Special Article

Moderate alcohol consumption and lipoprotein subfractions: a systematic review of intervention and observational studies

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Context: *Moderate alcohol consumption is associated with decreased risk of cardiovascular disease (CVD) and improvement in cardiovascular risk markers, including lipoproteins and lipoprotein subfractions.* ***Objective:*** *To systematically review the relationship between moderate alcohol intake, lipoprotein subfractions, and related mechanisms.* ***Data sources:*** *Following PRISMA, all human and ex vivo studies with an alcohol intake up to 60 g/d were included from 8 databases.* ***Data extraction:*** *A total of 17 478 studies were screened, and data were extracted from 37 intervention and 77 observational studies.* ***Results:*** *Alcohol intake was positively associated with all HDL subfractions. A few studies found lower levels of small LDLs, increased average LDL particle size, and nonlinear relationships to apolipoprotein B–containing lipoproteins. Cholesterol efflux capacity and paraoxonase activity were consistently increased. Several studies had unclear or high risk of bias, and heterogeneous laboratory methods restricted comparability between studies.* ***Conclusions:*** *Up to 60 g/d alcohol can cause changes in lipoprotein subfractions and related mechanisms that could influence cardiovascular health.*
Systematic Review Registration: *PROSPERO registration no. 98955*

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide.[1] In observational studies, regular moderate consumption of alcohol (ethanol) has long been linked to a reduced risk of CVD, especially coronary heart disease (CHD).[2] A J- or U-shaped relationship between alcohol intake and incidence of CVD and type 2 diabetes has been reported in several studies.[2–4] This association is mainly seen in middle-aged men and postmenopausal women,[4,5] with a relative risk for CVD among moderate drinkers vs non-drinkers of approximately 0.80.[2,6,7] The lowest risk has been observed at intakes between 2.5 g and 14.9 g of alcohol a day ($\leq$ 1 drink/d) for most CVD outcomes spanning both sexes. However, the risk of CHD might also be reduced at higher intakes.[2,5] In some analyses, the lowest risk is observed at intakes of 1–2 drinks per day for men and 0.5–1 drink per day for women, which is sometimes termed "light-to-moderate drinking."[8] This definition is supported by the National Institute of Alcohol Abuse and Alcoholism[9] and is in concordance with the maximum recommended intake level in most countries.[10]

Data from observational studies show that the type of alcoholic beverage appears to be less critical,

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Key words: apoB-containing lipoproteins, cardiovascular disease, cholesterol efflux capacity, high-density lipoprotein cholesterol, paraoxonase.

https://doi.org/10.1093/nutrit/nuab102

indicating that ethanol itself has biological activity.[11–13] A recent meta-analysis reported similar protective effects of beer and wine consumption but not of spirits.[14] This discrepancy could be related to a higher frequency of binge drinking in people who drink spirits rather than beer and wine.[14] The effect of alcohol on high-density lipoprotein (HDL) cholesterol, proposed as a group-level alcohol intake biomarker,[15] does not seem to differ according to beverage type.[16]

The underlying, potentially protective mechanisms of alcohol intake have not been fully elucidated.[17] Among the effects of moderate alcohol consumption are changes in circulating lipoproteins,[17] which have been associated with reduced risk of CHD in both clinical and observational studies.[18] An overview of the effect of moderate alcohol consumption on the overall classes of lipoproteins and related apolipoproteins as reported in published studies, is provided in Table 1.[17,19–23]

Lipoproteins are a heterogeneous group of lipid-carrying particles in the blood that differ in size, density, composition, metabolism, and biological activity. Within each overall class of lipoprotein, there are several subfractions.[24] Findings from some studies suggest the relationship between lipoproteins and CVD risk differs according to the distribution of subclasses.[25–27] Depending on the method, subfractions are classified according to various characteristics, including density, charge, apolipoprotein composition, and particle number.[28] This heterogeneity makes interpretation of the relationship between CVD and subfractions troublesome.

There is currently no universally accepted definition of lipoprotein subfractions (LPSFs), and comparability between studies is complicated by the use of different methodologies for separating and measuring these structures. These methodologies include analytical ultracentrifugation, gradient gel electrophoresis, nuclear magnetic resonance (NMR), and ion mobility (IM) spectrometry.[24] An expert group recently suggested a uniform nomenclature for the HDL subfractions that includes definitions of subfractions measured as particle number and size, but characterization by cholesterol content or apolipoprotein composition is not included.[29]

Knowledge about the effect of moderate alcohol consumption on the LPSFs could give more insight into the mechanisms involved and provide hypotheses for a potential causal role of alcohol consumption in CVD. Studies of LPSFs are not included in the most recent meta-analysis, which covered the effect of alcohol on 13 biological markers related to CVD risk.[17] Current narrative reviews describing the relationship between alcohol intake and lipoproteins with atherosclerosis provide only a few comments on LPSFs.[22,23,27,30,31]

Nevertheless, several observational studies and short-term intervention trials have investigated the effect of alcohol consumption on LPSFs in both younger and older populations.[32–36] A thorough systematic overview of these effects, including the mechanisms by which alcohol potentially alters the LPSFs, has not been published previously, to our knowledge. The primary aim of this systematic review, therefore, was to investigate the influence of moderate alcohol consumption and regular intakes up to 60 g/d on LPSF changes and related mechanisms, and secondarily, whether changes were influenced by study design or health status.

METHODS

Review protocol and registration

This systematic review was conducted in accordance with established Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)[37,38] and reported in the PROSPERO database before the systematic search (registration no. 98955).

Eligibility criteria

English-language studies published in peer-reviewed journals were included and assessed for eligibility according to predefined PICOTS criteria (Table 2). Studies investigating oral alcohol intakes ≤ 60 g/d in human adults were included. A broad interval of alcohol intake was chosen to avoid excluding studies in which "moderate" intake was up to 60 g/d. The comparator intervention included no or low alcohol intake. There were no restrictions on diet or medication, but comparable background diets and medication protocols in the intervention and control groups were required. Studies in patients with cancer, genetic lipid disorders, or kidney, pancreatic, and liver diseases, were excluded. Studies in people with alcoholism or heavy drinkers (>60 g/d) were also ineligible.

Eligible outcomes included all types of LPSFs related to the overall classes of lipoproteins: LDL, HDL, chylomicrons, very-low-density lipoprotein (VLDL), and intermediate-density lipoprotein. The LPSF definitions described in the individual studies were used. All these definitions are listed in Table S6 in the Supporting Information online. In addition, outcomes related to the mechanisms by which alcohol could modulate any LPSF were included. Studies with LPSFs defined according to apolipoprotein content were also eligible, but overall classes of lipoproteins and apolipoproteins were excluded. Quantitative changes in the LPSFs were the primary outcome, and related mechanisms were the secondary outcome. All types of human study designs longer than 3 days and investigating the effect of the exposure or intervention were included.

Table 1 **Results from meta-analyses of the effect of moderate alcohol intake on the overall classes of lipoproteins and associated apolipoproteins**

Lipoprotein	Huang et al[19,a]	Brien et al[17,b]	Rimm et al[20,c]	Spaggiari et al [21,d]	Hannuksela et al [22,23,e]
VLDL-TG	–	–	–	–	↔ or ↑
TG[f]	↔[g]	↔	↑	↔	↔ or ↑
HDL-C	↑	↑	↑	↑	↑
LDL-C	↓	↔	↔	↔	↔ or ↓
Lp(a)[h]	–	↔	↔	–	↓
ApoA-I	↑	↑	↑	↑	↑
ApoA-II	–	–	–	–	↑
ApoB[h]	–	–	↔	–	↔ or ↓
TC	↔	↔	–	↑	–
VLDL-C	–	–	–	–	↔
IDL-C	–	–	–	–	↔

[a]Meta-analysis of intervention studies: no diagnosed CVD, diabetes, or alcohol dependence; ≤ 30 g/d alcohol (n = 2 with 0.19–0.81 g/kg/d and 0.75 g/kg/d) for ≥ 7 d; TC: n = 17, HDL-C: n = 22, apoA-I: n = 11, LDL-C: n = 17, TG: n = 22.
[b]Meta-analysis of intervention studies: No diagnosed CVD and no heavy drinking; ≤ 90 g/d alcohol for ≥ 7 d; LDL-C and TGs nonsignificantly reduced; TGs increased at > 60 g/d; TC: n = 26, HDL-C: n = 33, apoA-I: n = 16, LDL-C: n = 24, TG: n = 31.
[c]Meta-analysis of intervention studies: no diagnosed CHD, diabetes, or alcohol dependence; <100 g/d alcohol for ≥ 7 d (predicted mean change after 30 g/d used in analysis); HDL-C level increased by 0.103 mmol/L per 30 g of alcohol consumed per day; Lp(a) (n = 4) nonsignificantly decreased; HDL-C: n = 25 (36 data records), apoA-I: 24 data records. ApoB and LDL-C analyses not specified.
[d]Meta-analysis of beer consumption in controlled intervention studies: Healthy, overweight, high cardiovascular risk, hypertension, or healthy; ≤ 41 g/d beer intake; acute studies (n = 5) and ≥ 3 wk; TC: n = 14, HDL-C: n = 18, apoA-I: n = 5, LDL-C: n = 12.
[e]Results from 2 extensive narrative reviews with different study designs. No dose definition available. ApoB might only be reduced at higher intakes.
[f]A review reports a J-shaped relationship to alcohol intake, nadir at intakes of 10–20 g/d (~1–2 drinks).[238]
[g]→: unchanged, ↑: increased, ↓: reduced, –: not investigated.
[h]Investigated in few studies only.
Abbreviations: Apo, apolipoprotein; C, cholesterol concentration; CVD, cardiovascular disease; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); VLDL, very-low-density lipoprotein; TC, total cholesterol; TG, triglycerides.

Table 2 **PICOTS criteria for eligibility of studies**

Criterion	Description
Population	Human adults ≥ 18 years of age, including healthy people and those at high risk of cardiovascular disease (eg, people with type 2 diabetes, hyperlipidemia, increased waist circumference, increased fasting blood glucose or reduced glucose tolerance, atherosclerosis, hypertension, metabolic syndrome. Individuals taking lipid-lowering drugs were eligible.
Intervention/exposure	Oral consumption of ethanol ≤ 60 g/d. Comparable background diet and medication use in compared groups
Comparison	No or low alcohol intake
Outcomes	Quantitative changes in lipoprotein subfractions and related physiological mechanisms
Timing	Any intervention or exposure period > 3 days, any follow-up period
Study design	All types of designs in humans

Despite being part of the initial aim and search strategy (Supplementary Methods in the Supporting Information online), we chose to exclude studies in which overall levels of apolipoproteins were investigated, in addition to animal, cell, and postprandial studies, because of the vast number of additional articles. This decision was made prior to data extraction and before the authors had any knowledge about the study outcomes.

Literature search strategy

A comprehensive systematic literature search was performed in April 2018 of 8 bibliographic databases (Figure 1) according to the predefined eligibility criteria. The search was updated in March 2021, spanning 2018 through March 2021. An email alert was created in the central databases, continuously informing the researchers of recent publications according to the search criteria. In addition, we screened the ClinicalTrials.gov database (www.clinicaltrials.gov) for unpublished literature. The search terms were constructed in blocks and related to exposure (alcohol consumption) and outcomes (LPSFs and mechanisms). Hand searching of reference lists in the included reviews was also performed. The complete search strategy is provided in the Supplementary Methods in the Supporting Information online. The process was verified by an experienced health sciences research librarian and agreed upon by all 4 authors.

