Associations of High-Density Lipoprotein Particle and High-Density Lipoprotein Cholesterol With Alcohol Intake, Smoking, and Body Mass Index  
— The INTERLIPID Study —

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**Background:** Recently, high-density lipoprotein particles (HDL-P) have been found to be more strongly inversely associated with coronary artery disease (CAD) risk than their counterpart, HDL cholesterol (HDL-C). Given that lifestyle is among the first targets in CAD prevention, we compared the associations of HDL-P and HDL-C with selected lifestyle factors.

**Methods and Results:** We examined 789 Japanese participants of the INTERLIPID Study: men (n=386) and women (n=403) aged 40–59 years in 1996–1998. Participants treated for dyslipidemias were excluded. Lifestyle factors included alcohol intake, smoking amount, and body mass index (BMI). Multivariable linear regression was used for cross-sectional analyses of these factors with HDL-P, HDL-C, HDL-P size subclasses (small, medium and large) and mean HDL-P size. In men, higher alcohol intake was associated with higher HDL-P and higher HDL-C. The associations of alcohol, however, were strongest with HDL-P. A higher smoking amount tended to be associated with lower HDL-P and HDL-C. In contrast, BMI was not associated with HDL-P, but was strongly inversely associated with HDL-C. While alcohol intake favored larger mean HDL-P size, smoking and BMI favored a lipid profile with smaller HDL-P subclasses and overall smaller mean HDL-P size. Similar, but generally weaker results were observed in women.

**Conclusions:** Although both HDL-P and HDL-C are parameters of HDL, they have different associations with alcohol, smoking and BMI.

**Key Words:** Alcohol; Body mass index; High-density lipoprotein cholesterol; High-density lipoprotein particle; Smoking

Higher circulating high-density lipoprotein (HDL) cholesterol (HDL-C) is well-known to be associated with lower risk of coronary artery disease (CAD); this has led to the suggestion that by increasing HDL-C, a lower risk of CAD may be achieved. Most drugs targeted to increase HDL-C, however, did not reduce CAD risk or its precursor, subclinical atherosclerosis. This has attracted renewed interest in other features of HDL that may provide insight into its association with CAD. Rather than the total cholesterol carried by HDL, the number of circulating HDL particles (HDL-P) that carry...
this cholesterol may be an important link to CAD. Recently, HDL-P have been shown to have inverse associations with CAD risk and subclinical atherosclerosis, independent of HDL-C, while such associations of HDL-C were not independent of HDL-P.7,8

Given that modifiable behavioral and dietary factors are among the first targets in the prevention of CAD, assessing and comparing their associations with HDL-P and HDL-C could be informative. Modifiable lifestyle factors, such as alcohol intake, smoking, and body mass index (BMI), are not only related to CAD risk, but are also known to influence HDL-C level. Few studies have assessed the relationships of lifestyle factors to circulating HDL-P levels, especially in healthy individuals. Moreover, no studies have directly evaluated the associations of HDL-P and HDL-C with these lifestyle factors. Thus, the aim of this study was to investigate associations of alcohol intake, smoking, and BMI with HDL-P and HDL-C.

Methods

Subjects

The INTERLIPID Study* is an ancillary study of INTERMAP, the International Study of Macronutrients and Blood Pressure.9,10 For the present study, only Japanese subjects were used. Participants were men and women aged 40–59 years who were recruited in 1996–1998 from general and workforce populations near 4 study centers located in Toyama, Aito, Wakayama and Sapporo in Japan. Local factory workers were recruited by study centers in Toyama, Wakayama, and Sapporo, and local residents were recruited by the Aito center (n=1,136). Participants from Sapporo study center, however, were excluded from this analysis because plasma samples thawed during shipment (n=293), becoming unsuitable for measurement of HDL-P. Those on lipid-lowering medication and those missing information on either HDL-P or HDL-C were also excluded from this study (n=54). A total of 789 participants (386 men and 403 women) were included in the present study.

Written informed consent was obtained from all study participants. The study protocol conforms to the Declaration of Helsinki (1975) and was approved by the Institutional review boards and ethics committees of Shiga University of Medical Science, Kanazawa Medical University, and Wakayama Medical University.

