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Associations between Alcohol Consumption and HDL Subspecies Defined by ApoC3, ApoE and ApoJ: the Cardiovascular Health Study

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From the a From Department of Nutrition, Exercise and Sports, Section for Preventive and Clinical Nutrition, University of Copenhagen, Frederiksberg, Denmark, b From Department of Mathematical Sciences, Data Science Lab, University of Copenhagen, Copenhagen East, Denmark, c From Department of Nutrition, Harvard T.H. Chan School of Public Health, MA, d From Department of Public Health, Section of Epidemiology, University of Copenhagen, Copenhagen K, Denmark and e From Beth Israel Deaconess Medical Center, Division of General Medicine Research Section, Boston, MA.

Abstract: Alcohol consumption increases circulating high-density lipoprotein cholesterol (HDL-C), but HDL protein cargo may better reflect HDL function. This study examined the associations between alcohol intake and HDL subspecies containing or lacking apoC3, apoE, and apoJ in a well-phenotyped cohort. We performed a cross-sectional analysis of 2092 Cardiovascular Health Study participants aged 70 or older.

Abbreviations: Apo, apolipoprotein; CHD, coronary heart disease; CHS, Cardiovascular Health study; CVD, cardiovascular disease; HDL, high-density lipoprotein

# Shared senior authorship

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Dr. Jensen and Furtado have a patent issued for apoC3 in HDL: US8846321B2, and Dr. J.D. Furtado has an additional patent: US9494606B.Funding: This research was supported by contracts [HHSN268201200036C], [HHSN26820180001C], [N01HC55222], [N01HC85079], [N01HC85080], [N01HC85081], [N01HC85082], [N01HC85083], [N01HC85086], [75N92021D00006], and grants [U01HL080295] and [U01HL130114] from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by [K24AG065525] and [R01AG023629] from the National Institute on Aging (NIA). It was further supported by a Ph.D. scholarship sponsored in part by a Carlsberg Foundation Semper Ardens grant on biomarkers [CF15-0574], and in part by the National Institute of Alcohol Abuse and Alcoholism (NIAAA) [U10AA025286]. The lipoprotein and apolipoprotein measurements were supported by [R01NS089638] from NINDS: [R01AG023629]. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)

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with HDL subspecies measured in stored specimens from 1998 to 1999. Associations between alcohol intake and apoA1 defined HDL subspecies lacking or containing apoC3, apoE, and apoJ, and circulating levels of total apoA1, apoC3, apoE, and apoJ were examined. HDL subspecies lacking and containing apoC3, apoE, and apoJ were all positively associated with alcohol intake, with \( \sim 1\% \) per additional drink per week or \( \sim 7\% \) per additional drink per day (subspecies without the apolipoproteins, \( P \leq 2 \times 10^{-9} \), subspecies with the apolipoproteins, \( P \leq 3 \times 10^{-5} \)). Total apoA1 was also directly associated with alcohol consumption, with a \( 1\% \) increase per additional drink per week \( (P = 1 \times 10^{-14}) \). Total apoC3 blood levels were \( 0.5\% \) higher per additional drink per week \( (P = 0.01) \), but the association was driven by a few heavily drinking men. Alcohol intake was positively associated with HDL subspecies lacking and containing apoC3, apoE, or apoJ, and with total plasma apoA1. ApoC3 was directly, albeit not as robustly associated with alcohol intake. HDL protein cargo is crucial for its anti-atherosclerotic functions, but it remains to be determined whether HDL subspecies play a role in the putative association between limited alcohol intake and lower risk of coronary heart disease. (Curr Probl Cardiol 2023;48:101395.)

**Introduction**

Consumption of \( \frac{1}{2}-2 \) alcoholic drinks per day is associated with a lower risk of coronary heart disease (CHD),\(^1\) but the putative biological mechanisms are not well understood.\(^2\) In randomized trials, alcohol intake directly alters lipoprotein levels, with a particularly strong effect on high-density lipoprotein cholesterol (HDL-C).\(^3\)–\(^5\) Although HDL-C strongly associates with lower risk of CHD,\(^6\) clinical efforts to increase HDL-C levels have not consistently lowered CHD risk.\(^7\) Studies utilizing genetic correlates of HDL-C concentration also challenge the hypothesis that increasing HDL-C levels will reduce CHD risk.\(^8\)

This inconsistency may be explained by HDL molecules being highly heterogeneous with distinct structural and functional properties.\(^9\) HDL
subspecies based on apolipoprotein (apo) content may better reflect HDL function. The most well-known HDL related protein is apoA1, the main structural component of HDL particles. Proteomic studies have discovered more than 100 different proteins carried by HDL particles in various combinations. These include apoC3, apoE, and apoJ, all of which may influence the biological functions of HDL and its relationship with CHD risk. Furtado et al. recently found that pharmacological inhibition of cholesteryl ester transfer protein (CETP) increased HDL subspecies related to increased risk of CHD, such as HDL containing apoC3. Such results support the use of other HDL biomarkers than HDL-C.

HDL particles containing apoC3 have been positively and HDL lacking apoC3 negatively associated with CHD risk in several cohorts, indicating that apoC3 on HDL changes the function of HDL particles. An observational study found that HDL particles containing apoE but lacking apoC3 were inversely associated with CHD, suggesting interactions among the apolipoproteins. ApoC3 on VLDL or alone stimulate monocyte recruitment and adhesion, implying a crucial and direct role in atherogenesis. Animal and in vitro studies also suggest a role for apoE in HDL, such as stimulation of HDL biogenesis and maturation and hepatic uptake of HDL particles. The relevance of apoJ (Clusterin) in HDL is poorly understood, but insulin resistance and obesity is associated with reduced levels of apoJ in HDL, and apoJ alone may stimulate cholesterol efflux from foam cells.

