Associations of Age, Adiposity, Alcohol Intake, Menstrual Status, and Estrogen Therapy With High-Density Lipoprotein Subclasses

Paul T. Williams, Karen M. Vranizan, Melissa A. Austin, Ronald M. Krauss

We used nondenaturing polyacrylamide gradient gel electrophoresis to examine the associations of age, adiposity, alcohol intake, and exogenous estrogen with high-density lipoprotein (HDL) subclasses in 427 members of 51 principally Mormon kindreds. The absorbency of protein stain was used as an index of mass concentrations at intervals of 0.01 nm within five HDL subclasses: HDL_{7.2} (7.2 to 7.8 nm), HDL_{8.2} (7.8 to 8.2 nm), HDL_{8.8} (8.2 to 8.8 nm), HDL_{9.7} (8.8 to 9.7 nm), and HDL_{12} (9.7 to 12 nm). Age and alcohol intake were obtained from questionnaires, and body mass index was computed from clinic measurements as weight (kg)/height (m)^2. The results suggest that HDL_{8.2} concentrations were higher after menopause than before.

Adult men (≥18 years old) had significantly higher HDL_{7.8} and HDL_{8.2} and significantly lower HDL_{9.7} and HDL_{12} levels than younger boys. Compared with the women, adult men had higher levels of HDL_{8.2} and HDL_{9.7} and lower levels of HDL_{12}. There were no significant differences between the HDL profiles of women and younger boys, suggesting that the divergence in HDL occurs during puberty. Eighty-eight percent of the increase in HDL associated with estrogen replacement in postmenopausal women occurred within HDL_{8.2} and HDL_{9.7}. Reported alcohol intake in adult men correlated with two HDL subclasses: one within the HDL_{8.2} region and a second within the HDL_{9.7} region, whereas in women the positive correlation between alcohol intake and HDL levels was within the HDL_{9.7} region only. In both men and premenopausal adult women, increasing levels of body mass index were associated with higher levels of HDL_{8.2} and lower levels of HDL_{12}. Statistical adjustment for HDL cholesterol levels eliminated the significant relations of alcohol with HDL subclasses in both men and women. The adjustment did not eliminate the significant relations of age and body mass index to HDL_{8.2} and HDL_{9.7}. Thus, gradient gel electrophoresis of HDL subclasses appears to identify important physiological relations that are independent of HDL cholesterol levels. (Arterioscler Thromb. 1993;13:1654-1661.)

KEY WORDS • gradient gel electrophoresis • HDL • age • menopause • menstrual • puberty • alcohol • adiposity • HDL_{7.2} • HDL_{8.2} • apolipoprotein A-I • estrogen

Coronary heart disease (CHD) risk increases with age and adiposity and is reduced in association with alcohol intake and postmenopausal estrogen use.1.4 These relations may be attributable in part to changes in high-density lipoproteins (HDLs).3 Plasma concentrations of HDL cholesterol and apolipoprotein (apo) A-I, one of the principal HDL proteins, are inversely associated with CHD risk,6 aging,4 and adiposity2 and positively associated with alcohol consumption8 and postmenopausal estrogen use.9

HDLs include multiple distinct subclasses of particles that have different mobilities on nondenaturing polyacrylamide gradient gels. The different mobilities reflect the impeded migration of larger HDL through the gel. Gradient gel electrophoresis10 has identified five HDL subclasses: HDL_{7.2} (7.2 to 7.8 nm in diameter), HDL_{8.2} (7.8 to 8.2 nm), HDL_{8.8} (8.2 to 8.8 nm), HDL_{9.7} (8.8 to 9.7 nm), and HDL_{12} (9.7 to 12 nm).10 These subclasses appear to correspond to differing amounts of the apo A-I and apo A-II molecules per particle.11,12 Because all five subclasses contribute to total HDL cholesterol and total apo A-I levels, HDL cholesterol and apo A-I may be insensitive to effects involving individual subclasses. We have previously described the relations of HDL subclasses to exercise-induced and diet-induced weight loss and to plasma concentrations of other lipoproteins.7,13,14 We have also described the relations of HDL subclasses among family members.15 In all cases, the relations were found to involve specific HDL subclasses. Case-control and angiographic studies suggest that CHD risk may be increased when HDL_{2b} is reduced relative to HDL_{7.2} and HDL_{9.7}.16-18 Characterizing HDL by particle size could provide an important key to interpreting the effects of aging, weight gain, alcohol intake, and postmenopausal estrogen therapy on HDL-related CHD risk. Specifically, this report tests the hypotheses that (1) HDL levels exhibit subclass-specific correlations with age, adiposity, and alcohol intake in both men and women; (2) HDL levels exhibit subclass-specific mean differences between exogenous hormone users and nonusers in postmenopausal women; and (3) these subclass-specific correla-
tions and mean differences are partially independent of total HDL cholesterol or apo A-I measurements.

