but not internal oxidised lipids from oxidised low density lipoprotein. Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids. 2016;1861(2):69–77.

Reichel, M. et al. Alterations of plasma glycerophospholipid and sphingolipid species in male alcohol-dependent patients. Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids. 2015;1851(11):1501–1510.

van Roekel, E.H. et al. Circulating metabolites associated with alcohol intake in the european prospective investigation into cancer and nutrition cohort. Nutrients. 2018;10(5):654.

Rosales, C., Gillard, B.K., Gotto, A.M. and Pownall, H.J. The alcohol–high-density lipoprotein athero-protective axis. Biomolecules. 2020;10(7):1–16.

Saito, M., Saito, M., Cooper, T.B. and Vadasz, C. Ethanol-induced changes in the content of triglycerides, ceramides, and glucosylceramides in cultured neurons. Alcoholism: Clinical and Experimental Research. 2005;29(8):1374–1383.

Schmitz, G. and Ruebsaamen, K. Metabolism and atherogenic disease association of lysophosphatidylcholine. Atherosclerosis. 2010;208(1):10–18.

Spaulding, S.C. and Bollag, W.B. The role of lipid second messengers in aldosterone synthesis and secretion. Journal of Lipid Research. 2022;64(4):100191.

Spitaler, M. and Cantrell, D.A. Protein kinase C and beyond. Nature Immunology. 2004;5(8):785–790.

Stegemann, C. et al. Comparative lipidomics profiling of human atherosclerotic plaques. Circulation: Cardiovascular Genetics. 2011;4(3):232–242.

Stegemann, C. et al. Lipidomics profiling and risk of cardiovascular disease in the prospective population-based bruneck study. Circulation. 2014;129(18):1821–1831.

Thiele, M. et al. Sphingolipids Are Depleted in Alcohol-Related Liver Fibrosis. Gastroenterology. 2023;164(7):1248–1260.

Ward-Caviness, C.K. et al. Improvement of myocardial infarction risk prediction via inflammation-associated metabolite biomarkers. Heart. 2017;103(16):1278–1285.

Waśkiewicz, A. and Sygnowska, E. Alcohol intake and cardiovascular risk factor profile in men participating in the WOBASZ study. Kardiologia Polska. 2013;71(4):359–365.

Weltzien HU. Cytolytic and membrane-perturbing properties of lysophosphatidylcholine. Biochim Biophys Acta. 1979;559(2-3):259-287.

Whelan, J. and Fritsche, K. Linoleic acid. Advances in Nutrition. 2013;4(3):311–312.

Williams, K.J. and Tabas, I. Point of View The Response-to-Retention Hypothesis of Early Atherogenesis. Arterioscler Thromb Vasc Biol. 1995;15(5):551-561.

Wirtz, P.H. and von Känel, R. Psychological Stress, Inflammation, and Coronary Heart Disease. Current Cardiology Reports 2017;19(11):111.

Wood, P.L. et al. Targeted Lipidomics of Fontal Cortex and Plasma Diacylglycerols (DAG) in Mild Cognitive Impairment and Alzheimer's Disease: Validation of DAG Accumulation Early in the Pathophysiology of Alzheimer's Disease. Journal of Alzheimer's Disease. 2015;48(2):537–546.

You, M. and Arteel, G.E. Effect of ethanol on lipid metabolism. J Hepatol. 2019;70(2):237-248.

Zhao, S. et al. The Association Between Acylcarnitine Metabolites and Cardiovascular Disease in Chinese Patients With Type 2 Diabetes Mellitus. Frontiers in Endocrinology. 2020;11:212.