Although alcohol intake clearly raises total HDL-C, its associations with HDL subspecies and apolipoproteins are far less clear. Recently published mega-data epidemiological studies have questioned the lower risk of CHD in light-moderate drinkers relative to abstainers as shown in observational studies for decades. Therefore, it seems pertinent to investigate the relationship between alcohol consumption and novel cardiovascular risk markers. This study investigates associations between alcohol consumption and HDL particles lacking or containing apoC3, apoE, or apoJ in participants from the Cardiovascular Health Study (CHS).

**Materials and Methods**

**Study Population and Design**

CHS is a longitudinal cohort study of 5888 participants above 65 years of age and is described in detail elsewhere. In brief, participants were randomly sampled from Medicare eligibility lists in each of 4 regions in the U.S. An initial recruitment cycle was conducted in 1989-1990.
enrolling 5201 participants. In 1992-1993, the CHS recruited an additional 687 predominantly African Americans. Eligible participants were non-institutionalized community-dwelling individuals who did not require a wheelchair, expected to stay in their respective regions for the next 3 years, were not receiving cancer treatment, and were able to provide individual informed consent. Participants visited their respective field centers yearly through 1989-1999 for questionnaires, physical examinations, and phlebotomy. An Institutional Review Board in each region approved the study.25 The present cross-sectional study included 2,092 participants with plasma apolipoprotein measurements available from the annual visit in 1998-99.

**Alcohol Consumption**

Annually, the CHS participants reported their usual frequency of consumption of beer, wine, and liquor, and the amount consumed on each occasion. In these analyses, alcohol intake in 1998-1999 (ie, at the time of lipoprotein measurement) was the primary exposure variable. Intakes from 1992 to 1993 through 1997-1998 were used in sensitivity analyses, except for intakes in 1995-1996, where only a semi-quantitative food frequency questionnaire was administered. A drink was defined as a 12-ounce can or bottle of beer, 6-ounce glass of wine, or a shot of liquor, corresponding to ~15 g ethanol. The complete CHS nutritional questionnaire is accessible elsewhere.27

Habitual alcohol intake was analyzed as continuous measurements and grouped as: long-term abstainers; former drinkers; <1 drink/week; 1-7 drinks/week; >7-14 drinks/week; and >14 drinks/week. Former drinkers were defined as any current abstainers who drank more than once/month in at least one of the preceding 5 years or that they previously drank 5 or more drinks almost daily. Twelve participants reported abstention in 1998-1999 but did not have alcohol data available from the preceding years. These were classified as long-term abstainers.

**Apolipoprotein Measurements**

Protein-defined HDL subspecies were quantified in 2092 CHS participants from stored specimens collected in 1998 of 1999. Participants eligible for the present analysis had provided fasting blood samples, had no history of previous stroke, and completed a routine contemporaneous magnetic resonance imaging brain scan offered to all participants. HDL
subspecies lacking or containing apoC3, apoE, and apoJ were chosen \textit{a priori}. ApoA1 was chosen as it defines HDL.

The lipid laboratory at Harvard T.H. Chan School of Public Health, Boston, MA, measured all HDL subspecies. A patented modified sandwich ELISA method were used to measure the HDL subspecies and whole plasma apolipoproteins (R&D Systems, Minneapolis, MN; and Academy Biomedical Company Inc., Houston, TX). A detailed description has been published. In brief, total plasma concentrations of apoA1, apoC3, apoE, and apoJ were measured with sandwich ELISA using polyclonal antibodies. ApoB-containing lipoproteins (d$<1.063$ g/mL) were removed from whole plasma through dextran sulfate and magnesium chloride precipitation, and the apoB-depleted plasma was fractionated by immunoaffinity separation into lipoproteins containing apoC3, apoE, or apoJ. The concentrations of apoA1 and apoE in each of these subspecies were assessed by sandwich ELISA. ApoA1 levels in HDL that lacked apoC3, apoE, and apoJ were calculated by subtracting the measured concentration of apoA1 in HDL containing each apolipoprotein from the total apoA1 concentration. ApoE subspecies that lacked apoJ and apoC3 were similarly calculated by subtraction. Thus, the HDL subspecies are quantified as apoA1 in HDL that contains or lack apoC3, apoE, or apoJ; and apoE in HDL that contains or lack apoC3 or apoJ. A simplified overview of these HDL subspecies is available in Supplementary Figure A14.

Trained and blinded personnel collected the fasting samples and performed the measurements in duplicates. The samples were stored at -80°C, thawed at room temperature before apolipoprotein measurements, and corrected for batch variation. The average coefficients of variation were 4%-18% for all apolipoprotein measures.

\textbf{Other Covariates}

Trained staff members registered age, sex, self-reported race, marital status, and education at the baseline visit in 1989-1990 or 1992-1993. Other covariates were measured in 1998-1999 except for the dietary variables, which were recorded 3 years earlier. We grouped race into Caucasian, African American, or other, and modeled years of education continuously. Marital status (married, widowed, divorced, separated, never married) and smoking status (never smokers, quit >1 year ago, quit <1 year ago, current smoker) were categorized. Weight change was calculated as the mean body weight change (%) during the preceding 6 years. We used average blood pressure (mmHg) continuously but
dichotomized the use of antihypertensive medications, statins, or estrogens (yes, no). Diabetes status was based on blood glucose $\geq 126$ mg/dL corrected for fasting time or diabetes medications. We used blocks walked and flights of stairs climbed/week as measures of physical activity. The participants' $APOE \epsilon_4$ status was used to test for effect modification. Analyses including $APOE \epsilon_4$ status were limited to subjects who gave consent to DNA use in studies of cardiovascular disease (CVD).