Study participants visited the research centers 4 times: 2 pairs of consecutive days, approximately 3 weeks apart. Data collection personnel were trained and certified in the study methods and protocol at national training sessions by senior staff.11 A standardized questionnaire was given consisting of questions about lifestyle and behavior, such as smoking and drinking, medical history and medication use. In women, menopause status (pre/post/current) was also recorded.

A non-fasting blood sample was taken from each participant after the last meal on the second day. Height and weight (with light clothing) were measured twice, at the first and third visits. Blood pressure was measured by a trained technician according to standardized protocol,11 twice at each of the 4 visits. Blood pressure of each participant was measured on the right arm when possible, in a seated position with a random zero sphygmomanometer after a rest ≥5 min. Average height, weight, and blood pressure from all measurements were recorded. BMI was calculated as weight (kg)/[height (m)]². Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or the use of antihypertensive drugs. Diabetes was defined as a diagnosis of diabetes mellitus from a doctor. Overweight was defined as BMI ≥25 kg/m².

Dietary Recall

According to high-level standardized protocol, 4 detailed multi-pass 24-h dietary recalls were collected, 1 on each of the 4 days of study center visits.10 The dietary inclusion criteria have been described elsewhere.10,11 Briefly, trained interviewers used food and drink models and measuring devices to help participants with accurate dietary recall.

Mean alcohol intake (g/day) from the first two 24-h recalls was used to characterize 48-h alcohol intake (as non-fasting blood was drawn on the second visit). Two 7-day recalls on daily alcohol consumption at the first and third visit were also performed. Mean alcohol intake per day of the two 7-day recalls was defined as habitual alcohol intake. Current drinkers were defined as participants who considered themselves to be drinkers.

Total energy intake from the 24-h recalls was calculated from the following energy values per 1 g of dietary source: 9 kcal for fat, 4 kcal for protein, 4 kcal for available carbohydrate, and 7 kcal for alcohol. Mean total energy intake (kcal/day) of the four 24-h recalls was used.

Laboratory Measurements

Non-fasting blood was centrifuged ≤30 min after collection for obtaining serum and plasma samples.9 Plasma and serum samples were refrigerated immediately and were stored at −70°C ≤24 h after collection. Samples were shipped in dry ice to a central laboratory where they were randomly allocated for analysis to prevent systematic measurement bias. The central laboratory followed standardized protocol from the Lipid Standardization Program, Centers for disease Control and Prevention (Atlanta, GA, USA)12 and measured serum and plasma samples 6–12 months after collection. Serum concentration (mg/dL) of total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C) and HDL-C was measured using enzymatic techniques with an auto-analyzer (Hitachi 7107, Tokyo, Japan).9 To convert total cholesterol, LDL-C and HDL-C from mg/dL to mmol/L, divide by 38.67. To convert triglycerides from mg/dL to mmol/L divide by 88.57. Lipoprotein profile of plasma samples was measured on nuclear magnetic resonance (NMR) spectroscopy approximately 4 years after collection and storage at −70°C by Liposcience (NC, USA), recently acquired by LabCorp (NC, USA). The lipoprotein profile measurement has been previously described.13,14 Briefly, lipoproteins were identified using the distinct NMR signal of the lipid methyl group characteristic of each lipoprotein and its subclass. The concentration of each lipoprotein was calculated from the amplitude of the NMR signal and conversion factors obtained from a lipoprotein subclass reference library. For subclasses of HDL, small (7.3–8.2 nm), medium (8.2–8.8 nm), and large (8.8–13 nm) particle concentrations were measured. Mean HDL-P size was determined from the sum of the diameter of each subclass multiplied by its relative mass percentage (weighted).13

Statistical Analysis

Characteristics of male and female participants are
HDL Particle, Cholesterol and Lifestyle

Results

Characteristics of INTERLIPID men and women are listed in Table 1. Mean age was 49.6±5.4 years and 49.0±5.3 years for men and women, respectively. Men had higher mean SBP and were more likely to be hypertensive and diabetic compared with women. Men also had higher total energy intake and drank and smoked more than women. Men had higher serum triglycerides, but no significant differences in total cholesterol or LDL-C were observed between the sexes. HDL lipid and lipoprotein profile, however, were significantly different between the 2 sexes. Men had lower HDL-C, but higher HDL-P levels than women. HDL-P size subclasses also varied between the sexes, with men having higher concentrations of small and medium particles and women having higher concentration of large HDL-P than men. Overall, women tended to have higher mean HDL-P size.