SUPPLEMENT

Abbreviations

AC, Acylcarnitines

Acyl-alkyl PC, Acyl-alkyl-phosphatidylcholine

ApoA1, Apolipoprotein A1

ApoB, Apolipoprotein B

ASM, acid sphingomyelinase

C, Cholesterol

CE, Cholesteryl ester

Cer, Ceramide

CHD, Coronary heart disease

CVD, Cardiovascular disease

DAG, Diacylglycerols

GalCer, Galactosylceramide

Gb3, Globotriaosylceramide

Glc, Glucosyl

HDL, High-density lipoprotein

LacCer, Lactosylceramide

LDL, Low-density lipoprotein

LPC, Lysophosphatidylcholine

LPE, Lysophosphatidylethanolamines

oxLDL, Oxidised low-density lipoprotein

PA, Glycerophosphate

PC, Phosphatidylcholine

PC aa, diacyl-phosphatidylcholine

PC ae, acyl-alkyl phosphatidylcholine

PE, Phosphatidylethanolamine

PEMT, Phosphatidylethanolamine methyltransferase

PG, Phosphatidylglycerol

PI, Phosphatidylinositol

PKC, Protein kinase C

PKD, Protein kinase D

PS, Glycerophosphoserine

PUFA, Polyunsaturated fatty acid

SRM, Standard Reference Material

SM, Sphingomyelin

TAG, Triacylglycerols

Supplementary table. Age and sex adjusted linear regression results between serum lipids and alcohol consumption. StdErr, standard error. P-value, p-value before Bonferroni correction. Bonf P-value, Bonferroni corrected p-value.

Lipid	Estimate	StdErr	P-value	Bonf. P-value
AcylCarnitine(12:0)	0.127	0.023	6.81E-08	2.97E-05
AcylCarnitine(13:0)	0.108	0.023	3.46E-06	1.51E-03
AcylCarnitine(14:0)	0.144	0.023	6.36E-10	2.78E-07
AcylCarnitine(14:1)	0.134	0.023	1.35E-08	5.89E-06
AcylCarnitine(16:0)	0.202	0.022	8.62E-20	3.77E-17
AcylCarnitine(16:1)	0.152	0.023	1.20E-10	5.23E-08
AcylCarnitine(18:1)	0.156	0.023	1.42E-11	6.18E-09
CE 14:1	0.153	0.023	7.72E-11	3.37E-08
CE 15:0	-0.102	0.023	1.59E-05	6.95E-03
CE 16:1	0.297	0.023	1.81E-37	7.89E-35
CE 16:2	0.107	0.023	5.09E-06	2.23E-03
CE 17:1	0.141	0.023	1.85E-09	8.10E-07
CE 18:0	-0.090	0.022	5.58E-05	0.0244
CE 18:2 +1	0.108	0.024	4.57E-06	2.00E-03
CE 24:6	0.103	0.024	1.47E-05	6.40E-03
Cer(d18:1/18:0)	0.092	0.023	6.88E-05	0.0301
Cer(d18:1/24:0)	0.110	0.023	2.03E-06	8.85E-04
Cer(d18:1/24:1)	0.166	0.022	9.69E-14	4.24E-11
Cer(d18:1/26:1)	0.186	0.023	1.15E-15	5.04E-13
Cer(d18:2/26:1)	0.142	0.023	1.25E-09	5.48E-07
Cer(d20:1/22:0)	0.098	0.023	1.82E-05	7.97E-03
Cer(d20:1/24:0)	0.109	0.023	2.49E-06	1.09E-03
Cer(d20:1/24:1)	0.184	0.022	3.57E-16	1.56E-13
DAG(16:0/16:1)	0.188	0.023	7.49E-16	3.27E-13
DAG(16:0/18:1)	0.116	0.023	3.18E-07	1.39E-04
DAG(16:0/22:6)	0.119	0.023	3.43E-07	1.50E-04
DAG(16:1/16:1)	0.181	0.024	3.79E-14	1.66E-11
DAG(16:1/18:1)	0.181	0.023	8.93E-15	3.90E-12
DAG(18:0/18:1)	0.125	0.022	2.12E-08	9.28E-06
DAG(18:1/18:1)	0.104	0.023	4.85E-06	2.12E-03
DAG(18:1/20:3)	0.165	0.023	1.11E-12	4.83E-10
DAG(18:2/18:2)	-0.089	0.023	9.58E-05	0.0419
Gb3(d18:1/23:0)	-0.118	0.023	3.46E-07	1.51E-04
Glc/GalCer(d18:1/22:0)	-0.108	0.023	3.78E-06	1.65E-03
Glc/GalCer(d18:1/23:0)	-0.096	0.023	4.13E-05	0.0180
Glc/GalCer(d18:1/26:1)	0.109	0.024	4.36E-06	1.90E-03
Glc/GalCer(d18:2/22:0)	-0.115	0.024	1.12E-06	4.90E-04
Glc/GalCer(d18:2/23:0)	-0.145	0.023	8.54E-10	3.73E-07
LPC 15:0_sn1	-0.234	0.023	9.30E-24	4.06E-21
LPC 15:0_sn2	-0.207	0.023	6.53E-19	2.85E-16