**Statistical Methods**

Explanatory variables and model residuals were investigated for normal distribution in validation plots. We utilized multivariable linear regression models to investigate associations between alcohol consumption in drinks/week and the following HDL subspecies: apoA1 in HDL that contained or lacked apoC3, apoE, or apoJ, and apoE in HDL that contained or lacked apoC3 or apoJ. In addition, the outcomes included whole plasma apoA1, apoC3, apoE, and apoJ.

All models were adjusted for potential confounders; Model 1 included age; sex; study site; race/ethnicity; marital status; and years of education. Model 2 included Model 1 covariates plus waist circumference; weight change (year 5-11); smoking status; average systolic blood pressure; use of antihypertensive medications; prevalent diabetes; statin use; estrogen use; physical activity; and estimated intakes of saturated fat, cholesterol, dietary fiber, omega-3 fatty acids, and sucrose. Inclusion of additional covariates, for example, fibrates, niacin, or antidiabetics, or intake of fructose or monounsaturated fat, did not improve our models. We tested models with and without log-transformation of the HDL subspecies, and modelled alcohol consumption continuously and categorically according to the pre-specified groups. In the latter, abstainers without former alcohol intake were used as the reference category.

A multivariate analysis using a linear mixed model was performed to test if the 2 different complementary HDL subspecies, such as lacking or containing apoC3, were equally associated with alcohol consumption. In these models, the interaction between alcohol and lipoprotein type was set as a fixed effect and subject ID was included as a random effect. We used generalized additive models (GAMS) with untransformed and log-transformed lipoprotein outcomes and tested non-linearity on either scale with partial F-tests. Missing values for covariates and outcomes were imputed with the last observation carried forward method.

Models were tested for effect modification by age, sex, statin use, previous CVD, and the presence or absence of the $APOE \epsilon_4$ allele. In
sensitivity analyses, we excluded former drinkers and used averaged alcohol intake during the 6 preceding years. Former drinkers were excluded to define the abstainer group as clear as possible. Beverage type analyses were explored in individual beverage type models and full models controlling for the consumption of the other 2 beverages, followed by a partial F-test for the importance of specific beverage types. All $P$-values were two-tailed, and $P < 0.05$ was considered statistically significant. Due to the number of extra tests, the significance level for effect modification was set to 0.01. All analyses were performed in R (R Development Core Team, version 4.0.0) using the following packages: multcomp, mgcv, lme4, and lmerTest.

Results

Study Participants

Table 1 summarizes the background characteristics of all the included CHS participants according to alcohol intake. Among the 2092 participants, the mean (SD) age was 80 (4) years. Overall, almost 60% of the CHS population were abstainers or former drinkers. On average, drinking more than 14 drinks/week was associated with a higher chance of being a current smoker, white male, and having a lower physical activity level. Due to assay difficulties, estimates failed for apoE in HDL in 36 samples and apoE that contained and lacked apoC3 and apoJ in 37.

Alcohol Intake and ApoA1 in HDL Lacking or Containing ApoC3, ApoE, and ApoJ

The log-transformed models generally showed the best data fit, and these results are presented in Table 2. Table 2 and Table 3 provide the results in drinks/week and drinks/day, but the results given in drinks/week are presented in the text. Outcome results on an absolute scale are available from Supplementary Tables A1-A2.