In men, current drinkers compared with non-drinkers had higher mean age- and site-adjusted HDL-P (30.4 µmol/L presented as mean±SD, as percentage, or as median (IQR). Differences between men and women were analyzed using P-value, which was determined using Student’s t-test for continuous variables with bell-shaped distribution, by Wilcoxon rank sum test for continuous variables with skewed distribution, or by chi-squared test for percentages (proportions). Analysis of covariance was used to obtain age- and study site-adjusted HDL-P and HDL-C means between individuals with presence or absence of lifestyle factors. To visualize differences in HDL parameter per quartile of lifestyle factor, unadjusted mean HDL-P, HDL-C, and HDL-P size subclasses per quartile were also assessed. Values for P trend were obtained from simple linear regression of each lifestyle factor as a continuous variable with HDL-P or HDL-C. To compare the strength of the associations of HDL-P and HDL-C with lifestyle factors, regression estimates are presented in percent standard deviations (%SD) of HDL-P or HDL-C per 1-SD higher lifestyle factor. Study site had no evidence of interaction in the associations of HDL-P or HDL-C with any of the lifestyle factors of interest. All analyses were sex-stratified. Sensitivity analysis (with no sex stratification) of the linear relationship between lifestyle factors and HDL-P and HDL-C was also performed. Two-tailed P<0.05 was considered statistically significant. SAS version 9.4 (SAS Institute, Cary, NC, USA) was used to perform all analyses.

Table 1. Characteristics of Japanese INTERLIPID Subjects, 1996–1998

| Characteristics | Men (n=386) | Women (n=403) | P-value |
|-----------------|-------------|---------------|---------|
| Age (years)     | 49.6±5.4    | 49.0±5.3      | 0.121   |
| SBP (mmHg)      | 120.5±13.4  | 114.1±14.1    | <0.001  |
| BMI (kg/m²)     | 23.5±2.8    | 23.1±3.1      | 0.050   |
| Total energy intake (kcal/day) | 2,304±434 | 1,823±324 | <0.001 |
| Overweight      | 28.0        | 22.3          | 0.067   |
| Hypertension    | 16.1        | 10.4          | 0.019   |
| Diabetes mellitus | 4.9         | 0.7           | <0.001  |
| Premenopause status | –         | 46.4        | –       |
| Current drinker | 97.7        | 83.6          | <0.001  |
| Current smoker  | 55.7        | 5.2           | <0.001  |
| Habitual alcohol intake (g/day)† | 25.8 (9.0–46.4) | 0.5 (0.0–3.0) | <0.001 |
| 48-h alcohol intake (g/day)§ | 22.9 (4.8–45.3) | 0.0 (0.0–2.5) | <0.001 |
| Smoking amount (cigs/day) | 13 (0–20)  | 0 (0–0)       | <0.001  |
| Triglycerides (mg/dL) | 135 (91–188) | 95 (70–131)  | <0.001  |
| Total cholesterol (mg/dL) | 199.3±28.8 | 201.7±31.9 | 0.272   |
| LDL-C (mg/dL)   | 124.1±28.2  | 126.0±29.5    | 0.343   |
| HDL-C (mg/dL)   | 54.5±13.3   | 60.3±14.0     | <0.001  |
| HDL-P (µmol/L)  | 30.3±6.1    | 28.0±5.3      | <0.001  |
| Small HDL-P (µmol/L) | 19.9±4.9 | 16.7±4.4     | <0.001  |
| Medium HDL-P (µmol/L) | 1.1 (0.0–3.4) | 0.2 (0.0–1.7) | <0.001  |
| Large HDL-P (µmol/L) | 8.0±3.7 | 9.7±3.1     | <0.001  |
| Mean HDL-P size (nm) | 9.3±0.4 | 9.7±0.4      | <0.001  |