Study selection

The study selection was performed in 2 phases after duplicates had been removed in Endnote X8.2[39] and

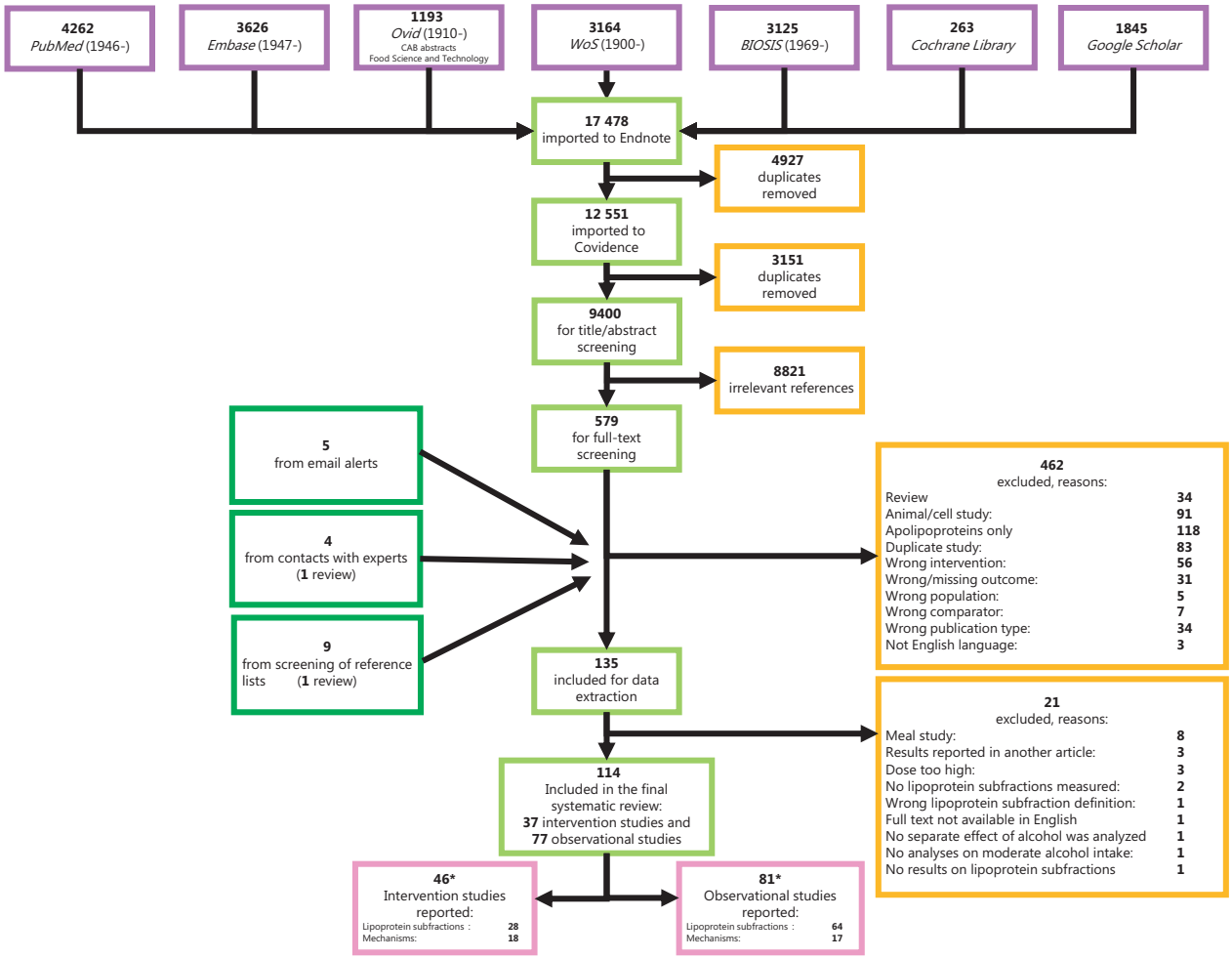

Figure 1 **Flowchart of the systematic literature process.** *Some articles include outcomes related to both lipoprotein subfractions and mechanisms (n = 13) and are reported twice in this review. Thus, 114 individual papers were included, but 127 data sets were reported. *Abbreviations:* EMBASE, Excerpta Medica Database; WoF, Web of Science.

Covidence.[40] Three independent researchers (T.L.W., J.N.E., and K.T.) screened all identified articles for eligibility on the basis of titles and abstracts. Articles that met the inclusion criteria, or articles with uncertain eligibility, were included for full-text screening. In the second phase, screening of articles at the full-text level was undertaken independently by 2 researchers (T.L.W. and K.T.). Any lack of consensus between the authors was settled by a third author (either J.N.E. or L.O.D.) until consensus was reached. Contact by a single email to study authors was made in case of missing or incomplete data.

Data collection and items

Two independent researchers (T.L.W. and K.T.) performed data extraction, and all 4 authors discussed any disagreements. A data extraction form was piloted on the different study designs before data collection. The following data were extracted: first author; publication year; country; sample size and dropouts; age distribution; sex distribution; health status; study design; alcohol intervention or exposure; laboratory measurements; statistical analyses of LPSFs and covariates; and LPSFs outcomes, including direction of the results. Missing data were retrieved via a single email to the authors; we state in the accompanying tables if data proved inaccessible.

Bias risk assessment

The methodological quality of the included articles was assessed independently by 2 researchers (T.L.W. and K.T.). Disagreements were discussed with the remaining authors (J.N.E. and L.O.D.). Assessment was performed at study level and according to the specific study design. For randomized controlled trials (RCTs), The Cochrane Collaboration tool for assessing the risk of bias was used.[41] The Risk of Bias Assessment tool for Nonrandomized Studies (RoBANS)[42] was used for

nonrandomized investigations, and the JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies[43] was used for cross-sectional studies. Blinding, by default, was scored as high risk of bias in all intervention studies, because this is not believed to be feasible for alcohol consumption.[44,45] Similarly, self-reported alcohol intake was classified as high risk of bias in observational studies because self-reported alcohol intake is regarded an inaccurate methodology, compared with objective biomarkers.[46]

Data synthesis and analysis

Alcohol intake in grams per day was used and recalculated from grams or ounces per week, when necessary. Amounts reported in grams per kilogram body weight were recalculated on the basis of a standard person weight of 70 kg. Frequency measures were not converted. In observational studies, the alcohol intake was classified into levels and handled as categorical or continuous variables. The lowest alcohol-intake group was often used as the reference group, except in studies using multivariate analysis. It was impossible to delimit the alcohol intake to exactly ≤ 60 g/d, because of the group classifications and the corresponding statistical analyses in the included observational studies. The individual LPSFs were not extracted in absolute values; instead they are described with arrows. Vertical arrows indicate significant increases or decreases ($P < 0.05$ or significance level used by the study). Horizontal arrows indicate nonsignificant effects. Arrows in brackets indicate trends defined by the review authors (P range, > 0.05 to ≤ 0.1). Outcomes were preferably based on both sexes, but results of each sex separately were included if a combined outcome was not available. Presumed multiple reports from the same study are reported individually but listed after each other in the final table. NMR- and IM lipoprotein outcomes are provided in particle number concentration, abbreviated -P.

RESULTS

Study selection

The number of articles retrieved from the individual databases is shown in Figure 1. A total of 17 478 articles were identified. After removing duplicates, and title and abstract screening was conducted, 579 articles were initially included for full-text screening. The interrater agreement at this stage was moderate ($\kappa = 0.59$). Additional articles were retrieved from various sources (Figure 1). Hence, 597 papers were screened at the full-text level, where the interrater agreement was high ($\kappa = 0.82$), and a total of 114 articles were included. These were 37 intervention studies and 77 observational studies, totaling 20 510 and 104 773 participants, respectively.

The characteristics of the included studies are outlined in Tables S1–S4 in the Supporting Information online. The vast number of citations in this review reduce readability; therefore, the reader is directed to Table S5 in the Supporting Information online, which lists specific statements supported by multiple articles included in this review, with the appropriate references. Table 3,[35,36,47–70] Table 4,[33,71–123] Table 5,[13,32,124–131] and Table 6[16,49,50,52,53,59–62,64,84,104,112,114,132–152] provide an overview of the outcomes from each study based on the distinct types of LPSFs, as illustrated in Figure 2.

Study characteristics and participants

Results from the individual LPSF studies are shown in Tables S1 and S2 in the Supporting Information online. LPSF outcomes were measured in 28 intervention studies, shared among 14 RCTs, 3 nonrandomized trials,[52,56,62] 9 nonrandomized studies with sequential crossover, and 2 Mendelian randomization studies (MRSs).[66,67] We included 64 observational LPSF studies, the majority of which were cross-sectional. Würtz et al[126] performed a cross-sectional analysis but also captured metabolic changes at a 6-year follow-up examination. In addition, 2 cohort studies[102,118] and 4 case-control studies were included. In all case-control studies and 1 cohort study,[102] the alcohol data extracted were from cross-sectional analyses. Last, 1 RCT study is listed among the observational studies, because the only available data on alcohol and LPSFs were cross-sectional and from baseline.[131] Multiple reports from the same study were identified; these redundant entries are combined in Table S2 in the Supporting Information online as Kee et al[107] and Marques-Vidal et al[108]; Luc et al[154] and Marques-Vidal et al (2001)[109]; Onat et al (2003)[105] and Onat et al (2009)[102]; and Rossouw et al[90] and Steenkamp et al.[76]

Intervention studies: characteristics. The intervention studies with LPSF outcomes were published between 1983 and 2017, and study size varied between 5 and 112 participants (Table S1 in the Supporting Information online). The 2 MRSs with 8,400–10,900 participants are listed with the intervention studies. Overall, the age of the participants ranged between 18 and 75 years, and the majority of studies included healthy participants only. Three studies included women only[48,51,57] and 17 studies included only men. The shortest duration of the alcohol interventions was 10 days; the longest was 6 months. Alcohol was provided in amounts from 12.6 to 60 g/d, but the majority of studies provided 20–

Table 3 **Summary of results of alcohol intake on lipoprotein subfractions from all intervention studies**

Lipoprotein subfraction	Increased	No change	Decreased
HDL_2-C	**Burr et al, 1986[36]; Clevidence et al, 1995[57]; Hartung et al, 1990[64,a]; Kaul et al, 2010[65]; Masarei et al, 1986[35,b]; Tabara et al, 2017[66,c]; Vu et al, 2016[67,d];** Hartung et al, 1993[68,e]; McConnell et al, 1997[69]	**Moore et al, 1988[70]; Pikaar et al, 1987[47,f]; Rumpler et al, 1999[48,g]; Senault et al, 2000[49,h];** Hagiage et al, 1992[50,i]; Hartung et al, 1986[51,j]; Nishiwaki et al, 1994[52]; Välimäki et al, 1988[53,k]; Välimäki et al, 1991[54]	**Cox et al, 1993[55,l]**
HDL_2-TM		**Haskell et al, 1984[34,m];** Fraser et al, 1983[56]; Hagiage et al, 1992[50,i]; Välimäki et al, 1988[53,k]; Välimäki et al, 1991[54]	
HDL_{2a}-TM			Bertière et al, 1986[58]
HDL_{2b}-TM		Bertière et al, 1986[58]	
HDL_3-C	**Clevidence et al, 1995[57]; Hartung et al, 1990[64,a]; Masarei et al, 1986[35,b]; Senault et al, 2000[49,h]; Tabara et al, 2017[66,c];** Nishiwaki et al, 1994[52]; Välimäki et al, 1988[53,k]; Välimäki et al, 1991[54]	**Burr et al, 1986[36]; Kaul et al, 2010[65]; Moore et al, 1988[70]; Pikaar et al, 1987[47,f]; Rumpler et al, 1999[48,g]; Vu et al, 2016[67,d];** Hartung et al, 1986[51,j]; Hartung et al, 1993[68,e]; McConnell et al, 1997[69]	**Cox et al, 1993[55,l];** Hagiage et al, 1992[50i]
HDL_3-TM	Bertière et al, 1986[58]; Välimäki et al, 1988[53,k]	Fraser et al, 1983[56]; Hagiage et al, 1992[50,i]; Välimäki et al, 1991[54]	**Haskell et al, 1984[34,m]**
VHDL	Bertière et al, 1986[58]		
Pre-β-HDL	**Beulens et al, 2004[59,n]**		
LpA-I	**Beulens et al, 2004[59,n]; Clevidence et al, 1995[57]; Senault et al, 2000[49,h];** Välimäki et al, 1991[54]	**Gottrand et al, 1999[60];** Hagiage et al, 1992[50,i]; Serdyuk et al, 2000[61,o]	

(continued)

Table 3 **Continued**

Lipoprotein subfraction	Increased	No change	Decreased
LpA-I:A-II	**Beulens et al, 2004**[59,n]**; Clevidence et al, 1995**[57]**; Gottrand et al, 1999**[60]**; Senault et al, 2000**[49,h]; Välimäki et al, 1991[54]	Hagiage et al, 1992[50,i], Serdyuk et al, 2000[61,o]	
HDL size	**Clevidence et al, 1995**[57]	**Mukamal et al, 2017**[44,p], De Oliveira E Silva et al, 2000[62]	
$VLDL_1$-C		**Kaul et al, 2010**[65]	
$VLDL_2$-C		**Kaul et al, 2010**[65]	
$VLDL_3$-C		**Kaul et al, 2010**[65]	
LDL size	Sharpe et al, 1995[63]	**Mukamal et al, 2017**[44,p]	
sdLDL-C	**Tabara et al, 2017**[66,c]		**Vu et al, 2016**[67,d]
lbLDL-C			**Tabara et al, 2017**[66,c]

Summary of results based on each lipoprotein subfraction investigated. Randomized controlled trials are indicated with bold text. A more detailed description of each study can be found in Table S1 in the Supporting Information online.

[a]Runners, inactive people, and drinkers of 15 g/d and 45 g/d alcohol. Participants were randomized to 1 or 3 beers in each activity category. Here, the results represent within-group changes (ie, the effect of moderate to low vs no alcohol consumption).

[b]Multiple linear regression analysis (adjusted for weight).

[c]A Mendelian randomization study; results represent men. Results for women were similar except no change in sdLDL was found.

[d]A Mendelian randomization study; results represent all drinkers compared with abstainers. From the 2-stage least squares regression analysis (adjusted for age and sex).

[e]Inactive participants. Results for runners (HDL2-C unchanged and HDL3-C increased) are reported in the text, but no *P* values are provided.