Methods

Subjects

The subjects were originally sampled as part of a family study of the inheritance of low-density lipoprotein (LDL) subclass phenotypes. The selection of kindreds took place primarily among the Mormon community in the San Francisco Bay Area. Additional subjects were recruited after the first report from this study. The kindreds were not selected for lipid disorders or family history of CHD, but sequential sampling of kindreds for expression of an LDL subclass phenotype expression was used. Persons were excluded from this analysis if they reported taking lipid-lowering drugs or other drugs known to affect plasma HDL.

Of the 427 subjects with complete HDL measurements, 27 were excluded for missing data on cigarette and alcohol usage. Few subjects reported using cigarettes or birth control pills. We did not test the relations of these variables to HDL subclasses because of their low statistical power. The 42 subjects who used cigarettes or birth control pills were excluded in the analyses to follow because these factors could potentially confound relations involving HDL subclasses. Three women were also excluded for not falling within one of three prescribed age categories: premenarchal girls ≤14 years old and not having periods, premenopausal women having periods (age range, 12 to 50 years), and menopause; women having periods ≥40 years old and not having periods.

Clinical Measurements and Questionnaires

Participants completed medical history questionnaires during their clinic visits, including questions on date of birth, drug and medication use, current and recent pregnancy, lactation, hormone use in women, cigarette use, and usual intake of alcoholic beverages. Ounces of alcohol consumed per week were calculated on the basis of 0.48 oz per 12-oz bottle of beer, 0.48 oz per 4-oz glass of wine, 0.60 oz per drink of hard liquor (including cocktails and mixed drinks), and 0.4 oz per after-dinner drink. Body mass index was calculated as the weight in kilograms divided by height in meters squared. Indices of android (upper body) versus gynoid (lower body) adiposity were not measured.

Laboratory Measurements

All participants provided blood samples after an overnight fast. HDL cholesterol was measured after precipitation with heparin MnCl₂. Plasma apo A-I concentrations were determined by radial immunodiffusion. Electrophoresis of HDL in the ultracentrifuge d≤1.20-g/mL fraction was performed on Pharmacia Electrophoresis Apparatus (GE 4-11) using slab gradient gels (PAA 4/30, Pharmacia, Piscataway, NJ) as described by Blanchet et al. The protein-stained gradient gels were scanned with a model RFT densitometer (Transidyne Corp, Ann Arbor, Mich) at a wavelength of 603 nm. A mixture of four globular proteins (High Molecular Weight Calibration Kit, Pharmacia) in the central lane was used to calibrate for particle diameter. The HDL migration distances (R₀) were measured relative to the migration distance of the peak of bovine serum albumin, one of the protein standards. Calculus (ie, transformation of variables) was used to convert the HDL distributions from the migration distance scale to the particle diameter scale. The coefficients of variations for HDL absorbency were 17.8 for HDL₁₀, 15.5 for HDL₁₂, 15.7 for HDL₁₆, 24.8 for HDL₂₀, and 29.5 for HDL₂₅.

Statistical Analysis

We used t tests and ANOVA to evaluate differences in mean HDL levels between age groups, and Pearson correlation coefficients to evaluate relations between HDL levels and age, body mass index, and alcohol intake. The correlations and differences were computed at each 0.01 nm between 7.2 and 12 nm. When assessing the effects of age, we excluded 87 persons who drank, smoked, or used estrogen. This was done to ensure that these variables did not confound the differences between age groups. The correlations between body mass index and HDL subclasses also excluded the 87 persons who drank, smoked, or used estrogen, and the analysis of estrogen therapy in postmenopausal women excludes smokers and drinkers.