ApoC3. Alcohol consumption was associated with about 1% increase in both apoA1 in HDL lacking or containing apoC3 for every additional drink/week (Table 2). The multivariate analysis confirmed the similarity between the subfractions with and without apoC3 in their association to alcohol intake ($P = 0.38$). Given the much higher concentration of apoA1 in HDL without apoC3 than with it, the 2 apoC3 fractions were differentially associated with alcohol on an absolute level ($P = 3 \times 10^{-5}$).
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Weekly number of drinks</th>
<th>Abstainers (n = 960)</th>
<th>Former (n = 275)</th>
<th>&gt; 0 - &lt; 1 (n = 348)</th>
<th>1.7 (n = 295)</th>
<th>&gt; 7.14 (n = 112)</th>
<th>&gt; 14 (n = 102)</th>
<th>All (n = 2092)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer, median drinks/wk (IQR)</td>
<td>0</td>
<td>0 (0.0)</td>
<td>0 (0.02)</td>
<td>0 (0.5)</td>
<td>0.3 (0.1)</td>
<td>0.3 (0.3)</td>
<td>0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Wine, median drinks/wk (IQR)</td>
<td>0</td>
<td>0 (0.0)</td>
<td>0.02 (0.02-0.3)</td>
<td>1 (0.1)</td>
<td>1 (0.02-0.7)</td>
<td>1 (0.04-1.4)</td>
<td>1 (0.004-0.04)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Liquor, median drinks/wk (IQR)</td>
<td>0</td>
<td>0 (0.0)</td>
<td>0 (0.02)</td>
<td>0.3 (0.1)</td>
<td>1 (0.7)</td>
<td>14 (1.14)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Age, mean years (SD)</td>
<td>80 (5)</td>
<td>79 (4)</td>
<td>79 (4)</td>
<td>79 (4)</td>
<td>80 (4)</td>
<td>80 (4)</td>
<td>80 (4)</td>
<td>80 (4)</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>696 (73)</td>
<td>113 (48)</td>
<td>224 (64)</td>
<td>139 (47)</td>
<td>41 (37)</td>
<td>32 (31)</td>
<td>1265 (60)</td>
<td>1477 (71)</td>
</tr>
<tr>
<td>Race,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- White, No. (%)</td>
<td>178 (19)</td>
<td>55 (20)</td>
<td>53 (15)</td>
<td>36 (12)</td>
<td>7 (7)</td>
<td>339 (16)</td>
<td>1742 (83)</td>
<td>1477 (71)</td>
</tr>
<tr>
<td>- Black, No. (%)</td>
<td>7 (1)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>11 (5)</td>
<td>11 (5)</td>
</tr>
<tr>
<td>- Other, No. (%)</td>
<td>643 (67)</td>
<td>195 (71)</td>
<td>237 (68)</td>
<td>223 (76)</td>
<td>95 (85)</td>
<td>84 (82)</td>
<td>1477 (71)</td>
<td>1477 (71)</td>
</tr>
<tr>
<td>Marital status, married, No. (%)</td>
<td>13 (5)</td>
<td>14 (5)</td>
<td>15 (4)</td>
<td>16 (4)</td>
<td>17 (4)</td>
<td>17 (4)</td>
<td>15 (5)</td>
<td>15 (5)</td>
</tr>
<tr>
<td>Waist circumference, mean cm (SD)</td>
<td>97 (13)</td>
<td>96 (12)</td>
<td>97 (13)</td>
<td>96 (12)</td>
<td>96 (12)</td>
<td>96 (12)</td>
<td>97 (89-105)</td>
<td>97 (89-105)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>52 (5)</td>
<td>22 (8)</td>
<td>22 (6)</td>
<td>29 (10)</td>
<td>4 (4)</td>
<td>14 (14)</td>
<td>143 (7)</td>
<td>143 (7)</td>
</tr>
<tr>
<td>- Current smoker, No. (%)</td>
<td>297 (31)</td>
<td>145 (53)</td>
<td>166 (48)</td>
<td>172 (58)</td>
<td>81 (72)</td>
<td>72 (71)</td>
<td>933 (45)</td>
<td>933 (45)</td>
</tr>
<tr>
<td>- Former smoker, No. (%)</td>
<td>610 (64)</td>
<td>107 (38)</td>
<td>160 (46)</td>
<td>94 (32)</td>
<td>27 (24)</td>
<td>16 (16)</td>
<td>1014 (48)</td>
<td>1014 (48)</td>
</tr>
<tr>
<td>- Never smoker, No. (%)</td>
<td>860 (89)</td>
<td>215 (80)</td>
<td>301 (78)</td>
<td>328 (76)</td>
<td>154 (46)</td>
<td>131 (52)</td>
<td>1435 (68)</td>
<td>1435 (68)</td>
</tr>
<tr>
<td>Sys. blood pressure, mean mmHg (SD)</td>
<td>135 (20)</td>
<td>135 (21)</td>
<td>132 (21)</td>
<td>133 (19)</td>
<td>133 (20)</td>
<td>139 (22)</td>
<td>135 (20)</td>
<td>135 (20)</td>
</tr>
<tr>
<td>Hypertension medication, No. (%)</td>
<td>607 (63)</td>
<td>160 (58)</td>
<td>199 (57)</td>
<td>158 (54)</td>
<td>53 (47)</td>
<td>49 (48)</td>
<td>1226 (59)</td>
<td>1226 (59)</td>
</tr>
<tr>
<td>Prevalent diabetes, No. (%)</td>
<td>202 (21)</td>
<td>60 (22)</td>
<td>48 (14)</td>
<td>44 (15)</td>
<td>5 (4)</td>
<td>16 (16)</td>
<td>375 (18)</td>
<td>375 (18)</td>
</tr>
<tr>
<td>Statins use, No. (%)</td>
<td>157 (16)</td>
<td>40 (15)</td>
<td>53 (15)</td>
<td>47 (16)</td>
<td>16 (14)</td>
<td>12 (12)</td>
<td>325 (16)</td>
<td>325 (16)</td>
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<tr>
<td>Estrogen use, No. (%)</td>
<td>125 (13)</td>
<td>27 (10)</td>
<td>44 (13)</td>
<td>22 (7)</td>
<td>13 (12)</td>
<td>6 (6)</td>
<td>237 (11)</td>
<td>237 (11)</td>
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<td>Physical activity</td>
<td>5 (1-24)</td>
<td>12 (1-24)</td>
<td>10 (2-24)</td>
<td>14 (3-36)</td>
<td>24 (4-60)</td>
<td>13 (4-60)</td>
<td>10 (1-24)</td>
<td>10 (1-24)</td>
</tr>
<tr>
<td>- Blocks walked/wk, median (IQR)</td>
<td>5 (1-20)</td>
<td>5 (0-21)</td>
<td>8 (1-28)</td>
<td>10 (2-25)</td>
<td>10 (2-20)</td>
<td>6 (1-30)</td>
<td>6 (1-24)</td>
<td>6 (1-24)</td>
</tr>
<tr>
<td>- Flights up stairs past wk, median (IQR)</td>
<td>223 (23)</td>
<td>73 (27)</td>
<td>64 (18)</td>
<td>76 (26)</td>
<td>22 (20)</td>
<td>19 (19)</td>
<td>477 (23)</td>
<td>477 (23)</td>
</tr>
<tr>
<td>HDL-C, median mg/dL (IQR)</td>
<td>58 (51-66)</td>
<td>57 (50-64)</td>
<td>57 (51-65)</td>
<td>59 (52-66)</td>
<td>58 (53-67)</td>
<td>62 (54-72)</td>
<td>58 (51-66)</td>
<td>58 (51-66)</td>
</tr>
<tr>
<td>Apolipoproteins, median mg/dL (IQR)</td>
<td>138 (114-164)</td>
<td>132 (109-160)</td>
<td>138 (113-161)</td>
<td>143 (116-170)</td>
<td>149 (120-178)</td>
<td>158 (136-177)</td>
<td>139 (115-165)</td>
<td>139 (115-165)</td>
</tr>
<tr>
<td>- Total ApoE in whole plasma</td>
<td>11 (9-14)</td>
<td>11 (9-14)</td>
<td>11 (9-14)</td>
<td>11 (9-13)</td>
<td>11 (8-13)</td>
<td>10 (8-12)</td>
<td>11 (9-14)</td>
<td>11 (9-14)</td>
</tr>
<tr>
<td>- Total ApoA1 in HDL plasma</td>
<td>5 (5-6)</td>
<td>5 (5-6)</td>
<td>5 (5-6)</td>
<td>6 (5-6)</td>
<td>5 (5-6)</td>
<td>5 (5-6)</td>
<td>5 (5-6)</td>
<td>5 (5-6)</td>
</tr>
<tr>
<td>- ApoA1 in HDL containing apoC</td>
<td>124 (104-145)</td>
<td>119 (103-141)</td>
<td>125 (104-145)</td>
<td>130 (108-153)</td>
<td>135 (116-156)</td>
<td>142 (122-163)</td>
<td>125 (105-147)</td>
<td>125 (105-147)</td>
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<tr>
<td>- ApoA1 in HDL lacking apoC</td>
<td>119 (101-141)</td>
<td>117 (99-138)</td>
<td>121 (101-140)</td>
<td>126 (103-151)</td>
<td>131 (110-153)</td>
<td>138 (122-160)</td>
<td>122 (102-144)</td>
<td>122 (102-144)</td>
</tr>
</tbody>
</table>
| - ApoA1 in HDL containing apoE | 15 (10-22) | 13 (9-18) | 14 (10-20) | 14 (10-20) | 16 (12-21) | 15 (12-20) | 14 (10-21) | 14 (10-21) | (continued on next page)
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Weekly number of drinks</th>
<th>0 - 1</th>
<th>1-7</th>
<th>&gt;7-14</th>
<th>&gt;14</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoA1 in HDL lacking apoJ</td>
<td>134 (114-156)</td>
<td>130 (112-153)</td>
<td>135 (113-157)</td>
<td>139 (117-164)</td>
<td>141 (127-167)</td>
<td>151 (134-174)</td>
</tr>
<tr>
<td>ApoA1 in HDL containing apoJ</td>
<td>3 (2-3)</td>
<td>3 (2-3)</td>
<td>3 (2-3)</td>
<td>3 (2-3)</td>
<td>3 (2-3)</td>
<td>3 (2-3)</td>
</tr>
<tr>
<td>ApoE in HDL lacking apoC3</td>
<td>5 (3-6)</td>
<td>5 (3-6)</td>
<td>4 (3-6)</td>
<td>4 (3-6)</td>
<td>4 (3-6)</td>
<td>4 (3-6)</td>
</tr>
<tr>
<td>ApoE in HDL containing apoC3</td>
<td>4 (2-5)</td>
<td>3 (2-5)</td>
<td>3 (2-5)</td>
<td>3 (2-5)</td>
<td>3 (2-5)</td>
<td>3 (2-5)</td>
</tr>
<tr>
<td>ApoE in HDL lacking apoJ</td>
<td>8 (6-10)</td>
<td>8 (6-10)</td>
<td>8 (6-10)</td>
<td>7 (6-9)</td>
<td>7 (6-9)</td>
<td>7 (6-9)</td>
</tr>
<tr>
<td>ApoE in HDL containing apoJ</td>
<td>0.3 (0.2-0.4)</td>
<td>0.3 (0.2-0.4)</td>
<td>0.3 (0.2-0.4)</td>
<td>0.3 (0.2-0.4)</td>
<td>0.3 (0.2-0.3)</td>
<td>0.2 (0.2-0.3)</td>
</tr>
</tbody>
</table>