Data given as mean±SD, %, or median (IQR). To convert total cholesterol, LDL-C and HDL-C from mg/dL to mmol/L, divide by 38.67. To convert triglycerides from mg/dL to mmol/L divide by 88.57. †BMI ≥25 kg/m². ‡Mean alcohol intake of two 7-day dietary recalls. §Mean alcohol intake of two 24-h recalls done consecutively. BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure.
Table 2. HDL-P and HDL-C vs. Lifestyle and Behavior Factors in Japanese Adults vs. Sex, 1996–1998

| Status                  | HDL-P (μmol/L) | HDL-C (mg/dL) |
|-------------------------|---------------|---------------|
|                         | Yes | No  | P-value | Yes | No  | P-value |
| **Men**                 |     |     |         |     |     |         |
| Current drinker         | 30.4±6.0 23.7±2.9 | 0.001 54.8±13.3 42.9±13.9 | 0.008 |
| Drinking during the past 48 h | 31.3±6.0 26.9±4.6 | <0.001 56.7±13.3 47.5±11.0 | <0.001 |
| Current smoker          | 30.1±6.4 30.5±5.7 | 0.550 53.9±13.6 55.3±13.0 | 0.323 |
| Overweight†             | 30.7±5.7 30.1±6.2 | 0.323 50.0±11.5 56.3±13.6 | <0.001 |
| **Women**               |     |     |         |     |     |         |
| Current drinker         | 28.1±5.3 27.1±5.3 | 0.101 60.8±14.2 58.1±12.5 | 0.158 |
| Drinking during the past 48 h | 28.7±5.8 27.6±5.0 | 0.046 63.6±15.0 58.7±13.2 | 0.001 |
| Current smoker          | 26.4±5.6 28.1±5.3 | 0.130 56.3±11.2 60.6±14.1 | 0.185 |
| Overweight†             | 28.6±6.4 27.8±5.0 | 0.135 57.0±16.5 61.3±13.0 | 0.011 |

Data given as mean±SD. Means were adjusted for age and study site in Japan (Toyama, Aito and Wakayama). †BMI ≥25 kg/m². BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle.

Figure. Mean high-density lipoprotein (HDL-P) particle (HDL-P), cholesterol (HDL-C), and HDL-P size subclass concentration per quartile for (A) alcohol intake, (B) smoking amount and (C) body mass index (BMI) in Japanese men and women, 1996–1998. In women, the first 3 quartiles of smoking amount are combined in 1 bar graph because of the low number of female smokers. *P<0.001 for the specified lifestyle factor with total HDL-P or HDL-C.
Table 3. Effect of 1-SD Increase in Lifestyle Factors on HDL-P and HDL-C in Japanese Adults vs. Sex, 1996–1998

| Lifestyle factor | HDL-P (µmol/L) | HDL-C (mg/dL) |
|------------------|----------------|---------------|
|                  | Estimate (%SD) | 95% CI P-value | Estimate (%SD) | 95% CI P-value |
| **Men**          |                |               |                |               |
| Habitual alcohol (g/day) | 41.1 | 31.9–50.3 <0.001 | 36.3 | 26.8–45.7 <0.001 |
| 48-h alcohol intake (g/day)† | 38.4 | 29.1–47.6 <0.001 | 31.5 | 21.9–41.0 <0.001 |
| Smoking (cigs/day) | −8.0 | −16.6–0.5 0.066 | −13.8 | −22.5–5.0 0.002 |
| BMI (kg/m²)      | −2.0 | −11.0–7.0 0.662 | −38.1 | −47.3–28.9 <0.001 |
| **Women**        |                |               |                |               |
| Habitual alcohol (g/day) | 15.5 | 6.6–24.3 <0.001 | 15.1 | 5.3–24.9 0.003 |
| 48-h alcohol intake (g/day)† | 16.1 | 7.3–25.0 <0.001 | 15.1 | 5.3–24.9 0.003 |
| Smoking (cigs/day) | −9.1 | −17.9–0.2 0.045 | −7.8 | −17.6–2.0 0.121 |
| BMI (kg/m²)      | 4.2  | −5.3–13.7 0.381 | −25.2 | −35.7–14.7 <0.001 |