Analyses are presented using more than one person per nuclear family (ie, parents and children) so as to enhance statistical power. Because HDL levels are positively correlated within families, the statistical significance may be underestimated when multiple family members are included. We therefore repeated the analyses using only one individual per nuclear family (more distant relations could be included in the same analyses). In selecting one subject per family, preference was given to the individual that would maximize the statistical power to detect differences or associations. This meant selecting from the least frequent age group and from the extremes of the distribution. For example, in comparing age groups, preference was given to selecting boys or adolescents over adult men because the sample contained far fewer younger males. When correlating adiposity with HDL levels, preference was given to selecting the more extreme values so as to maximize the variance of the independent variable. These manipulations of the independent variable do not bias the statistical test since, under the null hypothesis, the variables remain statistically independent (ie, the independent variable is presumed fixed). All significance levels are two-tailed but contain no correction for simultaneous inference. The results were also verified using the Mann-Whitney two-sample test and Spearman’s correlation to protect against the possible influences of nonnormality and the influence of outliers.

Conversion from absorbency to plasma concentration is not necessary for analyzing protein-stained HDL by particle diameter because the statistical tests used in this report (ie, t tests, ANOVA, Pearson’s correlation coefficients, and partial correlations) are invariant to translations of scale or location. That is, the significance levels for absorbency will be identical to those based on unknown plasma concentrations when the conversion involves the addition and/or multiplication of numerical constants. In fact, different constants may be used at each diameter, so that variation in chromogenicity across the HDL particle size spectrum will not affect the results.
TABLE 1. Mean Plasma Concentrations of HDL Cholesterol, Apo A-I, Triglycerides, and Total Cholesterol in Men and Women by Age Group

<table>
<thead>
<tr>
<th></th>
<th>HDL Cholesterol</th>
<th>Apo A-I</th>
<th>Total Cholesterol</th>
<th>Triglycerides</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤11 years (boys)</td>
<td>49.8±7.8</td>
<td>136.5±15.5</td>
<td>156.9±66.1</td>
<td>65.6±37.8</td>
<td>16</td>
</tr>
<tr>
<td>12-17 years (adolescents)</td>
<td>47.0±13.0</td>
<td>127.6±19.0</td>
<td>132.7±26.1</td>
<td>56.5±29.0</td>
<td>14</td>
</tr>
<tr>
<td>18 years and older (men)</td>
<td>40.4±8.5</td>
<td>123.0±18.7</td>
<td>178.0±39.2</td>
<td>116.7±69.7</td>
<td>115</td>
</tr>
<tr>
<td><strong>Differences±SE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men-boys</td>
<td>-9.4±2.1†</td>
<td>-13.5±4.4</td>
<td>21.0±16.9</td>
<td>51.1±11.5†</td>
<td></td>
</tr>
<tr>
<td>Men-adolescents</td>
<td>-6.6±3.6†</td>
<td>-4.6±5.4</td>
<td>45.3±7.9†</td>
<td>60.2±10.1†</td>
<td></td>
</tr>
<tr>
<td>Adolescents-boys</td>
<td>-2.8±4.0</td>
<td>-8.8±6.5</td>
<td>-24.2±17.9</td>
<td>-9.1±12.2</td>
<td></td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Means±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenarchal girls</td>
<td>48.3±7.7</td>
<td>134.0±25.1</td>
<td>143.0±27.3</td>
<td>61.5±22.0</td>
<td>16</td>
</tr>
<tr>
<td>Premenopausal women</td>
<td>48.7±10.9</td>
<td>135.6±17.5</td>
<td>170.9±39.9</td>
<td>84.5±43.4</td>
<td>88</td>
</tr>
<tr>
<td>Postmenopausal women</td>
<td>54.2±12.7</td>
<td>145.9±28.6</td>
<td>222.9±40.4</td>
<td>166.8±96.5</td>
<td>40</td>
</tr>
<tr>
<td><strong>Differences±SE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postmenopausal women-gals</td>
<td>5.9±2.8</td>
<td>11.9±8.2</td>
<td>79.9±9.3†</td>
<td>105.3±16.2†</td>
<td></td>
</tr>
<tr>
<td>Postmenopausal-premenopausal</td>
<td>5.5±2.3†</td>
<td>10.4±5.1*</td>
<td>52.0±7.7†</td>
<td>82.3±15.9†</td>
<td></td>
</tr>
<tr>
<td>Premenopausal women-girls</td>
<td>0.4±2.2</td>
<td>1.6±7.0</td>
<td>27.9±8.0†</td>
<td>23.0±7.2*</td>
<td></td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein and apo, apolipoprotein. All analyses exclude smokers and persons who consume alcohol or use birth control pills or postmenopausal estrogen therapy. Values are in milligrams per deciliter.