[f]Represent 23 g/d and 46 g/d vs 0 g/d alcohol.

[g]The low-fat diet group. The high-fat diet group: HDL2-C increased and HDL3-C unchanged.

[h]The red wine group: 30 g/d alcohol. LpA-I did not increase in the 30 g/d pure alcohol-with-mineral-water group. A randomized controlled trial, but only within-group results provided in the article.

[i]The nonobese subgroup. No significant changes in the obese participants. Results represent within-group changes. Changes between groups (obese vs control participants) were examined but not extracted.

[j]Inactive participants. Results for runners were similar.

[k]Represent 30 g/d and 60 g/d alcohol compared with abstention, respectively, because results are similar in both periods.

[l]Alcohol restriction compared with habitual, moderate alcohol intake.

[m]From 6 wk of intervention: 0 g/d vs 30 g/d alcohol.

[n]Brackets refer to a trend: $P > 0.05$ to ≤ 0.1.

[o]Subgroup with the lowest HDL level. The subgroup with high HDL ($\geq$ 50 mg/dL): LpA-I increased.

[p]A 6-month randomized controlled trial: no effect on any LPSF, including particle size (low compliance reported). Results on specific subfractions are not reported in the article but are reported for HDL size and LDL size here as examples.

Abbreviations: C, cholesterol concentration; HDL, high-density lipoprotein; lbLDL, large, buoyant low-density lipoprotein; LDL, low-density lipoprotein; LpA-I, HDL particles with apolipoprotein A1; LpA-I:A-II, HDL particles with apolipoprotein A1 and apolipoprotein A2; sdLDL, small, dense low-density lipoprotein; TM, total mass concentration; VHDL, very-high-density lipoprotein; VLDL, very-low-density lipoprotein.

Table 4 **Summary of results of alcohol intake effects on lipoprotein subfractions from observational studies with electrophoresis, ultracentrifugation, precipitation, immunologic, and enzymatic methods**[a]

Lipoprotein subfraction	Increased	No change	Decreased
HDL_2-C	Fulton-Kehoe et al, 1992[71,b]; Gardner et al, 2000[82,c]; Gaziano et al, 1993[93]; Ito et al, 1995[104]; Kim et al, 2014[115]; Lupien et al, 1988[120,d]; Meilahn et al, 1988[121]; Miller and Gilson, 1981[122,e]; Miller et al, 1988[123]; Moriyama and Takahashi, 2014[72,f]; Mänttäri et al, 1991[73]; Parlesak et al, 2014[33,g]; Patsch et al, 1992[74]; Robinson et al, 1987[75,h]; Steenkamp et al, 1990[76,i]; Rywik et al, 2011[77,ii]; Volcik et al, 2008[78,j]; Walton et al, 1995[79]	Andrade et al, 1990[80,k]; Bergmann et al, 1997[81,l]; Diehl et al, 1988[83,m]; Haffner et al, 1985[84,n]; Lakshman et al, 1996[85,o]; Marti et al, 1989[86,p]; Meilahn et al, 1991[87,]; Momoseet al, 1994[88,q]; Razay et al, 1992[89]; Rossouw et al, 1992[90,]; Sillanaukee et al, 1993[91,r]; Sillanaukee et al, 2000[92,s]; Vasisht et al, 1992[94,t]; Woo and Lam, 1990[95]	Skoczyńska et al, 2013[96]
HDL_{2a}-C	Schäfer et al, 2007[97]		
HDL_{2b}-C	Schäfer et al, 2007[97]		
HDL_2-TM		Williams et al, 1985[98,u]	
HDL_{2a}-TM	Williams et al, 1993[99]		
HDL_{2b}-TM		Williams et al, 1993[99]	
HDL_3-C	Bergmann et al, 1997[81,l]; Burke et al, 1992[100,uu]; Diehl et al, 1988[83,m]; Fulton-Kehoe et al, 1992[71,b]; Gardner et al, 2000[82,c]; Gaziano et al, 1993[93]; Haffner et al, 1985[84,n]; Ito et al, 1995[104]; Kim et al, 2014[115]; Lupien et al, 1988[120,d]; Meilahn et al, 1988[121]; Miller et al, 1988[123]; Momoseet al, 1994[88,q]; Moriyama and Takahashi, 2014[72,f]; Mänttäri et al, 1991[73]; Patsch et al, 1992[74]; Razay et al, 1992[89]; Robinson et al, 1987[75,h]; Steenkamp et al, 1990[76,i]; Rywik et al, 2011[77,ii]; Schäfer et al, 2007[97]; Sillanaukee et al, 1993[91,r]; Sillanaukee et al, 2000[92,s]; Volcik et al, 2008[78,j]; Walton et al, 1995[79]	Andrade et al, 1990[80,k]; Marti et al, 1989[86,p]; Meilahn et al, 1991[87,]; Miller and Gilson, 1981[122,e]; Parlesak et al, 2014[33,g]; Rossouw et al, 1992[90,]; Skoczyńska et al, 2013[96],Vasisht et al, 1992[94,t]; Woo and Lam, 1990[95]	
HDL_3-TM	Williams et al, 1985[98,u]		
HDL_{3a}-TM	Williams et al, 1993[99]		
HDL_{3b}-TM		Williams et al, 1993[99]	
HDL_{3c}-TM		Williams et al, 1993[99]	
HDL-C + apoC-III	Jensen et al, 2012[101]; Onat et al, 2003[105,v]; Onat et al, 2009[102,w]	Koch et al, 2017[103]	
HDL-C - apoC-III	Jensen et al, 2012[101]; Koch et al, 2017[103]		
Non-HDL + apoC-III	Onat et al, 2003[105,v]	Onat et al, 2009[102,w]	
LpA-I	Branchi et al, 1997[106,$; Kee et al, 1995107,x]; Marques-Vidal and Al, 1995[108]; Marques-Vidal et al, 2001[109,y]	Luc et al, 2002[110]; Lyu et al, 2018[111]; Mansfield, McPherson and Koski, 1999[112,z]; Perret et al, 2002[114,aa]; Steinmetz et al, 1990[113]	Puchois et al, 1990[116]
LpA-I:A-II	Branchi et al, 1997[106,bb]; Marques-Vidal and Al, 1995[108]; Luc et al, 2002[110]; Perret et al, 2002[114,aa]; Puchois et al, 1990[116]	Kee et al, 1995[107,x]; Lyu et al, 2018[111]; Steinmetz et al, 1990[113]	
LDL-IVB		Williams and Krauss, 1997[117,cc]	
LDL-IVA		Williams and Krauss, 1997[117,cc]	
LDL-IIIB		Williams and Krauss, 1997[117,cc]	
LDL-IIIA		Williams and Krauss, 1997[117,cc]	
LDL-II		Williams and Krauss, 1997[117,cc]	
LDL-I	Williams and Krauss, 1997[117,cc]		
ISL		Williams and Krauss, 1997 [117,cc]	

(continued)

Table 4 **Continued**

Lipoprotein subfraction	Increased	No change	Decreased
sdLDL-C			Parlesak et al, 2014[33,g]
lbLDL-C		Parlesak et al, 2014[33,g]	
LDL size			McNamara et al, 1992[118,dd]
LpE:B		Marques-Vidal and Al, 1995[108], Marques-Vidal et al, 2001[109,y]	Schiele et al, 2002[119,ee]
LpE:non-B			Schiele et al, 2002[119,ee]
LpC-III:B		Marques-Vidal and Al, 1995[108]	

Summary of results based on each lipoprotein subfraction investigated. A more detailed description on each study can be found in Table S2 (in the Supporting Information online).

[a] $P < 0.05$.

[b] Multiple regression (adjusted for age, body mass index, subscapular-to-triceps ratio, physical activity, smoking, fasting insulin, and use of β-blockers). Results represent female drinkers (intake, $\geq$ 50 g/d). In male drinkers of $\geq$ 50 g/d, HDL2-C results not available, HDL3-C increased. Alcohol intake of men and women $<$ 50 g/d compared with nondrinkers: no changes (results on HDL2-C not available for men).

[c] Women. In men, no change in HDL2-C, HDL3-C increased,

[d] Analysis of covariance (adjusted for age, weight, and smoking).

[e] Men. Results for women not available.

[f] Results from the multiple linear regression analysis (adjusted for sex, waist circumference, exercise, and smoking). Results from the analysis of variance were similar.

[g] Per response from study authors, there was no significant association between alcohol and HDL3-C.

[h] Multiple linear regression (adjusted for age, body mass index, waist-to-hip ratio, smoking, and physical activity). Results represent alcohol intake $<$ 13.4 g/d compared with abstention. Drinkers of $>$ 13.4 g/d compared with abstainers: HDL2-C was unchanged, HDL3-C increased.

[i] Multiple linear regression (adjusted for body mass index, triglycerides, smoking, CHO intake), in men. In women: HDL2-C increased, HDL3-C unchanged.

[ii] Men and women in the United States. Participants from Poland: in men, both subfractions were increased; in women, there was no change in any subfraction.

[j] White women and men. For the Black participants in all subgroups and for both sexes: HDL3-C increased, HDL2-C was unchanged.

[k] Similar results when those drinking alcohol $\geq$ 5 to $\leq$ 20 g/d and $>$ 20 to $<$ 70 g/d were compared with abstainers.

[l] Multiple linear regression (adjusted for age, body mass index, blood pressure, menopausal status, energy intake, cholesterol intake, saturated fatty acid, smoking, and physical activity).

[m] Multiple regression (adjusted for age, body mass index, smoking, physical activity, triglycerides, and social class). Results similar in men and women.

[n] Pearson's partial correlation and (adjusted for sex, body mass index, smoking, and VLDL-triglycerides), men and women combined.

[o] HDL3-C results not available.

[p] Women. In men, HDL3-C levels were increased, HDL2-C was unchanged.

[q] Men. Results for women were not available.

[r] Alcohol intake of $\geq$40 g/d compared with abstention. No association found when $<$ 40 g/d was compared with abstention.

[s] Results represent unpaired *t* tests: $>$20–40 g/d and $>$ 40 g/d compared with 0–10 g/d. When $>$ 10–20 g/d alcohol intake was compared with the 0–10 g/d, there was no change in HDL3-C levels and HDL2-C levels decreased.

[t] Alcohol intake of approximately 12 g/d (21 g/d for 3–5 d/wk) compared with abstention. Intakes of 44 g/d (62 g/d for 4–6 d/wk) compared with abstention: HDL2-C levels increased, HDL3-C levels were unchanged.

[u] Multiple regression analysis (adjusted for starch and sucrose). Partial Spearman's correlation analyses (adjusted for smoking, adiposity, and physical) returned similar results.

[uu] Results on HDL2-C not available.

[v] Results for men from the correlation analysis. In women, findings were not significant.

[w] Results for men and women.

[x] Alcohol intake $>$ 10 g/d compared with abstainers. Alcohol intake $<$ 10 g/d compared with abstainers: no change in any HDL subfraction.

[y] LpA-I increased in participants from France and participants from Northern Ireland, and by all beverage types (wine, beer, and spirits), except in drinkers of spirits in France. LpE:B levels were unchanged after all beverage types except beer. LpE:B levels increased after drinking beer.

[z] Model 1 (energy, protein, carbohydrates, fat [saturated, monounsaturated, and polyunsaturated], alcohol intake, waist-to-hip ratio, body mass index, and aerobic capacity). LpA-I:A-II result not available.

[aa] Alcohol intake $\leq$ 35 g/d compared with abstainers. Drinkers of $>$ 35 g/d compared with abstainers: no significant change was found in any HDL subfraction.

[bb] Multiple linear regression (adjusted for age, body mass index, physical activity, triglycerides, and dietary variables).

[cc] LDL-I: $\sim$ large LDL, LDL-II: $\sim$ intermediate LDL, LDL-III plus LDL-IV: $\sim$ small dense LDL.

[dd] Brackets refer to a trend: $P > 0.05$ to ≤ 0.1.

[ee] Women. In men, no change in any LPSF.

Pearson's partial correlation (adjusted for smoking, physical activity, blood pressure, triglycerides, and body mass index).

Linear regression analysis, in women. In men, HDL2-C and HDL3-C levels increased.

Abbreviations: C, cholesterol concentration; HDL, high-density lipoprotein; ISL, intermediate-size lipoprotein; lbLDL, large, buoyant low-density lipoprotein; LDL, low-density lipoprotein; LpA-I, HDL particles with apolipoprotein A1; LpA-I:A-II, HDL particles with apolipoprotein A1 and apolipoprotein A2; LpE:B, apoB-containing lipoproteins with apoE; LpE:non-B, non–apoB-containing lipoproteins with apoE; LpC-III:B, apoB-containing lipoproteins with apoC-III; LPSF, lipoprotein subfraction; sdLDL, small, dense low-density lipoprotein; TM, total mass concentration; VLDL, very-low-density lipoprotein.