All linear regression models included the following covariates: habitual alcohol, smoking (cigs/day), BMI, age, systolic blood pressure, hypertension (yes/no), diabetes (yes/no), study site, total energy intake and, for women only, menopause status (pre/current/post). †Model involved 48-h alcohol intake instead of habitual alcohol. In men: 1 SD HDL-P=6.1µmol/L; HDL-C, 13.3mg/dL; habitual alcohol, 24.5g/day; 48-h alcohol intake, 27g/day; smoking, 13.7cigs/day; BMI, 2.8kg/m². In women: 1 SD HDL-P=5.3µmol/L; HDL-C, 14mg/dL; habitual alcohol, 5.5g/day; 48-h alcohol intake, 6.2g/day; smoking, 2.4cigs/day; BMI, 3.1kg/m². Abbreviations as in Table 1.

Table 4. Effect of 1-SD Increase in Lifestyle Factors on HDL-P Size Subclasses in Japanese Adults vs. Sex, 1996–1998

| Lifestyle factor | Small HDL-P | Medium HDL-P | Large HDL-P | Mean HDL-P size |
|------------------|-------------|--------------|-------------|----------------|
|                  | Estimate (%SD) | 95% CI P-value | Estimate (%SD) | 95% CI P-value | Estimate (%SD) | 95% CI P-value | Estimate (%SD) | 95% CI P-value |
| **Men**          |             |               |             |               |             |               |             |               |
| Habitual alcohol (g/day) | 17.1 | 7.1–27.1 <0.001 | 23.2 | 12.9–33.4 <0.001 | 25.0 | 15.2–34.7 <0.001 | 10.5 | 1.0–20.0 0.031 |
| 48-h alcohol intake (g/day)† | 18.1 | 8.2–28.0 <0.001 | 21.0 | 10.8–31.2 <0.001 | 21.4 | 11.6–31.2 <0.001 | 7.9  | −1.6–17.4 0.101 |
| Smoking (cigs/day) | −2.2 | −11.5–7.1 0.639 | 7.7  | −1.9–17.2 0.115 | −15.6 | −24.7–6.6 <0.001 | −11.6 | −20.5–2.7 0.010 |
| BMI (kg/m²)      | 22.9 | 13.1–32.6 <0.001 | 9.8   | −0.2–19.8 0.054 | −39.7 | −49.2–30.2 <0.001 | −47.5 | −56.8–38.2 <0.001 |
| **Women**        |             |               |             |               |             |               |             |               |
| Habitual alcohol (g/day) | 5.1  | −4.1–14.3 0.278 | 6.2   | −3.9–16.2 0.230 | 13.9 | 4.3–23.5 0.005 | 7.9  | −1.4–17.2 0.097 |
| 48-h alcohol intake (g/day)† | −7.3 | −13.6–1.0 0.024 | 1.1   | −8.5–10.8 0.820 | 9.3  | −0.2–18.8 0.054 | 7.0  | −2.3–16.3 0.141 |
| Smoking (cigs/day) | −4.8 | −14.0–4.5 0.310 | 3.9   | −6.2–14.0 0.443 | −11.7 | −21.3–2.1 0.017 | −3.9  | −13.2–5.4 0.414 |
| BMI (kg/m²)      | 16.2 | 6.3–26.1 0.001 | 13.0  | 2.2–23.8 0.019 | −24.0 | −34.3–13.7 <0.001 | −36.9 | −46.8–26.9 <0.001 |