Results

Table 1 presents HDL cholesterol, apo A-I, total cholesterol, and triglyceride concentrations by age groups. ANOVA revealed significant group differences for HDL cholesterol, apo A-I, total cholesterol, and triglycerides (P<.05) by age group and menstrual status (results not displayed). The table shows that the men had lower HDL cholesterol and apo A-I and higher triglycerides than boys and lower HDL cholesterol and higher total cholesterol and triglycerides than adolescents. Menopause was associated with higher HDL cholesterol and apo A-I. Total cholesterol and triglycerides were higher after menopause and higher after menarche than before. In males, body mass index averaged 24.9±3.2 kg/m² in adults, 20.1±2.6 kg/m² in adolescents, and 16.5±1.6 kg/m² in boys. In females, body mass index averaged 27.4±4.5 kg/m² in postmenopausal women, 20.1±2.6 kg/m² in premenopausal women, and 16.5±1.6 kg/m² in premenarchal girls.

Table 2 examines the correlations of plasma HDL cholesterol and apo A-I with age, body mass index, and alcohol intake. In males, HDL cholesterol was nega-

TABLE 2. Pearson Correlations of HDL Cholesterol and Apo A-I With Age, Alcohol Intake, and Body Mass Index

<table>
<thead>
<tr>
<th>Correlation with age</th>
<th>HDL Cholesterol, mg/dL</th>
<th>Apo A-I, mg/dL</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (all ages)</td>
<td>-0.26†</td>
<td>-0.10</td>
<td>145</td>
</tr>
<tr>
<td>Females (all ages)</td>
<td>0.18*</td>
<td>0.22*</td>
<td>144</td>
</tr>
<tr>
<td>Correlation with body mass index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult males (18 years and older)</td>
<td>-0.18</td>
<td>-0.08</td>
<td>98</td>
</tr>
<tr>
<td>Adult premenopausal women</td>
<td>-0.23*</td>
<td>-0.02</td>
<td>83</td>
</tr>
<tr>
<td>Correlation with alcohol Intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult males (18 years and older)</td>
<td>0.38†</td>
<td>0.29†</td>
<td>151</td>
</tr>
<tr>
<td>Adult premenopausal and postmenopausal women</td>
<td>0.26†</td>
<td>0.22†</td>
<td>146</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein and apo, apolipoprotein. All analyses exclude smokers and persons who use birth control pills or postmenopausal estrogen replacement. The correlations with age also exclude drinkers.

Significance levels are coded *P<.05, †P<.01, and ‡P<.001.
Age and Menstrual Status

Fig 1. Graphs showing mean absorbency of protein-stained high-density lipoprotein (HDL) by particle size in boys (n=16), teenagers (n=14), and adult men (n=115, top) and the mean differences between age groups (bottom). The solid portions of the bar at the bottom of the figure designate the diameter values that achieve statistical significance between age groups (P<.05) for two-sample t test. Men who smoked or drank alcohol were excluded.