Table 5 **Summary of results on lipoprotein subfractions from observational studies with nuclear magnetic resonance and ion mobility methods**[a]

Lipoprotein subfraction	Increased	No change	Decreased
Total HDL-P	Mukamal et al, 2007[124]; Muth et al, 2010[32,b]; Zaid et al, 2018[125,c]		Millar et al, 2020[127]
Very large HDL-P	Du et al, 2020[13]; Würtz et al, 2016[126]	Si et al, 2021[128]	
Large HDL-P	Du et al, 2020[13,d]; Mukamal et al, 2007[124]; Muth et al, 2010[32,bSi et al, 2021128]; Sonestedt et al, 2012[129,e]; Würtz et al, 2016[126]; Zaid et al, 2018[125,c]	Bogl et al, 2013[130]; Tighe et al, 2013[131]	Millar et al, 2020[127]
Medium HDL-P	Bogl et al, 2013[130]; Du et al, 2020[13,d]; Mukamal et al, 2007[124]; Muth et al, 2010[32,b]; Si et al, 2021[128]; Tighe et al, 2013[131]; Würtz et al, 2016[126]	Zaid et al, 2018[125,c]	Millar et al, 2020[127]
Small HDL-P	Bogl et al, 2013[130]; Du et al, 2020[13,d]; Si et al, 2021[128]; Würtz et al, 2016[126]	Millar et al, 2020[127]; Muth et al, 2010[32,b]; Sonestedt et al, 2012[129,e]; Zaid et al, 2018[125,c]	Mukamal et al, 2007[124]; Tighe et al, 2013[131]
HDL particle size	Du et al, 2020[13,d]; Mukamal et al, 2007[124]; Muth et al, 2010[32,b]; Würtz et al, 2016[126] Zaid et al, 2018[125,c]	Bogl et al, 2013[130]; Si et al, 2021[128]	Millar et al, 2020[127]
HDL_2-C	Du et al, 2020[13,d]; Si et al, 2021[128]; Würtz et al, 2016[126]		
HDL_3-C	Si et al, 2021[128]; Würtz et al, 2016[126]	Du et al, 2020[13]	
Total LDL-P		Millar et al, 2020[127]; Mukamal et al, 2007[124],↻Δ	
Large LDL-P	Mukamal et al, 2007[124]	Bogl et al, 2013[130]; Millar et al, 2020[127]; Si et al, 2021[128]; Sonestedt et al, 2012[129,e]; Tighe et al, 2013[131]	Würtz et al, 2016[126]
Medium LDL-P	Bogl et al, 2013[130]	Du et al, 2020[13]; Si et al, 2021[128]; Würtz et al, 2016[126]	Sonestedt et al, 2012[129e]
Small LDL-P	Bogl et al, 2013[130]	Du et al, 2020[13]; Millar et al, 2020[127]; Si et al, 2021[128]; Sonestedt et al, 2012[129,e]; Tighe et al, 2013[131]; Würtz et al, 2016[126]	Mukamal et al, 2007[124]
Medium small LDL-P		Tighe et al, 2013[131]	
Very small LDL-P		Sonestedt et al, 2012[129e]; Tighe et al, 2013[131]	
LDL particle size	Mukamal et al, 2007[124]; Sonestedt et al, 2012[129e]	Millar et al, 2020[127]	Bogl et al, 2013[130]; Du et al, 2020[13]; Si et al, 2021[128]; Würtz et al, 2016[126]

(continued)

Table 5 **Continued**

Lipoprotein subfraction	Increased	No change	Decreased
Large IDL-P		Du et al, 2020[13]	
Total IDL-P		Si et al, 2021[128]	Du et al, 2020[13]; Würtz et al, 2016[126]
Total VLDL-P			Mukamal et al, 2007[124]
Extra-large VLDL-P	Si et al, 2021[128]; Würtz et al, 2016[126]	Du et al, 2020[13]	
Very large VLDL-P	Si et al, 2021[128]; Würtz et al, 2016[126]	Du et al, 2020[13]	
Large VLDL-P	Bogl et al, 2013[130]; Si et al, 2021[128]; Würtz et al, ., 2016[126]	Du et al, 2020[13]; Mukamal et al, 2007[124],↻Δ, Tighe et al, 2013[131]	Millar et al, 2020[127]
Medium VLDL-P	Bogl et al, 2013[130]; Si et al, 2021[128]; Würtz et al, 2016[126]	Millar et al, 2020[127]; Tighe et al, 2013[131]	Du et al, 2020[13,d]; Mukamal et al, 2007[124]
Small VLDL-P	Bogl et al, 2013[130]; Millar et al, 2020[127]; Si et al, 2021[128]	Tighe et al, 2013[131]; Würtz et al, 2016[126]	Du et al, 2020[13,d], Mukamal et al, 2007[124]
Very small VLDL-P		Si et al, 2021[128]; Würtz et al, 2016[126]	Du et al, 2020[13]
VLDL particle size	Bogl et al, 2013[130]; Mukamal et al, 2007[124]; Si et al, 2021[128]; Würtz et al, 2016[126]		Du et al, 2020[13]; Millar et al, 2020[127]

Summary of results based on each lipoprotein subfraction investigated. A more detailed description on each study can be found in Table S2 in the Supporting Information online.

[a]$P < 0.05$.

[b]Multiple linear regression analysis (adjusted for hormone replacement therapy, body mass index, diabetes, smoking, exercise, age). Results similar for men and women. Analysis of variance (ANOVA): Quadratic trend in medium HDL-P and HDL particle size (Δ) and in small HDL-P (↻). Total HDL-P was not included in the regression analysis, so ANOVA results are reported here.

[c]Women. In men, all HDL subfractions, total HDL-P, and mean HDL-P size increased. Brackets refer to a trend: $P > 0.05$ to ≤ 0.1.

[d]Significant changes in linear and polynomial regression and after adjustment for age, sex, socio-economic status, region of residence, education, occupation, marital status, smoking, physical activity, cardiorespiratory fitness, diet, depression, and anxiety (model 2).

[e]Women. In men, there was no change in medium LDL and LDL particle size; small HDL levels increased.

[f]Significant quadratic relationships and nonsignificant linear relationships in these outcomes. For some of the other outcomes, significant quadratic relationships are also found, but the linear relationship are reported here due to lower *P* values.

Abbreviations: C, cholesterol concentration; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; P, particle number concentration; VLDL, very-low-density lipoprotein.

Table 6 **Summary of results on mechanisms from intervention and observational studies**[a,b]

Mechanism	Increased	No change	Decreased
Cholesterol efflux capacity	**Beulens et al, 2004**[59,m]; **Králová Lesná et al, 2010**[132,c]**Padro et al, 2018**[133]; **Senault et al, 2000**[49,d]; **Sierksma et al, 2004**[134]; **van der Gaag et al, 2001**[135]; Hoang et al, 2008[136,e]; Koekemoer et al, 2017[137,f],Saleheen et al, 2015[138]	**Serdyuk et al, 2000**[61,g]; Perret et al, 2002[114]	
ABCA1 expression	Hoang et al, 2008[136,e]		
LCAT: activity	**Beulens et al, 2004**[59]; **Serdyuk et al, 2000**[61,g]	**Nishiwaki et al, 1994**[52,h]; Ito et al, 1995[104]; Riemens et al, 1997[139]	
LCAT: mass	Goto et al, 2003[140]	Albers et al, 1982[141,i]; Haffner et al, 1985[84]	
LCAT: serum/plasma cholesterol esterification	**van der Gaag et al, 2001**[135]; Perret et al, 2002[114]	**Sierksma et al, 2004**[134]	**Serdyuk et al, 2000**[61,g]
CETP: activity		**Beulens et al, 2004**[59]; **Nishiwaki et al, 1994**[52],h; **Serdyuk et al, 2000**[61,g]; Alarcon et al, 2004[142]; Vergeer et al, 2010[143]; Dullaart et al, 1998[144]; Ito et al, 1995[104]; Riemens et al, 1997[139]	Kinoshita et al, 1996[153]
CET activity: cholesterol transfer to apoB lipoproteins; process of CETP	**Serdyuk et al, 2000**[61,g]	**Sierksma et al, 2004**[134]; Perret et al, 2002[114]	**Hagiage et al, 1992**[50,j]
CETP: concentration		Senault et al, 2000[49,d]	
CETP: mass		Goto et al, 2003[140]; Mansfield, McPherson and Koski, 1999[112,k]	
PLTP activity		**Beulens et al, 2004**[59]; Alarcon et al, 2004[142]; Riemens et al, 1997[139]	
Lp-PLA$_2$ activity		**Beulens et al, 2008**[145]	Hatoum et al, 2010[146]
HL activity		**Hartung et al, 1990**[64]; **Nishiwaki et al, 1994**[52,h]; **Välimaki et al, 1988**[53]; Alarcon et al, 2004[142]	De Oliveira e Silva et al, 2000[62]
LPL activity	**Nishiwaki et al, 1994**[52,h]; Alarcon et al, 2004[142]; De Oliveira E Silva et al, 2000[62]	**Hartung et al, 1990**[64]; **Välimäki et al, 1988**[53]	
LPL mass	**Nishiwaki et al, 1994**a[52,h]	Goto et al, 2003[140]	
Specific activity of LPL[l]		**Nishiwaki et al, 1994**[52,h]	
ApoA-I TR	De Oliveira e Silva et al, 2000[62]		

(continued)

Table 6 **Continued**

Mechanism	Increased	No change	Decreased
ApoA-I PR		**Gottrand et al, 1999**[60]	
ApoA-I FCR		De Oliveira e Silva et al, 2000[62]; **Gottrand et al, 1999**[60]	
ApoA-II TR	De Oliveira e Silva et al, 2000[62]		
ApoA-II PR	**Gottrand et al, 1999**[60]		
ApoA-II FCR		De Oliveira e Silva et al, 2000[62]	**Gottrand et al, 1999**[60]
PON activity	**Rajdl et al, 2007**[147]; **Sierksma et al, 2002**[148]; **van der Gaag et al, 1999**[16]; Gruppen et al, 2018[149]; Rao et al, 2003[150]; Sirivarasai et al, 2011[151]	**Sarandöl et al, 2003**[152]	
PON mass		**Sierksma et al, 2002**[148]; **van der Gaag et al, 1999**[16]	

Summary of results based on each mechanism investigated. A more detailed description on each study can be found in Tables S3 and S4 in the Supporting Information online.

[a]Intervention studies are in bold text.

[b]$P < 0.05$.

[c]Brackets refer to a trend: $P > 0.05$ to ≤ 0.1.

[d]The red wine group: 30 g/d alcohol. The 30 g/d pure alcohol-with-mineral-water group: no change in CETP concentration and cholesterol efflux. A randomized controlled trial, but only within-group results provided in the article.

[e]Alcohol intake $>$ 11 g/d alcohol compared with abstention. Results from alcohol intake $<$ 11 g/d vs abstention analysis: cholesterol efflux and ABCA1 expression were unchanged.

[f]Multivariate analysis (adjusted for age, sex, smoking, systolic blood pressure, waist-to-hip ratio, albumin, HDL, LDL, TG, phospholipids, and lipoprotein(a)).

[g]Subgroup with the lowest HDL level. Subgroup with high HDL ($\geq$ 50 mg/dL): LCAT activity increased, no difference in CETP activity.

[h]The group that received alcohol (group 2).

[i]Men and women using hormones. In women not using hormones, LCAT mass decreased (P for trend = 0.07). Multiple linear regression analysis (adjusted for age, body mass index, and smoking).

[j]Nonobese subgroup. Obese subgroup: cholesteryl ester transfer activity unchanged. Results represent within-group changes. Changes between groups (obese vs control participants) were also examined but not extracted.

[k]Multiple regression analysis (adjusted for energy, protein, cholesterol, fat [saturated, monounsaturated, polyunsaturated acids], alcohol intake, waist-to-hip ratio, body mass index, and aerobic capacity). CETP increased in model 2 (adjusted for less covariates).

[l]Specific activity of LPL: amount of substrate that the enzyme converts per mg protein in the enzyme preparation, per unit of time.

[m]ABCA1-dependent cholesterol efflux.

Abbreviations: ABCA1, ATP-binding cassette transporter 1; apo, apolipoprotein; CET, cholesterol ester transfer; CETP, cholesteryl ester transfer protein; CHO, carbohydrates; FCR, fractional catabolic rate; HDL, high-density lipoprotein; HL, hepatic lipase; LCAT, lecithin-cholesterol acyltransferase; LDL, how-density lipoprotein; LPL, hipoprotein lipase; Lp-PLA2, lipoprotein-associated phospholipase A2; PLTP, phospholipase transfer protein; PON, paraoxonase; PR, production rate; TR, transport rate.