Apo, apolipoprotein; APOE, apolipoprotein E genes; HDL, high-density lipoprotein; IQR, interquartile range; sys, systolic; wk, week.
<table>
<thead>
<tr>
<th></th>
<th>ApoA1 in HDL lacking apoC3 (mg/dL)</th>
<th>ApoA1 in HDL containing apoC3 (mg/dL)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ApoA1 in HDL lacking apoC3</strong></td>
<td>Percentage increase (95% CI)</td>
<td>Percentage increase (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Model 1 (n = 2055)</td>
<td>Drinks/wk: 0.94% (0.71-1.18)</td>
<td>Drinks/wk: 0.96% (0.60-1.32)</td>
<td>3 x 10^-15</td>
</tr>
<tr>
<td></td>
<td>Drinks/d: 6.8% (5.1-8.5)</td>
<td>Drinks/d: 6.9% (4.3-9.6)</td>
<td>7 x 10^-13</td>
</tr>
<tr>
<td>Model 2 (n = 1934)</td>
<td>Drinks/wk: 0.86% (0.63-1.10)</td>
<td>Drinks/wk: 0.90% (0.53-1.27)</td>
<td>1 x 10^-6</td>
</tr>
<tr>
<td></td>
<td>Drinks/d: 6.2% (4.5-7.9)</td>
<td>Drinks/d: 6.5% (3.8-9.2)</td>
<td>1 x 10^-6</td>
</tr>
<tr>
<td><strong>ApoA1 in HDL lacking apoE</strong></td>
<td>Percentage increase (95% CI)</td>
<td>Percentage increase (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Model 1 (n = 2055)</td>
<td>Drinks/wk: 0.96% (0.69-1.24)</td>
<td>Drinks/wk: 1.10% (0.61-1.59)</td>
<td>8 x 10^-12</td>
</tr>
<tr>
<td></td>
<td>Drinks/d: 6.9% (4.9-9.0)</td>
<td>Drinks/d: 8.0% (4.4-11.7)</td>
<td>2 x 10^-9</td>
</tr>
<tr>
<td>Model 2 (n = 1934)</td>
<td>Drinks/wk: 0.86% (0.58-1.14)</td>
<td>Drinks/d: 1.16% (0.65-1.67)</td>
<td>9 x 10^-6</td>
</tr>
<tr>
<td></td>
<td>Drinks/d: 6.2% (4.1-8.3)</td>
<td>Drinks/d: 8.4% (4.6-12.3)</td>
<td>2 x 10^-6</td>
</tr>
<tr>
<td><strong>ApoA1 in HDL without apoJ</strong></td>
<td>Percentage increase (95% CI)</td>
<td>Percentage increase (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Model 1 (n = 2055)</td>
<td>Drinks/wk: 0.94% (0.72-1.17)</td>
<td>Drinks/wk: 0.84% (0.51-1.18)</td>
<td>&lt; 2 x 10^-16</td>
</tr>
<tr>
<td></td>
<td>Drinks/d: 6.8% (5.1-8.5)</td>
<td>Drinks/d: 6.0% (3.6-8.5)</td>
<td>5 x 10^-14</td>
</tr>
<tr>
<td>Model 2 (n = 1934)</td>
<td>Drinks/wk: 0.86% (0.64-1.09)</td>
<td>Drinks/d: 0.73% (0.39-1.07)</td>
<td>5 x 10^-14</td>
</tr>
<tr>
<td></td>
<td>Drinks/d: 6.2% (4.6-7.9)</td>
<td>Drinks/d: 5.2% (2.7-7.8)</td>
<td>3 x 10^-6</td>
</tr>
</tbody>
</table>