All linear regression models included the following covariates: habitual alcohol, cigarettes smoked per day, BMI, age, systolic blood pressure, hypertension (yes/no), diabetes (yes/no), study site, and total energy intake. †Model involved 48-h alcohol intake instead of habitual alcohol. In men: 1 SD small HDL-P=4.9µmol/L; medium HDL-P, 3.1µmol/L; large HDL-P, 3.7µmol/L; mean HDL-P size, 0.4nm; habitual alcohol, 24.5g/day; 48-h alcohol intake, 27g/day; smoking, 13.7cigs/day; BMI, 2.8kg/m². In women: 1 SD small HDL-P=4.4µmol/L; medium HDL-P, 2.4µmol/L; large HDL-P, 3.1µmol/L; mean HDL-P size, 0.4nm; habitual alcohol, 5.5g/day; 48-h alcohol intake, 6.2g/day; smoking, 2.4cigs/day; BMI, 3.1kg/m². Abbreviations as in Table 1.

vs. 23.7µmol/L, P=0.001) and HDL-C (54.8mg/dL vs. 42.9mg/dL, P=0.008), similar to those who drank alcohol in the past 48h compared with those who did not (Table 2). In women, neither HDL-P nor HDL-C were different according to current drinking status, but significantly higher HDL-P (28.7µmol/L vs. 27.6µmol/L, P=0.046) and HDL-C (63.6mg/dL vs. 58.7mg/dL, P=0.001) was seen in those who drank during the past 48h compared with those who did not. There were no significant differences in either HDL-P or HDL-C for current smokers compared with non-smokers in either men or women. Overweight individuals (BMI ≥25kg/m²) had significantly higher HDL-C than non-overweight individuals (men, 50.0mg/dL vs. 56.3mg/dL, P<0.001; women, 57.0mg/dL vs. 61.3mg/dL, P=0.011), but no significant differences in HDL-P were observed.

HDL-P and HDL-C were positively correlated with each other, with a Pearson correlation coefficient of 0.49 (P<0.001) and of 0.31 (P<0.001) in men and women, respectively. Correlations of these HDL parameters with
lifestyle factors are listed in Table S1. Unadjusted mean concentrations of HDL-P, HDL-C and mean HDL-P size subclasses (small, medium, and large) per quartile of lifestyle factor are presented in Figure. Higher HDL-P and HDL-C were observed with higher quartiles of alcohol intake (P<0.001) in both men and women. Generally, large and medium HDL-P levels were higher with higher alcohol intake. No trend of differing HDL-P or HDL-C with quartiles of smoking amount was found in either men or women. Large HDL-P appeared to slightly decrease and medium HDL-P to increase with higher quartiles of smoking amount. HDL-C had an inverse trend with higher BMI quartiles (P<0.001). Although total HDL-P did not change significantly, a trend towards higher levels of smaller and lower levels of larger HDL-P was observed with increasing quartiles of BMI.

Independent of other covariates, including smoking and BMI, a 1-SD higher habitual alcohol intake in men was associated with a 41.1% higher HDL-P and a 36.3% higher SD of HDL-P and HDL-C, respectively (Table S3). This is equivalent to a 2.5-µmol/L higher HDL-P and 4.8-mg/dL higher HDL-C. A similar positive association was found with 48-h alcohol intake. Smoking was significantly inversely associated only with HDL-P in women, while significantly inversely associated only with HDL-C in men. BMI was not associated with HDL-P, whereas it was significantly strongly associated with HDL-C in men (38.1% SD) and women (25.2% SD). Results of alcohol intake were similar in a sensitivity analysis of current drinkers only. The significant inverse association of smoking amount with HDL-C was attenuated, however, in an analysis of current smokers only (Table S2). Sensitivity analyses combining men and women, resulted in significant positive relationships of alcohol intake (habitual and 48-h) and inverse associations of smoking with both HDL-P and HDL-C (Table S3, P<0.05). BMI remained inversely related with HDL-C only.

All HDL-P size subclasses (small, medium and large) were positively associated with alcohol intake in men (Table 4). The association with alcohol intake was stronger for large HDL-P, and this was evident from the positive relationship of mean HDL-P size with alcohol intake. Findings in women were attenuated, but generally only large HDL-P were positively associated with alcohol intake. Small and medium HDL-P were positively associated with BMI, while large HDL-P was inversely associated with BMI and smoking amount. HDL-P size subclass findings were supported by the association of mean HDL-P size with each lifestyle factor.