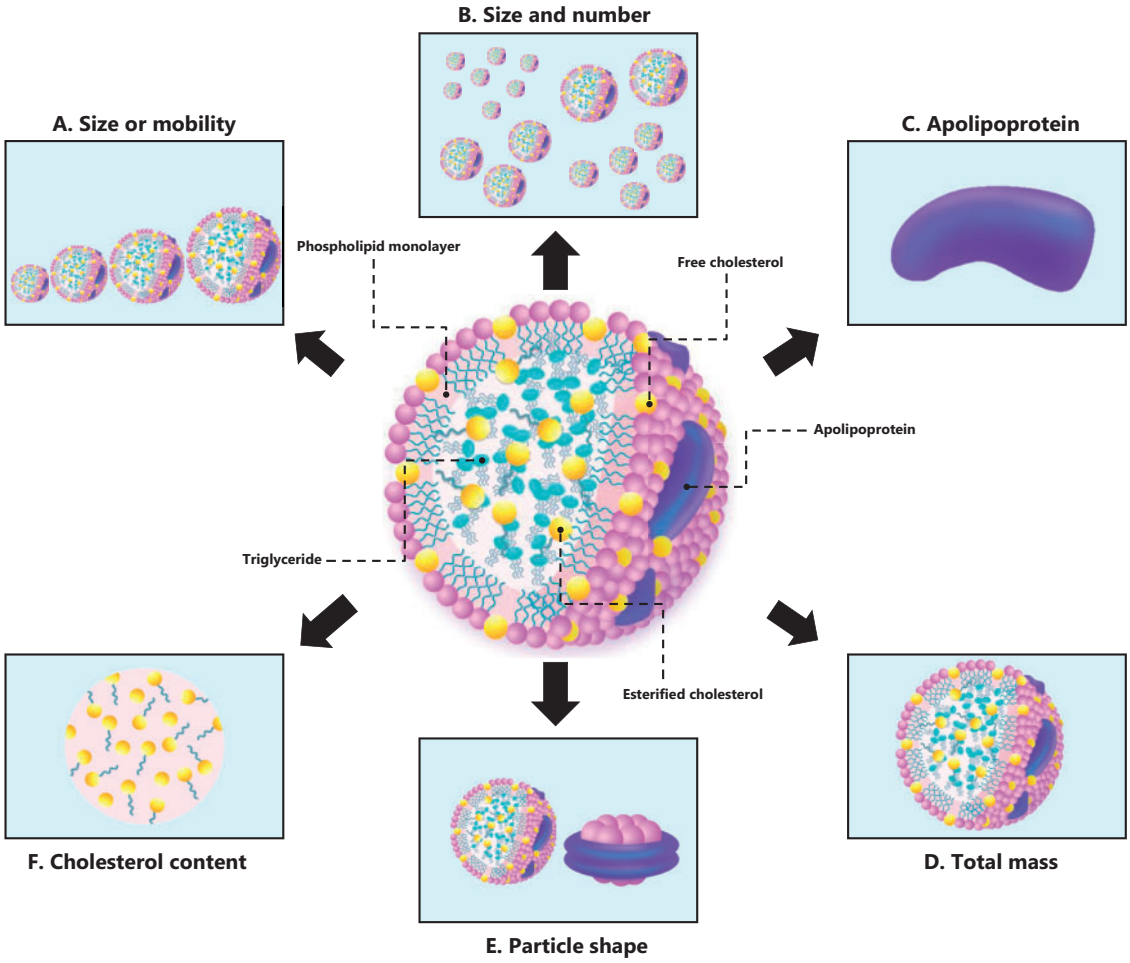

Figure 2. **Lipoprotein subfractions included in this review.** Lipoprotein subfractions are classified differently depending on the method of assessment. Some of the different types of lipoproteins subfractions included in this review are illustrated here. (A) Subfractions defined according to size or mobility with, for example, gel electrophoresis (eg, low-density lipoprotein [LDL]-I through LDL-IVB). (B) Subfractions defined according to size and particle number per unit volume, measured by nuclear magnetic resonance or ion mobility (eg, small, medium, and large HDL particle number concentration). (C) Subfractions defined by their apolipoprotein content (eg, apoA-I, apoA-II, or apoC-III) with immunoelectrophoresis (eg, LpA-I:A-II, LpC-III:B, or HDL with apoC-III). (D) Subfractions of distinct densities defined by their total mass of each subfraction with, for example, analytical ultracentrifugation or ultracentrifugation followed by enzymatic assays (eg, HDL_2-TM, HDL_3-TM). (E) Subfractions defined by their shape (spherical or discoidal) with 2-dimensional immunoelectrophoresis (eg, pre-β-HDL). (F) Subfractions of distinct densities defined by their cholesterol cargo; the density measured with ultracentrifugation and the cholesterol content in each subfraction measured with, for example, enzymatic assays (eg, HDL_2-C, HDL_3-C, $VLDL_1$-C). (Adapted from Camont et al[159] and Rizzo et al.[28] Illustrations by graphic designer Susanne Riber, www.susanneriber.dk)

40 g/d and lasted for at least 2 weeks. The type of alcohol consumed varied among studies. Several different laboratory techniques for LPSF analyses were used. Depending on the characterization metric, techniques for separation and quantification included different precipitation techniques, immunogenic assays, radiolabeling, ultracentrifugation techniques, chromatography methods, and more recent technologies such as NMR or IM.

Of the 28 intervention studies with LPSF outcomes, compliance information was found in 10 studies. This information was based on self-reports in 6 studies, on self-reports and blood biomarkers in 3 studies,[35,44,55] and on a blood biomarker alone in 1 study.[60] Alcohol was served at the study site in 3 studies.[56,59,62] Compliance was only explicitly described in 1 of these studies,[59] but compliance appears to have been closely monitored in the remaining 2 studies. In general, the studies reported good compliance with the alcohol intervention.

Observational studies: characteristics. The 64 observational studies investigating LPSF outcomes were published from 1981 to 2021, and number of participants ranged from 25 to 9778 (Table S2 in the Supporting Information online). Participants' age ranged from 18

to 85 years. Five studies included only women, and 23 studies included only men. The observational studies include 1 IM study[129] and 9 NMR studies. The period of self-reported alcohol exposure was highly diverse across studies, with a maximum of 1-year recall. However, exact duration was not consistently reported in all the articles. The results represent alcohol per se, because no specific type of alcohol was investigated in any study. Healthy populations were included in most studies. A range of laboratory methods was used, including enzyme-linked immunosorbent assay, immunoaffinity chromatography, immunoelectrophoresis, IM, or NMR.

Outcomes related to each LPSF

HDL subfractions: intervention studies. Measured subfractions of HDL included HDL_2 and HDL_3. The cholesterol content of HDL_2, HDL_2-C, was measured in 19 intervention studies, and increased HDL_2-C levels were found in 9 studies, whereas no change was reported in 9 studies. A decrease was shown in a single study in which the effects of alcohol restriction were compared with moderate intake (Table 3 and Table S1 in the Supporting Information online).[55] The reported increases in HDL_2-C concentration ranged from 0.039 to 0.155 mmol/L, with a median of 0.06 mmol/L. Studies that showed increasing concentrations of HDL_2-C had a median of 45 participants (range, 20–10 893), whereas the median was 25 participants (range, 10–60) in the remaining studies. Increasing HDL_2-C levels were shown in studies of both low (range, 13.5–19 g/d)[36,69] and higher (range, 45–50 g/d)[35,64] amounts of alcohol intake, but no effects on HDL_2-C were reported in other studies with alcohol intakes of 45–60 g/d.[47,53,54] Increases were observed in studies of shorter duration (2–4 wk) and studies with moderate duration (6–12 wk).[35,57,69] The majority of studies reporting increased HDL_2-C levels were RCTs or MRSs. Studies of women only[57] or men only[35,64,68] reported increased concentrations of HDL_2-C.

HDL_3-C was measured in the same 19 studies as HDL_2-C. Increased concentrations were found in 8 studies, and no effect was found in 9 studies. Decreased levels were reported in 2 studies,[50,55] 1 of which investigated alcohol restriction.[55] HDL_3-C was increased in the range of 0.049–0.132 mmol/L, with a median increase of 0.078 mmol/L. The median population size of the studies finding positive effects was 45.6 (range, 10–8364), whereas the median population was 36 (range, 12–10 893) in the studies with nonsignificant results. Alcohol doses >30 g/d were provided in 7 of the studies in which increased HDL_3-C concentrations were reported; none of the studies providing amounts <30 g/d reported any increases. Increased HDL_3-C level was reported in studies of 2–4 weeks' and studies of 6–12 weeks' duration.[35,57] The 9 studies in which researchers reported finding increased HDL_3-C concentrations included 1 study of women only[57] and 6 studies of men only, and 5 of the studies were RCTs or MRSs.

Total mass concentrations of HDL_2 and HDL_3 were measured in 6 studies; increased levels of HDL_3 were reported in 2 of these (Table 3).[53,58] The LpA-I and LpA-I:A-II subfractions were investigated in 7 studies (Table 3). Increased levels of LpA-I and LpA-I:A-II were found in 4 and 5 studies, respectively, whereas no change was found in 3[50,60,61] and 2 studies,[50,61] respectively. The increases ranged from 0.03 to 0.04 g/L (median, 0.035 g/L) for LpA-I, and from 0.03 to 0.248 g/L (median, 0.05 g/L) for LpA-I:A-II. The respective median population size was 34 (range, 10–56) and 12 (range, 5–20) for significant and nonsignificant results, respectively, for increased LpA-I. The corresponding, respective median population sizes for LpA-I:A-II were 24 (range, 5–56) and 17 (range, 14–20). Positive results were primarily found in studies with a randomized, controlled design.

Two intervention studies measured HDL subfractions and HDL size by NMR, with no effects reported for alcohol doses of 15 g/d or $\approx$30 g/d (Table 3).[44,62] Low compliance was reported in 1 of these studies.[44] A third study showed increased HDL size measured with gradient gel electrophoresis after a dose of 30 g/d.[57]

Overall, most intervention studies found increased levels of all types of HDL subfractions independent of analytical method, dose, and study duration. These findings were supported by 2 MRSs.[66,67] A limited number of studies included participants with disease; therefore, stratification by disease status was not possible. Most of the study populations were healthy, though participants with high CVD risk were included in 2 studes,[44,70] and participants with mixed health status were included in 1 study, with 9% having diabetes.[67]

HDL subfractions: observational studies. HDL_2-C was measured in 36 studies, 21 of which reported that higher alcohol intake was associated with higher HDL_2-C levels. No associations were found in 14 studies, and a negative association was found in 1 study (Tables 4 and 5).[96] Schäfer et al[97] found positive associations of alcohol intake with higher HDL_{2a}-C and HDL_{2b}-C levels. The median population size was 1032 (range, 32–9778) in studies with increasing concentrations of the HDL subfractions with alcohol intake, and 246 (range, 32–1386) in the studies that found no associations. Of the 5 studies conducted with women, a positive association between alcohol intake and HDL_2-C concentration was found in 1 study,[121] whereas higher

HDL_2-C levels were found in 7 of the 12 studies conducted with men.

HDL_3-C concentration was measured in 37 studies, of which 27 reported positive associations with alcohol intake, and 10 studies found no associations (Tables 4 and 5). The median population size was 932 (range, 30–9778) in studies with positive associations between alcohol intake and HDL_3-C, and 290 (range, 32–2044) in studies with no change in HDL_3-C. Four of the 5 studies with women only and 8 of the 24 studies with men only reported significant results for HDL_3-C relative to alcohol intake.

Two studies investigated the total mass of $HDL_{2a–2b}$ and $HDL_{3–3c}$, but the findings were inconsistent (Table 4).[98,99] In contrast, LpA-I was measured in 10 studies and positive associations between alcohol intake and LpA-I levels were found in 4. No relationships were found in 5 studies, and a negative association was found in 1 study (Table 4).[116] The studies that found positive associations between alcohol intake and LpA-I levels had a median size of 395 (range, 100–6729), compared with a median size of 409 (range, 25–8357) in the studies with nonsignificant results. LpA-I:A-II was examined in 8 studies, 5 of which reported positive associations with alcohol intake, and no relationships were reported in 3 studies.[107,111,113] The median population size was 344 (range, 46–8357) and 409 (range, 175–536) in the significant and nonsignificant studies, respectively. All except 2 LpA-I and LpA-I:A-II studies[111,113] were conducted with men only.

HDL subfractions were defined by the apolipoprotein C-III (apoC-III) content in 4 studies (Table 4 and Table S2 in the Supporting Information online). Koch et al[103] found that greater amount of alcohol intake was associated with higher levels of apolipoprotein A-I (apoA-I) in HDL without apoC-III, but not with apoA-I HDL containing apoC-III. Positive associations with HDL cholesterol with and without apoC-III were reported in the study by Jensen et al,[101] in which 50% of participants had CHD. Onat et al found positive correlations with both apoC-III in HDL and non-HDL in men but not in women in 1 study,[105] and positive associations with apoC-III in HDL in men, and men and women combined, in another study.[102]

HDL subfractions characterized by size and particle numbers were available from 1 IM study[129] and 9 NMR studies involving 233–9778 volunteers (Table 5 and Table S2 in the Supporting Information online). Alcohol intake was associated with increases in all types of HDL subfractions. Nonsignificant associations were found only for a few individual outcomes, and 2 studies reported negative associations between alcohol intake and small HDL-P.[124,131] The study by Millar et al[127] was the only study with contrasting results; they reported a negative association of alcohol intake with most HDL subfractions. Overall, with increased alcohol intake, the increase in the medium and larger HDL-P occurred more frequently than the increase in the smaller HDL-P, and increased average HDL particle size was reported in 5 studies. Total HDL-P were measured in 3 studies, all of which found increased levels with moderate alcohol intake.[32,124,125]

Sex-specific analyses were performed in 4 NMR studies. Minor differences between men and women were reported in some of these studies. However, women often had a lower average alcohol intake than did men.[32,125,129] Mukamal et al[124] found no substantial differences in HDL subfractions between men and women. Du et al[13] found no interaction with sex, and Würtz et al[126] found similar results for men and women.