Outcomes were log-transformed and reported in percentage change per additional drink per week or day.

Model 1: adjusted for age, sex, study site, race/ethnicity, marital status, and years of education.

Model 2: adjusted for Model covariates and waist circumference, weight change (year 5-11), smoking status, average systolic blood pressure, antihypertensive medications, prevalent diabetes, statin use, estrogen use, physical activity measures (blocks walked, flights up stairs), and dietary factors (saturated fat, cholesterol, dietary fibers, omega-3, sucrose).

Apo, apolipoprotein; HDL, high-density lipoprotein.
<table>
<thead>
<tr>
<th></th>
<th>ApoA1 in whole plasma (mg/dL)</th>
<th>ApoC3 in whole plasma (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percentage increase or decrease (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Model 1 (n = 2055) Drinks/wk</td>
<td>1.01% (0.77-1.25)</td>
<td>&lt; $2 \times 10^{-16}$</td>
</tr>
<tr>
<td>Drinks/d</td>
<td>7.3% (5.5-9.1)</td>
<td>0.94% (0.70-1.18)</td>
</tr>
<tr>
<td>Model 2 (n = 1934) Drinks/wk</td>
<td>6.8% (5.0-8.6)</td>
<td>-0.02% (-0.28-0.25)</td>
</tr>
<tr>
<td>Drinks/d</td>
<td>-0.1% (-2.0-1.8)</td>
<td>-0.09% (-0.35-0.19)</td>
</tr>
<tr>
<td>Model 1 (n = 2055) Drinks/wk</td>
<td>-0.6% (-2.5-1.3)</td>
<td>0.1% (-1.2-1.4)</td>
</tr>
</tbody>
</table>

Outcomes were log-transformed and reported in percentage change per additional drink per week or day.
Model 1: adjusted for age, sex, study site, race/ethnicity, marital status, and years of education.
Model 2: adjusted for Model 1 covariates and waist circumference, weight change (year 5-11), smoking status, average systolic blood pressure, antihypertensive medications, prevalent diabetes, statin use, estrogen use, physical activity measures (blocks walked, flights up stairs), and dietary factors (saturated fat, cholesterol, dietary fibers, omega-3, sucrose).
Apo, apolipoprotein.

The outcomes were log-transformed. The models were adjusted for age, sex, study site, race/ethnicity, marital status, and years of education, waist circumference, weight change (year 5-11), smoking status, average systolic blood pressure, antihypertensive medications, prevalent diabetes, statin use, estrogen use, physical activity measures (blocks walked, flights up stairs), and dietary factors (saturated fat, cholesterol, dietary fibers, omega-3, sucrose). Apo, apolipoprotein. HDL, high-density lipoprotein. (Color version of figure is available online.)
Analyses with GAMs showed that both fractions tend to increase linearly with alcohol consumption until ~20-30 drinks/week, but then stabilize (Figure 1). Significant non-linearity was found only for apoA1 in HDL lacking apoC3 on the absolute scale ($P = 0.035$). In categorical analyses, apoA1 in HDL lacking and containing apoC3 were both progressively higher in heavier drinking categories above 1 weekly drink, compared with abstainers. The increase was strongest for apoA1 in HDL lacking apoC3 (Supplementary Table A3).

**ApoE.** Results for apoA1 in HDL lacking or containing apoE were somewhat similar to those for the apoC3 fractions; both were increased by about 1% and not different in the multivariate analysis ($P = 0.08$) (Table 2). The GAM plots confirmed non-linearity on an absolute scale only for apoA1 in HDL without apoE ($P = 0.035$) and a linear relationship to the apoE containing fraction ($P = 1.0$) (Supplementary Figures A1-A2).