Fig 2. Graphs showing mean absorbency of protein-stained high-density lipoprotein (HDL) by particle size in premenarchal girls (n=16), menstruating women (n=88), and postmenopausal women (n=40, top) and the mean differences between age groups (bottom). The solid portions of the bar at the bottom of the figure designate the diameter values that achieve statistical significance (P<.05) for two-sample t test. Women who smoked, drank alcohol, or took birth control pills or postmenopause estrogen replacement were excluded.

Figures 1 and 2 display the average distribution of HDL protein by age in men and by menstrual status in women. The curves were computed by averaging the heights of the individual subject’s HDL distributions at each diameter value. The mean differences between age groups are provided for individual diameter values from two-sample t tests (indicated by the solid portions of the bar at the bottom of the graph). The approximate subclass interctions of the bar at the bottom of the figure designate the diameter values that achieve statistical significance (P<.05) for two-sample t tests (indicated by the solid portions of the bar at the bottom of the graph). The approximate subclass intervals described by Blanche et al14 are provided for reference. In nondrinking males, HDL3a and HDL3b levels were significantly higher in adults ≥18 years old than in boys 5 to 11 years old or in adolescents 12 to 17 years old. Plasma HDL3a levels were higher in boys than adolescents or men, and HDL3a was higher in boys than men. Nondrinking postmenopausal women had higher HDL3b than premenarchal girls or premenopausal women. ANOVA revealed significant differences among age groups for males (significant between 7.37 and 8.33, 8.69 and 9.52, and 9.79 and 12 nm) and by

menstrual status for females (significant between 7.96 and 8.54 nm).

To determine at what age the HDL levels of men and women diverge, we combined HDL profiles of the premenarchal girls and the premenopausal women to enhance statistical power (there were no significant differences between the women’s and girls’ HDL subclass levels). Fig 3 shows that compared with the women, adult men had higher levels of HDL3a and HDL3b and lower levels of HDL2a, HDL2b, and larger-diameter HDL2c, and adolescent males had lower levels of HDL2a, HDL2b, and HDL2c. There were no significant differences between the women’s and the boys’ HDL profiles. Thus, these cross-sectional data suggest that divergence in HDL subclasses occurs during puberty.

Postmenopausal Estrogen Therapy

Among nondrinking, nonsmoking postmenopausal women, HDL3a and HDL2a levels were significantly higher in 6 women taking estrogens than in 40 who did not (specifically, between 8.41 and 9.47 nm, analyses not displayed). None of these women were using progestins. These differences were confirmed by the Mann-Whitney test (a nonparametric test used in this case to ensure that the differences were not due to outliers). They were also confirmed for the larger sample of 16 postmenopausal women on estrogen replacement therapy (12 estrogen only, 4 estrogen with progestins) when adjusted by analysis of covariance for reported cigarette,
alcohol, and progestin usage (significantly higher HDL between 8.16 and 9.37, analyses not displayed).

**Drinkers Versus Nondrinkers**

Thirty-two men (≥18 years old) reported drinking more than 1 oz of alcohol per week. Fig 4 shows that their plasma levels of larger HDL$_{3a}$ and HDL$_{2a}$ were higher than those of the 118 men who reported no alcohol consumption. The mean differences in HDL$_{2b}$

---

**Fig 3.** Graph showing mean differences in absorbency of protein-stained high-density lipoprotein (HDL) by particle size between premenopausal females (premenarchal girls and menstruating women combined, n=104) and boys (n=16), teenagers (n=14), and adult men (n=115). A positive-differences test indicates that the males have a higher value than the females. The solid portions of the bar at the bottom of the figure designate the diameter values that achieve statistical significance (P≤.05) for two-sample t test. Males or females who smoked, drank alcohol, or took birth control pills or postmenopause estrogen replacement were excluded.

**Fig 4.** Graph showing mean absorbency of protein-stained high-density lipoprotein (HDL) by particle size in male drinkers (>1 oz/wk, n=32) and nondrinkers (top, n=118) and the mean differences between age groups (bottom). The solid portions of the bar at the bottom of the figure designate the diameter values that achieve statistical significance (P≤.05) for two-sample t test. Men who smoked cigarettes were excluded.