In summary, alcohol intake was associated with increased levels of almost all types of HDL subfractions (Tables 4 and 5). The evidence for HDL subfractions characterized by total mass or apoC-III content was sparse (Table 4). Populations of mixed health status were included in 18 studies, and participants with dyslipidemia[73] and hypertension[85] were included in 2 (Tables S1 and S2 in the Supporting Information online). A health status description was missing in 12 of the included studies. No pattern between disease status and outcomes was found.

LDL subfractions: intervention and observational studies. LDL subfractions were investigated relative to alcohol intake in 2 intervention studies[44,63] and 2 MRSs[66,67] (Table 3). No effect on any NMR-measured LPSFs was found in a 6-month RCT with presumed low compliance regarding alcohol intake.[44] An increased ratio of LDL-C to apolipoprotein B (apoB) was found in the other study, implying an increase in LDL size with moderate alcohol intake.[63] One of the 2 MRSs found decreased levels of large, buoyant LDL cholesterol in Japanese men and women and increases in small, dense LDL cholesterol (sdLDL-C) in men.[66] In that study, the aldehyde dehydrogenase 2*1 allele was used as a proxy for alcohol consumption in men but not in women. The other MRS found a trend toward decreased sdLDL-C in both men and women, using 5 different single-nucleotide polymorphisms on different alcohol dehydrogenase genes as exposure markers.[67]

LDL subfractions were included in 6 observational studies, excluding NMR and IM studies (Table 4). No consistent pattern was observed, but one study found a positive association between alcohol intake and large LDLs[155] in men,[117] and another study found a decrease in sdLDL-C in association with alcohol intake.[33] A trend toward decreased LDL particle size was found in a third study.[118] Five studies investigated LPSFs, defined

by apolipoprotein E[108,109,119] or apoC-III[102,105,108] content, but reported inconsistent results. Results from 8 NMR and IM studies were heterogeneous (Table 5); associations in opposite directions and nonsignificant or even nonlinear relationships were reported relative to alcohol intake. Mukamal et al[124] found a U-shaped association of alcohol intake with total LDL-P, with a different pattern depending on the particle size. The concentration of large LDL-P was highest in consumers of ≥ 1 drink/wk, and that of small LDL-P was lowest in consumers of 7–13 drinks/wk. The net result was increased average LDL size. The LDL-C measure did not capture the shift in the distribution of LDL subfractions. Generally, the associations were similar in men and women, but stronger in women. Würtz et al[126] reported complex relationships to apoB-carrying lipoproteins. Most of these associations had a U-shaped pattern in the first segment of the slopes up to 100 g/wk and lowest lipoprotein levels at ∼ 50 g/wk (7 g/d). The decreasing limb of these curves was generally steeper in women than in men.

VLDL subfractions: intervention and observational studies. VLDL subfractions were only measured in 1 intervention study, which found no effect of alcohol at a dose of 20 g/for 2 weeks or 35 g/d for 1 week (Table 3).[65] Inconsistent results were found in 7 observational NMR studies. Positive and negative associations of alcohol intake with all types of VLDL subfractions and VLDL size were found, but positive associations were reported most frequently (Table 5). Positive associations of alcohol intake with medium and large VLDL-P plus average VLDL particle size was observed in the largest NMR study by Würtz et al[126] (N = 9778). The relationship to medium VLDL was nonlinear, with a U-shaped curve at alcohol intakes between 0 and 100 g/wk. In partial agreement with this, Mukamal et al[124] reported a quadratic or U-shaped relationship of alcohol intake to large VLDL-P and larger average VLDL particle size, but inverse associations with small, medium, and total VLDL-P. Consumers of 1–13 drinks/wk had the lowest level of large VLDL-P, and the decreased total number of VLDL-P was driven by reduced levels of medium and small VLDL-P.

Mechanisms. Results from individual studies investigating potential mechanisms for the relationship between alcohol intake and LPSFs are presented in Tables S3 and S4 in the Supporting Information online, and a summary is provided in Table 6. Most intervention and observational studies examined cholesterol ester transfer protein (CETP), hepatic lipase, lecithin-cholesterol acyltransferase (LCAT), phospholipid transfer protein (PLTP), and lipoprotein-associated phospholipase A2 ($Lp\text{-}PLA_2$). No consistent relationships with alcohol intake were found for any of these. Results were more robust for cholesterol efflux capacity (CEC) and paraoxonase (PON). CEC was increased with alcohol intake in 9 studies, whereas 2 studies found no change.[61,114] A positive association with the cholesterol efflux regulatory protein, ATP-binding cassette transporter A1 (ABCA1) was reported in 1 study.[136] PON activity and PON mass were increased in 6 studies, and no change was found in 3.[16,148,152] Three studies found increased levels lipoprotein lipase (LPL) activity and mass with alcohol intake,[52,62,142] whereas no such changes were reported in 3 other studies.[53,64,140]

Bias risk assessment of individual studies

Bias risk assessment results for all the included studies are shown in Figures S1–S6 in the Supporting Information online. Several of the RCTs with LPSF outcomes were older and inadequately reported, and none of the studies were of high quality in terms of bias risk (Figure S1 in the Supporting Information online). For example, randomization procedures were not described adequately in any of the included RCTs, resulting in unclear risk of selection bias. Likewise, several studies had unclear or high risk of reporting bias caused by a lack of consistency between planned and reported outcomes. Because of the nature of alcohol interventions, the risk of performance bias is high in all RCTs. Last, all RCTs had unclear or high risk of other bias due to shortcomings such as unidentified carryover effects in cross-over studies[57,60]; baseline imbalances[55]; potential selection bias due to insufficient description of participant recruitment[36,60,64]; or potential confounding from changes in body weight, physical activity, smoking status, or dietary intake. The background diet was only fully controlled in 4 studies and partially in 1.[59] In the remaining studies, participants were asked not to change their dietary intakes.

The different nonrandomized investigations (Figure S2 in the Supporting Information online) were a mix of (1) non-RCTs with a crossover or parallel design, (2) nonrandomized studies with sequential crossover, (3) case-control studies, and (4) cohort studies.[102,118] Most of these studies had unclear or high risk of selection bias due to inadequate description or selection of participants, compromising external validity, or insufficient consideration or handling of potential confounders, compromising internal validity. A priori, all the studies were assessed with high risk of performance bias due to inadequate possibilities for blinding for the drinking of alcohol. In contrast, the risk of detection bias was consistently considered low due to the objective nature of lipoprotein outcomes.

Only a few studies had unclear risk of attrition bias or unclear or high risk of reporting bias. Overall, only 2 of the randomized studies were of high quality regarding bias risk.[101,115]

Overall, many observational studies were assessed to have lower risk of bias, including the most recently published NMR studies. However, some of these studies were older and did not follow current reporting standards (Figure S3 in the Supporting Information online). The descriptions of eligibility criteria and study populations were generally insufficient. Alcohol exposure was measured via self-report and scored with high risk of bias on the validity and reliability of the exposure variable. In contrast, all studies except 1[69] identified potential confounders and adjusted for them in their analyses. In a few included studies, researchers performed inappropriate statistical analyses or did not describe them. The LPSFs were generally measured reliably (ie, with laboratory methods found reliable when the study was conducted), but some included studies did not specify the laboratory method used.[81,100,120]

No obvious systematic patterns relating risk of bias with study outcomes in any study designs were observed. Only a few studies had low risk of bias, and these appeared more frequently among the more recent observational studies.

DISCUSSION

To our knowledge, this systematic review is the first to examine LPSFs after moderate alcohol intake. Most intervention studies provided alcohol in an amount of 20–40 g/d for at least 2 weeks, even though the limit for eligibility was set to $\leq$ 60 g/d. The reviewed articles explored several different LPSFs, mainly characterized according to total size and mobility; size and number; apolipoprotein content; total mass; shape; or cholesterol content and size (Figure 2). Alcohol in amounts up to 60 g/d was related to increased levels of almost all HDL subfractions measured, independent of the analytical approach. Results on LDL subfractions were sparse, especially from intervention studies. However, a few observational studies and MRSs found reduced levels of sdLDL-C, increased average LDL size, and U-shaped or other nonlinear relationships for alcohol intake and apoB-containing lipoproteins. Mechanistic studies show a pretty clear pattern of increased CEC and PON activities across all study designs. Because these was an insufficient number of relevant studies, it was impossible to fulfill the aim of analyzing data according to disease status.

Comparison with other studies

High-density lipoproteins. The overall classes of lipoproteins were not included in the search strategy because they have been covered in 3 previous meta-analyses of intervention studies summarizing the effect of moderate drinking on several biological markers of CVD risk (Table 2). These meta-analyses mainly included healthy individuals, and the dose of alcohol provided was up to 100 g in some of the included studies.[17,20] They all reported increased levels of HDL-C and apoA-I (Table 2), in concordance with findings in several nonsystematic reviews.[22,23,27,30] On the basis of these results, we hypothesized that the increase in HDL could be explained by a more robust increase in some types of HDL subfractions than in others. Most studies in the present review do not support this hypothesis, because an increase with moderate alcohol intake is commonly reported for all the HDL subfractions. That said, increased levels of HDL_3-C were reported more frequently than for the larger HDL_2-C, especially in observational studies (Tables 3–5).

On the other hand, increases in medium and larger HDL-P compared with the smaller HDL-P occurred more often in the NMR and IM studies (Table 5). Würtz et al[126] found more pronounced associations of alcohol intake and the medium and small HDLs in the largest NMR study, underlining that there are no consistent differences between the effects of moderate alcohol intake on the different HDL subfractions. Similarly, no clear distinction was found for LpA-I compared with LpA-I:A-II, and the reported effect sizes of HDL_2-C and HDL_3-C do not differ substantially. These findings are further corroborated by Hannuksela et al[22] in their narrative review, whereas Brinton et al[27] reported more significant increases in HDL_3 compared with HDL_2. In neither of these reviews was the evidence gathered systematically, and the definition of moderate alcohol intake was not clear in these reports, which undermines the weight of their conclusions.

Low-density lipoproteins. The LDL subfractions have not been included in previous meta-analyses of moderate drinking, and results on total LDL-C are inconsistent.[17,19,20] No effect on LDL-C was found in 2 of these meta-analyses,[17,20] whereas Huang et al[19] showed decreased levels in a meta-analysis of studies with alcohol doses up to only 30 g/d. The inconclusive findings on total LDL could be due to opposing changes in small and large LDL subfractions or to nonlinear dose-response relationships masking physiologically relevant findings. The evidence is sparse in the present review, but some studies indicate decreased levels of smaller LDLs,[33,67,124] increased levels of lbLDL,[117,124] and an increase in

overall LDL particle size (Tables 3–5).[63,124,129] However, this pattern needs further substantiation. In addition, standardization of subfraction classification is required, because a direct comparison of different types of smaller LDLs, such as sdLDL-C[33,67] and small LDL-P[124] is troublesome. Results on LDL subfractions were not included in 2 narrative reviews, but the authors reported decreased or unchanged total mass of LDL or total LDL-C with moderate drinking.[22,27] No clear definition of types of LDL particles, alcohol dose, or duration of alcohol intake was made in these reviews, which complicates direct comparison. It has been suggested that changes in LDL particles seem to vary more with population, sex, and drinking pattern than changes in HDL particles.[27] Despite the original aim, too few studies investigating LDL subspecies were included to allow for stratified analyses on population characteristics.

Very-low-density lipoproteins. Results on VLDL subfractions come primarily from NMR studies and are sparse and inconsistent (Table 5). VLDL was not included in the meta-analyses of alcohol intervention studies.[17,19,20] Under normal circumstances, VLDL particles carry most of the plasma triglycerides (TGs) in their core,[156] and larger VLDL particles generally contain more TG.[157] Overall TG levels were unchanged after moderate alcohol intake in 2 meta-analyses of intervention studies.[17,19] In contrast, increased TG levels were shown with alcohol intake in an older meta-analysis (Table 2).[20] Brien et al[17] reported increased TG levels only when alcohol intake exceeded 60 g/d. Likewise, in 1 included observational study, the authors found decreased levels of all VLDL subfractions and overall TG levels in drinkers of 7–13 drinks/wk compared with abstainers.[124] Results from 2 of the largest observational NMR studies found positive or U-shaped associations of alcohol with medium or large VLDLs.[124,126] Taken together, TG levels could be unaltered or decreased after moderate alcohol consumption and increased at higher intakes. A decreased TG level would be in line with the well-known inverse relationship between TG levels and HDL-C.[158] However, if the HDL-C level increases in a linear manner with alcohol consumption,[20] and the relationship to TG is U-shaped, there is no longer concordance with the inverse-relationship hypothesis. Effects on VLDL subfractions need further examination. Even though U-shaped patterns with alcohol intake were observed, results were inconsistent.