Categorical analyses confirmed a progressive increase in apoA1 in HDL lacking apoE in drinkers above 1 drink/week compared with abstinence, which was less consistent for apoA1 in HDL containing apoE. Former drinking was associated with lower levels of apoA1 in HDL containing apoE (Supplementary Table A3).

**ApoJ.** One additional weekly drink also increased both apoA1 in HDL lacking and containing apoJ by about 1% (Table 2), and the similarity was confirmed in the multivariate analysis ($P = 0.11$). The associations to both apoJ fractions were stronger on a percentage scale (Table 2) than on an absolute scale (Supplementary Table A1), but GAM plots only implied significant non-linearity on both scales for apoA1 in HDL lacking apoJ (Supplementary Figures A3-A4).

Whereas apoA1 in HDL lacking apoJ increased progressively with greater alcohol intake compared with abstention, circulating levels of apoA1 in HDL containing apoJ were only positively associated with alcohol intake for drinkers of 1-7 or >14 drinks/week (Supplementary Table A3).

**Alcohol Intake and Total ApoA1, ApoC3, ApoE, and ApoJ in Whole Plasma**

Alcohol intake was strongly associated with whole plasma apoA1 with an increase of about 1% per weekly drink (Table 3). The association with total apoC3 levels was also positive but the increase was smaller and weaker (Supplementary Figure A8), and it did not persist when compared
with abstention in the categorical alcohol analysis (Supplementary Table A4).

Sex-specific post hoc analyses showed that total apoC3 was not significantly increased in women (n = 1265): $\beta$: -0.02 (-0.12-0.08), $P = 0.73$, but was significant in men (n = 827) $\beta$: 0.11 (0.05-0.18), $P = 0.0005$. After removal of 3 outliers representing heavily drinking men (Supplementary Figure A9), the overall positive relationship between alcohol intake and total apoC3 levels in both sexes was substantially attenuated: $\beta$: 0.03 (-0.02-0.09) $P = 0.25$.

We found no association of alcohol consumption with overall levels of apoE or apoJ, but total plasma apoJ levels were higher among drinkers of 1-7 drinks/week than abstainers (Supplementary Table A4). Non-linearity was not evident on either scale for any of the total apolipoprotein concentrations (Supplementary Figures A5-A7).

**Alcohol Intake and ApoE In HDL Lacking or Containing ApoC3 and ApoJ**

Increasing alcohol intake was associated with decreased levels of apoE in HDL without apoC3 and apoE in HDL containing apoJ. The results can be found in the Supplementary Tables A9-A12 and Figures S10-S13.

**Sensitivity Analyses**

Excluding former drinkers and averaging the alcohol intake from year 5-11 did not change our overall conclusions (Supplementary Tables A5-A6). There was no effect modification by age, sex, statin use, previous CVD, and the presence or absence of APOE $\varepsilon4$ on a significance level of 0.01.

**Beverage Type**

We tended to observe stronger associations with liquor compared with wine and beer for apoA1 in HDL lacking or containing apoC3, apoE, and apoJ. This tendency reached significance for apoA1 in HDL containing apoC3 (Supplementary Table A7). Beer and wine were more robustly associated with increases in apoA1 in HDL lacking apoC3 ($P \leq 0.01$) compared with the corresponding fractions containing apoC3 ($P > 0.05$), whereas liquor was strongly associated with both fractions (Supplementary Table A7). In contrast to beer and liquor, wine was not positively associated with total apoA1 and total apoC3. Whole plasma apoC3 was negatively but non-significantly associated with wine intake. Beer was
more strongly associated with total apoC3 than were wine and liquor ($P = 0.03$) (Supplementary Table A8).

**Discussion**

In this cross-sectional analysis of older adults, alcohol consumption was directly associated with all HDL subspecies quantified by the concentration of apoA1 in HDL and categorized by presence or absence of apoC3, apoE, and apoJ. All subfractions increased to a somewhat similar extent of $\sim 1\%$ per additional drink/week ($\sim 7\%$ per additional drink/day). The increase was similar to the increase in total apoA1 levels, whereas total apoC3 increased about $0.5\%$ per additional weekly drink. The associations were generally higher in absolute terms and more statistically robust for the more prevalent HDL fractions lacking apoC3, apoE, and apoJ than the fractions containing those apolipoproteins.

Increased circulating levels of total apoA1 following alcohol intake are well-known.\(^3\) ApoA1 is an essential regulator of reverse cholesterol transport through activation of the membrane transporter, ATP-binding cassette transporter A1 and lecithin:cholesterol acyltransferase.\(^{11}\) Its increase following alcohol consumption has been ascribed to stimulated hepatic apoA1 synthesis and secretion in vitro.\(^{29,30}\)