LPC 16:0_sn1 +1	0.137	0.022	3.86E-10	1.69E-07
LPC 16:0_sn2 +1	0.127	0.022	6.96E-09	3.04E-06
LPC 17:0_sn1	-0.199	0.022	4.99E-19	2.18E-16
LPC 17:0_sn2	-0.090	0.023	7.57E-05	0.0330
LPC 17:1_sn1	0.159	0.022	8.76E-13	3.83E-10
LPC 17:1_sn2	0.158	0.022	1.41E-12	6.17E-10
LPC 18:1_sn1	0.154	0.022	4.35E-12	1.90E-09
LPC 18:1_sn2	0.168	0.022	4.20E-14	1.84E-11
LPC 19:0_sn1	-0.195	0.023	2.03E-17	8.85E-15
LPC 19:0_sn2	-0.104	0.023	7.81E-06	3.41E-03
LPC 20:0_sn1	-0.102	0.023	1.03E-05	4.49E-03
LPC 20:0_sn2	-0.093	0.023	5.01E-05	0.0219
LPC 20:4_sn1	0.095	0.022	1.18E-05	5.17E-03
LPC 20:4_sn2	0.114	0.022	1.85E-07	8.07E-05
LPC 20:5_sn1	0.130	0.022	8.06E-09	3.52E-06
LPC 20:5_sn2	0.130	0.023	9.75E-09	4.26E-06
LPC 22:4_sn1	0.125	0.022	1.24E-08	5.40E-06
LPC 22:4_sn2	0.129	0.022	9.33E-09	4.08E-06
LPC O-16:0	-0.109	0.022	1.21E-06	5.29E-04
LPC O-18:0	-0.189	0.023	1.77E-16	7.73E-14
LPC O-18:1	-0.138	0.023	1.83E-09	8.02E-07
LPC O-20:0	-0.200	0.023	1.38E-17	6.03E-15
LPC O-20:1	-0.177	0.023	5.54E-14	2.42E-11
LPC O-22:0	-0.155	0.023	3.72E-11	1.63E-08
LPC O-22:1	-0.170	0.023	5.20E-13	2.27E-10
LPC O-24:2	-0.153	0.024	1.10E-10	4.82E-08
LPE 16:0_sn1	0.104	0.024	1.12E-05	4.89E-03
LPE 16:0_sn2	0.107	0.024	6.43E-06	2.81E-03
LPE 18:1_sn1	0.104	0.023	7.71E-06	3.37E-03
LPE 18:1_sn2	0.111	0.023	1.62E-06	7.08E-04
LPE 20:4_sn1	0.107	0.023	3.76E-06	1.64E-03
LPE 20:4_sn2	0.114	0.023	1.03E-06	4.49E-04
LPE 22:6_sn1	0.103	0.023	1.25E-05	5.47E-03
LPE 22:6_sn2	0.110	0.023	2.91E-06	1.27E-03
LacCer(d16:1/16:0)	-0.119	0.024	4.71E-07	2.06E-04
LacCer(d18:1/16:0)	-0.098	0.023	3.28E-05	0.0143
LacCer(d18:1/23:0)	-0.113	0.023	1.75E-06	7.67E-04
LacCer(d18:2/24:1)	-0.102	0.024	1.78E-05	7.80E-03
PC 30:0	0.102	0.023	1.30E-05	5.70E-03
PC 30:2	-0.097	0.024	4.07E-05	0.0178
PC 31:0	-0.103	0.023	1.00E-05	4.38E-03
PC 31:1	0.118	0.023	3.60E-07	1.57E-04
PC 32:2	0.165	0.023	2.02E-12	8.81E-10
PC 32:3	0.298	0.023	3.32E-38	1.45E-35
PC 33:2	-0.231	0.023	8.74E-24	3.82E-21
PC 33:3	-0.119	0.023	3.42E-07	1.49E-04