**Discussion**

In this cross-sectional study of Japanese men and women, we observed varying associations of HDL-P and HDL-C with the lifestyle factors investigated. Higher alcohol intake was more strongly positively associated with HDL-P than HDL-C. Smoking was generally inversely associated with both HDL parameters, although BMI was strongly inversely associated only with HDL-C.

**Alcohol and HDL**

Both habitual and 48-h alcohol intake were positively associated with HDL-P and HDL-C. In a meta-analysis of experimental studies on moderate alcohol intake and lipid profile, alcohol intake led to higher HDL-C and its main protein component, apolipoprotein AI (apoAI).19 Alcohol was also found to increase HDL-C and apoAI levels in a dose-dependent manner,16 and to be positively associated with total HDL-P.17 When considering the different HDL-P sizes, we observed that all subclasses were positively associated with dietary alcohol amount in men. In women, subclass associations with alcohol intake were not as strong, likely due to the lower amounts and narrow range of alcohol intake. Large HDL-P appeared to have the strongest relationship with alcohol. This was highlighted by the positive association between alcohol and mean HDL-P size. Alcohol intake has also been found to favor a shift in lipid profile towards larger HDL sizes, such as large and medium HDL-P.17,18 Overall, in terms of %SD of the HDL parameters investigated, alcohol intake was most strongly associated with total HDL-P.

Alcohol intake has been suggested to influence HDL-C by increasing lipase activity and decreasing cholesteryl ester transfer protein (CETP),19 thereby increasing cholesterol transfer to, while reducing cholesterol transfer from, HDL, respectively.20 These pathways, however, are under debate because inconsistent findings have been reported.21 Alcohol increases circulating apoAI and, in combination with other pathways,22 can lead to the formation of HDL-P. A U- or W-shaped association between alcohol intake and CAD has been reported, in which light-moderate drinkers have the lowest risk of CAD23,24 and prevalence of subclinical atherosclerosis.25 Given that alcohol intake also decreases LDL-C,26 this may be related to the lower risk of CAD observed with light–moderate alcohol intake. The high CAD risk in non-drinkers has been partly attributed to higher prevalence of comorbidities, but also to lower HDL-C and higher LDL-C compared with light–moderate drinkers.17,26,27

**Smoking and HDL**

Although smoking appeared to be not significantly associated with either HDL factor, after adjustments for important confounders, such as drinking, inverse associations were generally observed. Moreover, sensitivity analyses combining men and women resulted in stronger statistical power to identify the inverse association of smoking with both HDL-P and HDL-C. We found that smoking more cigarettes per day was associated with a lower level of larger HDL-P. Smoking has been found to decrease HDL-C and apoAI,28,29 and smoking cessation reverses these changes30,31 and increases the concentrations of HDL-P and large HDL-P, as well as mean HDL-P size.30

Smoking can reduce apoAI, increase the activity of CETP, and decrease the activity of lecithin-cholesterol acyltransferase (LCAT), an enzyme crucial for the storage of cholesterol in lipoproteins.32 Through these effects, HDL-C and HDL-P levels can be reduced.

Smoking is a significant risk factor for CAD33 and a predictor of atherosclerosis.34 Smoking leads to the formation of reactive oxygen species that oxidize HDL to possibly promote atherogenesis,35 given that the oxidation affects the cholesterol efflux ability of HDL.36

**BMI and HDL**

We found a strong inverse association between BMI and HDL-C as well as with HDL-P size, although no association was identified with total HDL-P. Higher BMI appeared to favor a shift towards smaller HDL-P: inverse associations with mean HDL-P size and large HDL-P and positive relationships of alcohol intake (habitual and 48-h) analyses combining men and women, resulted in significant
associations with small HDL-P were identified. BMI, independent of potential confounders, is inversely associated with HDL-C in obese and non-obese individuals across a broad range of BMI. In a meta-analysis of lifestyle intervention studies in obese individuals ≥35 kg/m², however, lowering of BMI, due to exercise and various lifestyle interventions, had no significant effect on HDL-C, although triglycerides, LDL-C, and total cholesterol were affected. BMI was inversely associated with HDL size and apoA1 level. Although body weight gain was inversely associated with larger mean HDL-P size, it was not significantly correlated with total HDL-P. The differences in the associations of HDL-P and HDL-C with BMI may be due to the activity of important enzymes involved in HDL metabolism, such as hepatic lipase and endothelial lipase.