Clinical implications

HDL and CVD. HDL subfractions not classified solely by their dynamic cholesterol content and mechanistic or functional outcomes of HDL have been suggested as potential biomarkers for assessing cardiovascular risk.[159,160] Traditionally, HDL-C has been used in cardiovascular risk equations.[161] The inverse association between HDL-C and CHD is well known[162] and has been ascribed to the significant role of HDL in reverse cholesterol transport.[159] In recent years, the causal role of HDL-C in the development of atherosclerosis has been questioned.[163] Several clinical drug trials have shown no or negative effects on CVD outcomes despite increased HDL-C levels,[164,165] and an MRS did not prove a causal relationship between HDL-C and myocardial infarction.[166] In addition, the association of HDL-C with all-cause mortality and CVD has been shown to be J-shaped, with a minimum in risk at 54–58 mg/dL and 68–71 mg/dL, respectively,[167] and the relationship between alcohol and HDL-C is linear.[20] High levels of dysfunctional HDL-C may even increase the risk of CVD.[168] When investigating the potential beneficial effects of moderate drinking on cardiovascular health, it would seem, therefore, too simplistic to include HDL-C exclusively.[160] Several antiatherogenic roles of HDL that are not captured by measuring the cholesterol content have also been described. These include anti-inflammatory, vasodilatory, and antioxidative functions, and they may be subfraction specific.[159] Examining the effect of alcohol on HDL subfractions, therefore, is relevant.

In this review, it was not confirmed that increases in the small rather than the large HDLs could explain a potential cardiovascular benefit of moderate drinking. Some studies found that HDL_3-C is more frequently increased than HDL_2-C, but increases in the medium and large HDL-P were reported more often in other studies. In addition, increased HDL size was reported by several studies (Tables 3 and 5) Authors of a previous nonsystematic literature review concluded that HDL_2-C and HDL_3-C did not significantly improve risk prediction over HDL-C but also pointed to the inadequacy of cholesterol measurements to sufficiently identify HDL heterogeneity and CVD risk.[25] Several larger, secondary analyses examining the relationship between the different types of HDL subfractions and CVD risk have been conducted.[169–174] With 1 exception,[172] their results indicate stronger inverse associations of CVD with smaller HDL subfractions, such as HDL_3-C and small HDL-P, compared with larger HDL subfractions and overall HDL-C. That said, only a few of these analyses were adjusted for apoB.[170] Findings relating to the role of LpA-I and LpA-I:A-II in CVD risk are less convincing,[29,110,175] and these subfractions were not included in a proposed new nomenclature of HDL subfractions.[29] Authors of a narrative review suggested a more beneficial role of smaller HDLs and refer to their importance

in CEC and their antioxidant, anti-inflammatory, and antithrombotic actions.[159] However, the clinical relevance of specific HDL subfractions still needs to be determined.[159]

LDL and CVD. LDL-C has been firmly established as a causal factor for atherosclerosis and CVD, with convincing evidence from genetic studies, prospective epidemiological studies, MRSs, and RCTs of LDL-lowering therapies.[176] The alcohol-induced change in LDL subfractions is much less clear than for the HDL subfractions. Still, some evidence for reduced levels of smaller LDL, increased levels of larger LDL, and increases in overall LDL particle size was seen. Whether LDL subfractions are better predictors of risk than LDL-C is debatable.[24] Small LDLs enter the arterial wall more easily[177] and bind more avidly to arterial wall macrophages and proteoglycans and glycosaminoglycans.[178] They have a lower affinity for the LDL receptor, causing longer residence time.[179] Overall, the majority of studies have associated smaller LDL with higher CVD risk,[24,180] compared with larger LDL.[181,182] A recent narrative review found independent associations to CVD clinical outcomes for small LDL subfractions and LDL-P,[180] despite minor inconsistencies between studies. Smaller LDLs do often coexist with higher TG levels and lower levels of HDL-C (the atherogenic lipid triad), however, so disentangling an independent effect of small LDLs is difficult.[24]

Quantification of LDL subfractions may strengthen our understanding of the association between moderate alcohol intake and CVD, but improved risk predictions compared with standard lipid measurements have yet to be made.[180] Several studies reported elevated levels of sdLDL in disorders such as diabetes and metabolic syndrome,[183] which are metabolic states with a high prevalence of the atherogenic lipid triad.[184] Several alcohol studies have shown inverse associations between moderate alcohol intake and metabolic syndrome,[185] glycemic markers,[186] and diabetes.[3,187] These results strengthen the hypothesis that moderate alcohol intake could have beneficial health effects partially due to changes in lipoproteins such as smaller LDLs. That said, a cause-and-effect relationship is still missing.

In this review, interesting post hoc results on overall apoB levels were also observed. All lipoprotein particles carrying apoB, and the cholesterol content within them, play a central role in atherosclerosis.[177,188] ApoB has been proposed as a better marker of atherogenic risk than HDL-C and LDL-C,[189] and apoB measurements are now included in clinical guidelines.[18,190] LDL-P can be used as a surrogate for apoB under normal circumstances.[189] One of the included NMR studies found a negative association of alcohol with apoB,[13] and another study showed a U-shaped relationship, with a nadir at intakes between 50 and 100 /wk.[126] Total LDL-P was inversely related to alcohol consumption in a quadratic pattern in another study, with the lowest level at 1–6 drinks/wk.[124] Conversely, older intervention studies have found unchanged apoB levels,[20] but results on apoB were not included in the 2 most recent meta-analysis of intervention studies (Table 2).[17,19] These results might have clinical implications for moderate alcohol intake and should be investigated further.

VLDL and CVD. Large and total VLDL-P have been associated with insulin resistance,[191,192] and increased large VLDL-P levels have been positively associated with markers of CVD severity in smaller cohorts.[193,194] A higher concentration of large VLDL-P is also associated with a decrease in small LDL-P levels and an overall increase in the number of LDL particles, changes that are important for CVD risk.[192] Equally, all VLDL-P measured by NMR and a higher average VLDL particle size were positively associated with a higher risk of incidence of CVD in the Women's Health Study.[172] Positive associations to all types of VLDL subfractions and increased VLDL size were seen here (Table 5), implying a potential downside of moderate drinking. At the same time, nonlinear results were found in some observational NMR studies. A positive association to large VLDL-P and a U-shaped relationship to medium VLDL-P was found in 1 study,[126] and Mukamal et al[124] found a quadratic or U-shaped relationship to large VLDL-P. As for the TGs described previously in the present review, these results could indicate either decreased or unchanged VLDL subfractions after moderate alcohol intake with increased levels at higher intakes, though this is speculative. Mukamal et al[124] also found negative associations to small, medium, and total VLDL-P, which could be beneficial (Table 5), but the changes in VLDL subfractions after moderate drinking, and the clinical relevance, still have to be clarified.

Apolipoprotein C-III. ApoC-III resides on lipoproteins like HDL, LDL, and VLDL,[195] and stimulates atherogenesis directly via mechanisms such as recruitment of monocytes and activation of endothelial cells.[196] In this review, results on HDL with and without apoC-III were limited and inconsistent, but alcohol intake was positively associated with HDL lacking apoC-III in 2 studies (Table 4).[101,103] On the other hand, 3 studies found higher levels of HDL containing apoC-III.[101,102,105] These studies included diverse population types and different laboratory procedures for HDL subclassification, making direct comparison troublesome. One of the studies classified the HDL subfractions by cholesterol content[101] (Table S2 in the Supporting Information

online). Another study that was excluded from this review because the analysis included people with alcoholism also reported increasing levels of non-apoB lipoproteins (HDLs) containing apoC-III, termed LpC-III, with higher levels of alcohol intake ($P \leq 0.001$).[197]

The clinical impact of apoC-III in HDL on CVD risk is not fully clarified. However, analyses from the Nurses' Health Study and the Health Professionals Follow-up Study showed that HDL-C lacking apoC-III was inversely associated with CHD risk, and HDL-C containing apoC-III was directly associated with CHD risk. The results remained significant after adjustment for TGs and apoB.[101] These results were confirmed in another analysis of 4 different cohorts.[198] When residing on apoB-containing lipoproteins, apoC-III modulates TG metabolism through delayed TG lipolysis and inhibited catabolism of TG-rich lipoproteins.[199] In the present review, 3 observational studies investigated apoB-containing lipoproteins containing apoC-III and found positive associations[105] or nonsignificant results[102,108] with alcohol intake (non-HDL plus apoC-III and LpC-III:B) (Table 4). A study not included here found more or less similar levels of apoC-III in apoB-containing lipoproteins (LpC-III:B) even though the levels were significant in the overall analysis of variance. In summary, the evidence is limited, and the influence of moderate alcohol intake on apoC-III levels should be addressed in future studies.

Biological mechanisms

The metabolism of lipids and lipoproteins is complex, and only a limited number of regulating factors have been examined after moderate alcohol intake.[200] The primary proteins and enzymes involved (ie, LCAT, CETP, PLTP, LPL, and hepatic lipase)[22] were systematically included in this review (Table 6). No evident increase in LCAT was observed after moderate alcohol intake. LCAT is responsible for the maturation of the HDLs. After activation by apoA-I, and in cooperation with the ABCA1 transporter, LCAT converts free cholesterol from blood or tissues into cholesteryl esters, making them ready for storage in the core of lipoproteins.[201] The small, discoidal pre-β-HDL is the primary substrate for LCAT, an enzymes that catalyzes the conversion and maturation of small HDLs into larger, spherical HDLs. On this basis, it has been postulated that LCAT is a critical enzyme in reverse cholesterol transport, where cholesterol is removed from the periphery and transported back to the liver.[201]

Another regulatory protein in this pathway is CETP. CETP transfers cholesteryl esters from HDL to apoB-containing lipoproteins in exchange for TGs.[202] Inhibition of CETP can increase HDL-C levels[165] and would be a plausible mechanism for how moderate drinking could increase HDL levels. However, this is not supported by findings of the present review (Table 6). PLTP is also unchanged after moderate drinking in studies from the present review. PLTP is another member of the lipid transfer protein family and mediates the exchange of phospholipids and other lipids between lipoproteins.[203] PLTP mediates the transfer of phospholipids from apoB-containing lipoproteins to HDL, and PLTP deficiency reduces HDL-C levels. In addition, PLTP might also have a role in macrophage cell cholesterol efflux, although results from animal studies have been conflicting.[203]

Likewise, the literature on moderate alcohol intake and LPL activity or mass is conflicting (Table 6). LPL hydrolyses TG in chylomicrons and VLDL and plays an essential role in TG metabolism.[200] In the present review, we found evidence for increasing or unchanged LPL activity, which is concordant with decreased or unchanged TG levels, respectively.[17,124] A previous narrative review reported increased LPL in heavy drinkers and unchanged or increased levels in moderate drinkers.[22] At excessive alcohol intakes, TG synthesis may exceed LPL activity, whereas this might be more or less outbalanced in moderate drinkers.[204] The same review found unchanged or reduced hepatic lipase activity after moderate alcohol intake.[22] Hepatic lipase is a lipolytic enzyme that catalyzes the hydrolysis of TGs and phospholipids in IDL, LDL, and HDL, leading to smaller particles.[200] Hepatic lipase also stimulates hepatic uptake of cholesteryl esters from larger HDLs, thereby converting them to smaller HDL particles that can potentially take up more cholesterol from other cells.[205] This review demonstrates increased levels of smaller HDLs after alcohol intake, which could theoretically be explained by increased hepatic lipase activity, but the current evidence does not confirm this (Table 6).

Because the initial step in HDL synthesis requires its main structural component, apoA-I, increased flux of apoA-I could explain the increased HDL levels. An increased apoA-I transport rate was only found in 1 of the studies reviewed here (Table 6).[62] More convincing results have been noted for CEC, the initial step in reverse cholesterol transport, and a measure of HDL function.[206] Moderate alcohol intake consistently induced increased CEC, which was shown for doses of 15–40 g/d in the intervention studies (Table 6). The increase in CEC could explain the concomitant increase in HDL-C and HDL-C subfractions after moderate drinking. However, the metabolic fate of cholesterol after removal from the arterial wall is complex.[200] The role of CEC has been reviewed in detail elsewhere.[159] Briefly, small and larger HDLs are probably capable of promoting

CEC, depending on the efflux regulatory protein: ABCA1, ATP-binding cassette transporter G1, or scavenger receptor class B type 1.[159] For example, smaller HDLs, such as apoA-I and pre-β-HDL, are inducers of CEC via interaction with ABCA1, and both of these are increased after moderate alcohol intake.[17,19,20,59]

Adiponectin, which is also increased with moderate alcohol intake,[17] upregulates ABCA1 expression and thus facilitates apoA-I–mediated CEC.[207] In addition, the phospholipid content of HDL has been associated with the capacity of HDL to promote CEC.[206] Compositional changes in HDL are beyond the scope of this review. However, it is worth noting that some of the included studies found increased phospholipid content of the HDL molecule after moderate alcohol intake.[33,97,134,208] Thus, specific particle characteristics of the HDL subfraction, stimulated by moderate drinking, could potentially be inducers of cholesterol efflux.