PC 34:4	0.267	0.023	2.19E-30	9.58E-28
PC 34:5	0.135	0.024	1.14E-08	4.98E-06
PC 34:1 +1	0.258	0.022	3.63E-30	1.59E-27
PC 34:2 +2	0.106	0.023	6.52E-06	2.85E-03
PC 34:3b	0.096	0.023	4.39E-05	0.0192
PC 35:0	-0.125	0.024	1.16E-07	5.06E-05
PC 35:5	-0.174	0.023	1.87E-13	8.17E-11
PC 35:2a	0.104	0.024	1.047E-05	4.57E-03
PC 35:2b	-0.116	0.024	7.89E-07	3.45E-04
PC 35:3a	-0.131	0.023	2.54E-08	1.11E-05
PC 36:6	0.155	0.023	4.18E-11	1.83E-08
PC 36:1 +1	0.094	0.024	6.95E-05	0.0304
PC 36:2 +2	0.109	0.024	4.54E-06	1.98E-03
PC 36:3b +1	0.195	0.023	1.16E-16	5.09E-14
PC 36:4a	0.181	0.023	6.38E-15	2.79E-12
PC 36:5b +1	0.302	0.023	1.15E-38	5.02E-36
PC 37:2	-0.139	0.024	4.38E-09	1.91E-06
PC 37:3	-0.150	0.024	2.40E-10	1.05E-07
PC 37:6	-0.146	0.023	1.68E-10	7.36E-08
PC 38:3 +1	0.132	0.024	2.02E-08	8.82E-06
PC 38:4c +1	0.178	0.023	2.13E-14	9.33E-12
PC 38:5a +1	0.100	0.023	1.74E-05	7.58E-03
PC 38:5b +1	0.108	0.023	3.74E-06	1.63E-03
PC 38:6c +1	0.218	0.023	1.82E-21	7.95E-19
PC 39:4	-0.117	0.024	7.50E-07	3.28E-04
PC 39:6	-0.144	0.023	6.70E-10	2.93E-07
PC 40:2	0.149	0.023	1.13E-10	4.95E-08
PC 40:4	0.145	0.023	4.07E-10	1.78E-07
PC O-32:0	-0.107	0.023	5.17E-06	2.26E-03
PC O-32:1	0.117	0.022	1.23E-07	5.38E-05
PC O-34:0	-0.175	0.023	9.78E-14	4.27E-11
PC O-34:1	-0.092	0.023	5.26E-05	0.0230
PC O-36:1	-0.148	0.023	3.41E-10	1.49E-07
PC O-36:5	-0.098	0.023	1.92E-05	8.37E-03
PC O-36:2a	-0.174	0.023	1.13E-13	4.93E-11
PC O-36:2b	-0.105	0.024	7.35E-06	3.21E-03
PC O-38:0	-0.099	0.024	2.89E-05	0.0126
PC O-38:1	-0.132	0.024	2.39E-08	1.05E-05
PC O-38:2	-0.190	0.023	1.39E-16	6.07E-14
PC O-38:3	-0.111	0.024	2.59E-06	1.13E-03
PC O-38:4b	-0.148	0.023	3.22E-10	1.41E-07
PC O-40:3	-0.144	0.023	3.76E-10	1.64E-07
PC O-40:4	-0.132	0.023	1.98E-08	8.65E-06
PC O-40:5	-0.111	0.023	2.33E-06	1.02E-03
PC P-32:1	0.246	0.021	2.32E-29	1.02E-26
PC P 36:2b	0.127	0.023	4.26E-08	1.86E-05