Higher BMI (obesity) was associated with significant changes in catabolic and production rates of apoA1 molecules. How BMI affects HDL-C level and HDL-P size is unclear. Associations of BMI with HDL-C and larger HDL-P may be a result of several biological pathways. BMI is also positively associated with circulating total cholesterol, LDL-C and triglycerides, which may directly influence HDL-C and the size distribution of HDL-P.

Similar to the other lifestyle factors investigated, BMI has been linked to CAD, in which, independent of other confounding factors, such as hypertension, diabetes, and total energy intake, it can predict risk of future CAD.

HDL and CAD Risk
The presumed role that HDL-C plays in CAD risk reduction has been under debate recently. Most clinical trials involving significant increases in HDL-C (via CETP inhibition) did not lead to reductions in CAD risk. Although, recently, HDL-C-increasing drug led to modest reductions in CAD risk, it was believed that this was largely due to the non-HDL-C-lowering action of the drug and not particularly to its HDL-C-increasing ability. This suggests that HDL-C may not be causally protective against CAD, but may possibly be an indicator of other cardioprotective mechanisms or a byproduct of other pathways pertinent to developing CAD. Similarly, the lifestyle factors investigated in the present study may have affected other metabolic pathways that are related to CAD. Having indirect effects on HDL-C, indeed, we identified a strong inverse relationship of BMI with HDL-C, but not with HDL-P.

HDL-P may provide an explanation for the oft-documented inverse association of HDL-C with CAD risk. Recently, HDL-P have been shown to be inversely associated with subclinical atherosclerosis and incident cardiovascular disease, independent of HDL-C. In contrast, the relationship of HDL-C with reduced CAD risk was not independent of HDL-P. HDL-P may represent the activity of the reverse cholesterol transport pathway, such that higher total HDL-P and larger HDL-P may indicate higher efflux of cholesterol from peripheral cells to the liver, a process believed to prevent atherosclerosis. This is only speculation, however, given that HDL-P and HDL-C are both parameters representing the same lipoprotein and a more intricate mechanism may be at play.

Limitations and Strengths
Due to the cross-sectional design of this study, we cannot assume causality of the lifestyle factors on the HDL profile. Plasma samples were stored for 4 years prior to NMR measurement. We are unaware of whether such long-term storage affects HDL-P and its subclass measurements. Furthermore, we cannot fully eliminate potential confounding from other factors (such as diet) that may influence or explain the associations between HDL-P and HDL-C and the lifestyle factors assessed. We attempted to reduce this, however, by including multiple potential confounding lifestyle factors in multivariable models. Finally, the present subjects were limited to Japanese men and women, and generalization to other populations requires some caution.

The present study, however, is not without its strengths. We have used high-quality standardized dietary data and NMR, which is known to have higher precision than traditionally used gradient gel electrophoresis. No studies that we are aware of have compared the strengths of associations of HDL-P and HDL-C with modifiable lifestyle factors that are known to influence HDL-C, CAD risk, or both. We showed this in a relatively healthy population of Japanese people with a wide range of habitual and 48-h alcohol intake, smoking amount, and BMI prior to onset of CAD.

Conclusions
Although HDL-P and HDL-C are parameters of HDL, they have different associations with lifestyle factors related to CAD. Given that, independent of and more strongly than their cholesterol counterpart, HDL-P are associated with CAD and subclinical atherosclerosis, there is a need for investigation into factors that affect the total circulating level of HDL-P.

Disclosures
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**Supplementary Files**

**Supplementary File 1**

Table S1. Lifestyle and CVD risk factors: correlations with HDL-P and HDL-C

Table S2. Effect of 1-SD increase in alcohol drinking and smoking on HDL-P and HDL-C in Japanese adults vs. sex

Table S3. Effect of 1-SD increase in lifestyle factors on HDL-P and HDL-C in Japanese adults, 1996–1998

Please find supplementary file(s);
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