The methods for CEC estimation have several limitations because ex vivo experiments may not entirely resemble a living organism.[206] However, in theory, CEC may be a more relevant marker than HDL-C for removal of cholesterol from macrophages, and it has been suggested to be the key pathway by which HDL reduces the cholesterol content of the arterial wall and thus prevents atherosclerosis.[159] CEC has also been inversely associated with CVD risk in several studies, independent of HDL-C and apoA-I.[138,206] However, measurement of CEC says little about the fate of the cholesterol molecules following efflux from macrophages or other cells. Nonetheless, the potential cardioprotective effects of moderate drinking might involve stimulation of the initial step in reverse cholesterol transport. This function may be more relevant than the overall abundance of HDL-C.

Antiatherogenic effects of HDLs may not only be attributable to the reverse cholesterol pathway, because antioxidant effects have also been described.[159] In this context, the esterase enzyme PON may be important. PON is attached to HDL and protects LDL and HDL from oxidative damage.[209] PON activity or mass was consistently increased in the reviewed studies and could be among the mechanisms underlying the reduced CVD risk in moderate drinkers. Its clinical relevance is still questionable, but a negative association with CVD risk has been shown.[210] Furthermore, PON has been suggested to be a stimulator of CEC.[211] Another enzyme attached to both LDL and HDL is Lp-PLA_2. Lp-PLA_2 has been suggested to be an inflammatory marker that contributes to atherogenesis by increasing inflammatory processes in the arterial intima,[212] and Lp-PLA_2 has independently been associated with CHD and ischemic stroke.[213] Unlike PON, Lp-PLA_2 is unchanged, according to the results reviewed here, but the evidence is limited.

Limitations of the study

This review presents an LPSF perspective of the relationship between moderate drinking and CVD, and other biological markers and mechanisms besides lipoproteins may be equally important.[17] In addition, our results apply primarily to healthy men and women aged 18–85 years, whereas the assumed beneficial effect of moderate drinking generally concerns middle-aged individuals.[5]

Furthermore, the upper limit of $\leq$ 60 g/d of alcohol is high compared with current recommendations for alcohol intake.[10] This limit was set to avoid excluding important studies using this threshold as moderate alcohol intake and because some studies used >1 alcohol dose or increasing doses. Higher doses than 60 g/d have also been included in other meta-analyses investigating moderate alcohol intake.[17,20] The cutoff was also set as a compromise between the available evidence and the nadir of the U-shaped risk estimate; 1 of the most recent meta-analyses showed higher all-cause mortality risk compared with abstaining only when the alcohol dose exceeded 60 g/d.[2] On the other hand, it is acknowledged that such drinking limits may have changed over the years. The broad definition could have compromised our comparisons between the intervention studies, but most studies provided 20–40 g/d alcohol (Tables S1 and S4 in the Supporting Information online).

In the PICOTS criteria, the comparator intervention is defined as "no" or "low" alcohol intake. Three RCTs with alcohol intakes in the comparators groups >0 g/d were included because these studies did not use the control group in their analyses but analyzed the results according to a before-and-after design.[35,55,64] In addition, the reference groups in few observational studies were defined as individuals drinking a range of alcohol amounts, including 0 g/d, but with upper limits of 5 g/d,[97] 10 g/d,[92,96] 22 g/d,[113] or 28 g/d.[85] Removing these studies from our analyses did not change our overall conclusions. Of note, many observational studies used regression analyses without reference groups and thus did not include any comparator group. In addition, several observational studies exceeded 60 g/d because of the use of regression analyses without dose restriction.

This review does not include potential differential effects of specific beverage types, and data from the majority of the observational studies did not allow for such an analysis. Ethanol per se has been suggested to be the element primarily responsible for the potential benefits of moderate intakes.[11,12] However, a recent, large observational analysis of spirits drinkers suggested no causal relationship between moderate drinking and CVD.[214] Besides the type of beverage, drinking pattern is also crucial because of the toxic effects of higher doses of

alcohol.[215] Regular drinking vs binge drinking was not examined in this review and was not part of the aim.

The included studies also defined the LPSFs differently, complicating comparability (Table S6 in the Supporting Information online). For example, LpA-I is found in both the HDL_2 and HDL_3 density range, and LpA-I:A-II is mainly found in the HDL_3 density range.[216] The investigated LPSFs were categorized (Figure 2), but definitions still differ between analytical methods.[99,124] Even definitions of LPSFs in studies using the same methodology varied.[13,124] This variation also involved HDL definitions, and some studies[48,58,70] used a broader density range than the more commonly used range of 1.063–1.210 g/mL.[159]

The analytical methods for LPSF measurements have limitations, too. Ultracentrifugation has been the gold standard, although outcomes are operator dependent and the procedures time consuming.[217,218] In addition, ultracentrifugation may cause loss of apolipoproteins and redistribution of subspecies due to buffer components and shear force.[218,219] Compared with precipitation, losses of apoA-I are assumed to be higher by ultracentrifugation.[220] Other laboratory methods focus on particle number concentrations based on size and density, such as NMR and IM. The lipoprotein particle number concentration has been suggested as a better measure of risk than the cholesterol cargo,[221] and clinical studies have indicated that HDL-P may provide more information of CVD status than HDL-C.[222] NMR lipoprotein analyses are less labor-intensive, high-throughput measurements.[223] Ultracentrifugation is often used for calibration of the NMR spectral model, so the method basically predicts what would be measured by ultracentrifugation, improving comparability between these methods.[223] Overall, standardization of the different NMR methods, including using a standard, unique reference material, is needed.[224]

Discrepancies between NMR and IM measurements have also been found. NMR studies in healthy cohorts have predicted an average HDL particle concentration of approximately 32–34 μmol/L,[225,226] whereas IM studies have reported average levels of approximately 5–6 μmol/L.[227,228] Such differences are critical for the validity and comparability of the particle number measurements as a clinical metric. Although these differences indicate that comparison of absolute quantities is compromised in this review, a comparability study showed that the positive association between sdLDLs and coronary artery stenosis was consistently found by 4 independent methods (ie, NMR, IM, gradient gel electrophoresis, and vertical auto profile), although the correlations among the methods varied significantly.[181] Taken together, differences between laboratory methods and their limitations may explain some of the inconsistency in results between studies and underline the need for standardization.

Another limitation is the high frequency of unclear or high risk of bias in eligible studies, particularly in the intervention studies and trials. More specifically, older studies had short descriptions of the applied methods and results, complicating their interpretation. Compliance was not often measured, and suitable biomarkers of alcohol intake still need to be implemented. Compliance was not even discussed in several intervention studies, and only a few studies reported control of diet, physical activity, body weight, or smoking habits. Furthermore, not all the crossover studies examined carryover effects. Last, it is impossible to judge to what extent results were influenced by study size and power, because none of the studies reported any power calculations for LPSFs.

Although the observational studies generally had lower bias risk, they were prone to intentional and unintentional recall bias due to self-reported alcohol intake. Also, the definition of a standard drink differed among studies. Therefore, data on alcohol intake were extracted in grams per day or week. Furthermore, nonlinear relationships between alcohol intake and LPSFs were only directly explored in 3 observational studies.[13,32,124] It has also been suggested that LPSF analyses should be adjusted for other LPSFs and overall classes of lipoproteins due to collinearity,[24] which was generally not practiced.

The included MRSs also had limitations. Even though MRSs are assumed to be less prone to confounding and reverse causation than conventional epidemiologic studies, such limitations may exist.[229] For example, the instrumental variables used in the 2 MRSs[66,67] included here, alcohol dehydrogenase and aldehyde dehydrogenase, could potentially directly influence the outcome, because the enzymes are involved in the metabolism of alcohol. In Japanese men that the aldehyde dehydrogenase 2 variant is related to reduced alcohol intake and lower body weight, a potential consequence of lower calorie intake from alcohol.[230] No bias-risk assessment tool specific to MRSs is available; thus, the results of these studies may be biased.

Future perspectives

High-quality RCTs that target LPSFs, apoB, and apoB-containing lipoproteins after moderate alcohol intake are needed. Studies should include individuals at elevated risk of CVD, because lipoprotein structure and function may change in disease states.[168] Studies investigating mechanisms within these types of populations are few. From the reviewed studies, a daily alcohol

intake of 20 g/d might be sufficient to cause changes in LPSFs, though few studies showed changes in specific subfractions at lower intake levels (Table S1 in the Supporting Information online). In recent years, observational studies questioning any safe level of drinking have been published.[231,232] Intervention studies, therefore, should aim for low amounts of alcohol, preferably no more than 1 drink/d (10–15 g/d). Standardization of laboratory methods to ease comparison within and between methodologies is needed,[233] and consensus nomenclatures for all LPSFs are crucial.

HDL and its subspecies may still be of interest in cardiovascular research, even if HDL-C is not casually related to atherosclerotic CVD.[234] Focus has shifted from the HDL-C hypothesis to an HDL function hypothesis. Among the HDL functions of interest are mechanisms related to reverse cholesterol transport.[160] Studies tracking cholesterol from macrophages to hepatic uptake and onward to fecal excretion have been conducted in animals but need confirmation in humans.[235] Furthermore, "omics" analyses of the HDL lipidome and proteome may support future CVD diagnostics and provide more information on the composition of a broader range of subpopulations.[168,236]

LPSFs may eventually be included as cardiovascular biomarkers in risk prediction models relevant for moderate drinkers. A recently developed diabetes risk index comprises several NMR-measured LPSFs and has been associated with insulin resistance and increased risk of developing type 2 diabetes.[237] Such risk markers will likely expand to other diseases soon.

CONCLUSIONS

Alcohol in doses from 12–60 g/d is related to higher levels of all types of HDL subfractions, independent of study design. Effects on total HDL-C, therefore, seem unrelated to any specific subfraction. The influence of moderate drinking on LDL and VLDL subfractions is still speculative; however, some observational studies found nonlinear associations of alcohol intake with potential beneficial associations in the moderate drinking range. A few studies of different designs found reduced levels of small LDLs, higher levels of large LDLs, and increased LDL particle size. Moderate alcohol intake consistently increases CEC and PON activity, both of which have been associated with reduced CVD risk and with HDL's antiatherosclerotic functions. More research is needed to study effects in women and in people with diabetes and other cardiometabolic conditions. At present, evidence is lacking on the influence of moderate drinking on functional metrics of HDL, apoB-containing lipoproteins, and subfractions classified by their content of essential apolipoproteins, such as apoC-III.

Acknowledgments

We thank especially Anne Berit Bagger and Anne Cathrine Trumpy, librarians at Copenhagen University Library, for support during the systematic search. We are very grateful for their help in handling bibliographic databases and retrieving articles for full-text screening.

Author contributions. T.L.W. wrote the protocol, supervised by J.N.E. and L.O.D. T.L.W. conducted the literature search, and T.L.W., K.T., and J.N.E. screened the literature and agreed on exclusion and inclusion of studies. T.L.W. and K.T. performed the data extraction. T.L.W. wrote the manuscript; all authors revised and provided intellectual feedback regarding the manuscript. All authors approved the final manuscript.

Funding. This work was supported by a PhD scholarship sponsored in part by a Carlsberg Foundation Semper Ardens grant on biomarkers to L.O.D. [grant no. CF15-0574], and in part by National Institute of Alcohol Abuse and Alcoholism to L.O.D. [grant no. U10AA025286]. Salaries for T.L.W., J.N.E., and L.O.D. were partly supported by grants from the Carlsberg Foundation and National Institute of Alcohol Abuse and Alcoholism.

Declaration of interest. The funders had no role in the planning or conduct of the systematic search or interpretation of the results. The authors have no other conflicts of interest to report.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Table S1 **Lipoprotein subfractions in intervention studies**

Table S2 **Lipoprotein subfractions in observational studies**

Table S3 **Mechanisms in intervention studies**

Table S4 **Mechanisms in observational studies**

Table S5 **Statements from the manuscript including citations**

Table S6 **Definitions of lipoprotein subfractions as provided in the individual intervention and observational studies**

Figure S1 **Bias risk assessment from randomized controlled trials on lipoprotein subfractions**

Figure S2 **Bias risk assessment from nonrandomized investigations on lipoprotein subfractions**

Figure S3 **Bias risk assessment from cross-sectional studies on lipoprotein subfractions**

Figure S4 **Bias risk assessment from randomized controlled trials on mechanisms**

Figure S5 **Bias risk assessment from nonrandomized investigations on mechanisms**

Figure S6 **Bias risk assessment from cross-sectional studies on mechanisms**

Text S1 **Systematic literature search strategy**

Text S2 **PRISMA checklist moderate alcohol consumption and lipoprotein subfractions**

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