PC P-38:6	0.109	0.023	3.79E-06	1.66E-03
PC P-40:2	-0.149	0.023	1.26E-10	5.52E-08
PC P-40:3	-0.147	0.023	4.31E-10	1.88E-07
PC P-40:4	-0.133	0.024	2.05E-08	8.97E-06
PE 32:1	0.235	0.023	6.21E-24	2.71E-21
PE 34:1	0.172	0.023	3.45E-13	1.51E-10
PE 34:2	0.115	0.023	1.07E-06	4.67E-04
PE 36:1	0.113	0.024	1.64E-06	7.18E-04
PE 36:2	0.107	0.024	6.54E-06	2.86E-03
PE 36:4	0.159	0.023	1.23E-11	5.37E-09
PE 36:5	0.189	0.023	5.59E-16	2.44E-13
PE 38:1	0.100	0.024	2.62E-05	0.0115
PE 38:6	0.142	0.024	2.21E-09	9.65E-07
PE 38:4b	0.150	0.023	2.17E-10	9.47E-08
PE 38:5a	0.165	0.023	2.56E-12	1.12E-09
PE 38:5b	0.140	0.023	1.18E-09	5.16E-07
PE 40:4	0.161	0.023	6.81E-12	2.97E-09
PE 40:5	0.149	0.023	2.40E-10	1.05E-07
PE 40:6	0.131	0.023	2.16E-08	9.44E-06
PE 40:7	0.165	0.023	1.08E-12	4.74E-10
PE P-36:4	0.090	0.023	8.70E-05	0.0380
PE P-38:4b	0.113	0.023	1.40E-06	6.11E-04
PG 34:1	0.150	0.024	2.46E-10	1.07E-07
PG 36:1	0.183	0.023	1.12E-15	4.89E-13
PI 32:1	0.223	0.023	1.29E-21	5.63E-19
PI 34:1	0.126	0.024	8.74E-08	3.82E-05
PI 36:4	0.112	0.024	2.43E-06	1.06E-03
PI 38:3a	0.171	0.023	4.21E-13	1.84E-10
PI 38:3b	0.116	0.023	6.65E-07	2.91E-04
PI 40:4a	0.123	0.024	2.08E-07	9.10E-05
PI 40:5a	0.149	0.023	1.21E-10	5.28E-08
PI 40:5b	0.144	0.023	7.51E-10	3.28E-07
SM 30:2	-0.182	0.022	3.97E-16	1.73E-13
SM 31:1	-0.166	0.023	4.73E-13	2.07E-10
SM 31:2	-0.143	0.022	1.75E-10	7.67E-08
SM 33:1	-0.139	0.023	2.78E-09	1.22E-06
SM 33:2	-0.113	0.023	6.56E-07	2.87E-04
SM 34:0	0.095	0.023	4.57E-05	0.0200
SM 35:1	-0.146	0.023	5.21E-10	2.28E-07
SM 35:2	-0.193	0.023	1.06E-16	4.61E-14
SM 36:0	0.166	0.023	1.61E-12	7.02E-10
SM 36:3b	-0.167	0.023	7.42E-13	3.24E-10
SM 39:1	-0.135	0.023	9.53E-09	4.16E-06
SM 40:2b	-0.123	0.023	1.39E-07	6.09E-05
SM 41:1	-0.101	0.024	1.91E-05	8.34E-03
SM 41:2b	-0.179	0.023	7.96E-15	3.48E-12

SM 44:2	0.160	0.023	3.35E-12	1.46E-09
SM 44:3	0.100	0.024	2.38E-05	0.0104
TAG(14:0/16:0/18:2)	0.097	0.023	3.55E-05	0.0155
TAG(14:1/16:0/18:1)	0.107	0.024	5.85E-06	2.56E-03
TAG(14:1/16:1/18:0)	0.167	0.023	1.58E-12	6.93E-10
TAG(16:0/16:0/16:0) +1	0.155	0.023	1.54E-11	6.73E-09
TAG(16:0/16:0/18:0)	0.159	0.022	2.26E-12	9.89E-10
TAG(16:0/16:0/18:2)	0.136	0.023	3.62E-09	1.58E-06
TAG(16:0/16:1/18:1) +1	0.113	0.023	1.58E-06	6.90E-04
TAG(16:0/17:0/18:2)	0.110	0.023	1.48E-06	6.45E-04
TAG(16:0/18:0/18:1) +1	0.148	0.022	4.86E-11	2.13E-08
TAG(16:0/18:1/18:1) +2	0.095	0.023	4.00E-05	0.0175
TAG(16:1/16:1/16:1)	0.105	0.024	9.09E-06	3.97E-03
TAG(16:1/16:1/18:1) +1	0.160	0.023	1.02E-11	4.45E-09
TAG(16:1/18:1/18:1)	0.112	0.023	2.04E-06	8.91E-04
TAG(18:0/18:0/18:1)	0.099	0.022	9.23E-06	4.03E-03
TAG(18:1/18:1/22:6)	0.100	0.023	2.00E-05	8.73E-03