In the CHS, a minor increase in whole plasma apoC3 per extra weekly drink was seen in the primary analysis. However, no positive association was found in neither the categorical analysis nor in women alone. In men, it was sensitive to removal of heavy drinking outliers. A previous study of 32 healthy and 97 hospitalized subjects before and after 21 days of alcohol withdrawal found higher total apoC3 with increasing levels of alcohol intake. The apoC3 levels reversed after alcohol withdrawal.\(^{31}\) In 2 cross-sectional studies, alcohol intakes associated positively with total apoC3 in men but not in women.\(^{32,33}\) Total apoC3 was unchanged in a third cross-sectional study.\(^{34}\) ApoC3 adversely affects lipoprotein metabolism via inhibition of lipoprotein lipase (LPL)\(^{35}\) and hepatic uptake of triglyceride-rich lipoproteins.\(^{36}\) ApoC3 also has a direct influence on atherosclerosis via mechanisms such as endothelial cell activation and monocyte recruitment.\(^{17,37}\) *APOC3* loss-of-function mutations are associated with a lower risk of ischemic CVD,\(^{38}\) implying a crucial role for apoC3 in CVD. Even though this cross-sectional study did not find a strong and consistent positive association to total apoC3, and thus cannot conclude any adverse effect of alcohol consumption, its relationship with alcohol consumption in different populations and in subjects with different drinking patterns requires further study.
Results similar to ours were found in previous cross-sectional studies, where 2 studies found higher levels of HDL or HDL-C lacking apoC3 \cite{15,21} and 4 found higher levels of HDL or HDL-C containing apoC3 \cite{15,31-33}. In 2 of the latter studies, the increases were only significant in men. \cite{32,33} Distinct laboratory modalities and population groups may explain these discrepancies. In 1 study, 42% had metabolic syndrome, a prevalent condition in obesity. \cite{39} Obesity itself has been linked to higher levels of HDL containing apoC3 on a percentage scale. \cite{40} Unlike these other analyses, we explored associations to the HDL fractions on both an absolute and a relative level. The higher increase in apoA1 in HDL lacking apoC3 compared with the HDL fraction containing apoC3 may imply a higher cardiovascular benefit than a disadvantage, given that circulating levels of apoA1 in HDL lacking apoC3 are inversely associated with insulin resistance, \cite{41} subclinical atherosclerosis, \cite{42} and CHD risk. \cite{14,15} Still, the clinical relevance of each of these measures needs to be clarified.

The relationship between alcohol and HDL subspecies with and without apoE and apoJ has not been investigated before. Animal and in vitro studies support a role for apoE in HDL in atheroprotection, such as stimulation of HDL biogenesis and maturation plus hepatic uptake of cholesterol and HDL particles. \cite{18,19,43} On the other hand, the apoE concentration in HDL has been associated with higher CHD risk, \cite{44} and proteomics analyses have shown apoE enrichment of HDL3 in patients with established CHD compared with controls. \cite{45} A higher prevalence of small HDL particles with apoE has also been shown in obesity compared with normal weight individuals. \cite{40} These results suggest apoE as a CHD risk marker. \cite{16}

It is still speculative whether the increase in apoA1 in HDL lacking apoE in our study mediates the lower CHD risk shown for moderate drinking, \cite{1} and we did not measure HDL size. However, the categorical analysis confirmed robust positive associations to apoA1 in HDL lacking apoE in drinkers of 1-7 and 8-14 units/week, and the GAM model implies an inverse J-shaped relationship with the highest levels in drinkers of about 10-20 weekly drinks (Supplementary Table A3 and Figure A1). Nonetheless, the overall higher levels of apoA1 in HDL with or without apoE may be simply a reflection of increased overall HDL levels.

ApoJ (Clusterin) is primarily found in HDL or unbound, \cite{46} and reduced levels of apoJ in HDL is found in obesity, insulin resistance, dyslipidemia, and CHD. \cite{47,48} In hypertriglyceridemia, apoJ may shift from HDL to apoB-containing lipoproteins, and lower levels of HDL apoJ in CHD impair potential anti-atherosclerotic functions of HDL. \cite{48} In light of these
results, the higher absolute level of apoA1 in HDL lacking apoJ compared with the HDL fraction containing apoJ may have no cardiovascular benefit. On the other hand, we found that total apoJ levels were significantly higher in drinkers of 1-7 drinks/week compared with abstention implying that this group could benefit metabolically (Supplementary Tables A5).

Among the strengths of this study are the large sample size, repeated measures of alcohol intake, analyzed continuously and categorically, and outcome reporting on different levels. However, due to the observational cross-sectional design, the study is prone to residual confounding; and causality between alcohol intake and HDL subspecies cannot be assumed. We could not perform longitudinal analyses, as the HDL subspecies were only available in 1998–99. Although our method of lipoprotein assessment is well-established,\textsuperscript{14,42,49,50} potentially alternative measures of HDL content and function are available\textsuperscript{44,50} and should be further investigated in relation to alcohol intake. Among our limitations is also the alcohol intake and age distribution exemplified with the CHS, where more than half were abstainers and all >65 years.

In conclusion, alcohol consumption was positively associated with apoA1 in HDL lacking apoC3, apoE and apoJ to a similar extent as the complementary HDL subspecies on a percentage scale. Due to higher blood concentrations, the HDL fraction without apoC3, apoE, and apoJ increased more compared with the HDL subspecies containing these apolipoproteins per additional weekly drink. The increases of about 1% were similar to the increase in the overall apoA1 levels, suggesting an increase in overall HDL levels rather than an apolipoprotein redistribution among HDL particles. Alcohol was also positively but weakly associated with whole plasma apoC3 levels, which was not supported in a categorical analysis comparing drinkers with abstainers. The HDL protein cargo appears to modify the potential cardioprotective effects of HDL substantially, but whether these results reflect possible benefits or harms of alcohol intake in relation to risk of coronary heart disease remains to be determined.

Author contributions

Trine L. Wilkens: conceptualization (lead), data curation (lead), formal analysis (lead), methodology (lead), project administration, software (lead), visualization, writing - original draft preparation, writing - review and editing (lead). Helle Sørensen: software (equal), formal analysis (equal), supervision (equal), writing - review and editing (equal). Majken
Acknowledgement

We thank the CHS investigators, staff, and participants for their contributions, which are highly appreciated. More information of the CHS personnel is accessible at https://chs-nhlbi.org.

Data statement

The datasets created and analyzed during this study are available from the CHS Coordinating Center upon request and completion of an approved data distribution agreement.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.cpcardiol.2022.101395.

REFERENCES