---

**Fig 5.** Graphs showing Pearson correlation coefficients between levels of high-density lipoprotein (HDL) protein and plasma HDL cholesterol, age, body mass index (BMI), and amount of alcohol consumed. The solid portions of the bars at the bottom of the graphs designate the range of diameter values that correlate significantly at P≤.05 and the range of values where the correlation is significantly different in men and women. The correlations for age and BMI exclude drinkers. Smokers are excluded from all correlations.

between male drinkers and nondrinkers did not achieve statistical significance. Premenopausal and postmenopausal women were combined in the analyses of alcohol intake because there were few women drinkers. The mean differences for HDL subclasses in female drinkers and nondrinkers did not achieve significance (results not displayed).

**Correlations With HDL Cholesterol, Age, Body Mass Index, and Alcohol Intake**

Fig 5 displays the correlations of protein-stained HDL with HDL cholesterol (milligrams per deciliter), age (years), body mass index (kilograms per meters squared), and alcohol intake (ounces per week) by particle diameter. Significant associations (P≤.05) in men and women are designated by the solid portions of the bars at the bottom of each graph. A third bar
designates those diameter values at which the men's correlations are significantly different from the women's correlations. The following results were observed.

**HDL cholesterol.** In both men and women, HDL cholesterol correlated positively with HDL$_{3a}$, HDL$_{2a}$, and HDL$_{3b}$, however, the correlation with HDL$_{2a}$ is significantly stronger in men than women.

**Age.** In both men and women, age is positively correlated with plasma levels of HDL$_{3a}$ and HDL$_{3b}$. Age correlates negatively with HDL$_{3b}$ in men only.

**Adiposity.** In nondrinking men and women, increasing levels of body mass index were associated with higher levels of HDL$_{3b}$ and larger-diameter HDL$_{3c}$ and lower levels of HDL$_{2b}$.

**Alcohol intake.** In men, alcohol intake was positively correlated with two distinct regions: one within the HDL$_{2b}$ region and a second achieving a maximum value at approximately 9 nm (between HDL$_{2a}$ and HDL$_{2b}$). In women, the correlation with alcohol intake was significant within HDL$_{2b}$ only. The men's correlation of alcohol intake with HDL$_{3a}$ and HDL$_{2a}$ was significantly greater than the correlation in women.

**Partial Correlations**

Fig 6 displays the significance levels for the correlations of protein-stained HDL levels with age, body mass index, and alcohol intake when adjusted for HDL cholesterol and apo A-I concentrations. The wide solid and dashed portions of the bars designate the range of diameter values that correlate significantly at $P<.05$ when adjusted.

**Excluding First-Degree Relatives**

The analyses of Figs 1, 2, 4, and 5 were repeated using only one subject per nuclear family. The results were consistent with those presented in the figures. Adult nondrinking males had higher levels of HDL$_{3b}$ and larger-diameter HDL$_{3c}$ than boys (specifically, between 7.57 and 8.19 nm) and higher HDL$_{3b}$ levels (between 7.85 and 8.09 nm) than adolescent males. The adult males also had significantly lower HDL$_{2a}$ and HDL$_{2b}$ than boys (between 8.77 and 11.93 nm; compare with Fig 1). Nondrinking postmenopausal women had significantly higher HDL$_{3b}$ and HDL$_{3a}$ than premenarchal girls and nondrinking premenopausal women (between 8.01 and 8.50 for both, compare with Fig 2). Adult male drinkers had significantly higher HDL$_{3a}$ and HDL$_{3b}$ than nondrinking males (specifically, between 8.61 and 9.43 nm; compare with Fig 4). Fig 6 displays the significance levels for correlations between HDL levels and age, BMI, and alcohol, using one person per family. The results are consistent with the complete sample, except that alcohol intake was unrelated to HDL$_{2b}$ levels in men.

**Discussion**

Our results suggest that age, adiposity, alcohol, and postmenopause estrogen therapy may involve more specific changes than revealed by measurements of HDL cholesterol or apo A-I. Sex, age, adiposity, alcohol, and estrogen are related to levels of specific HDL subclasses even after adjustment for total HDL cholesterol or apo A-I concentrations. Importantly, factors that increase total HDL cholesterol do not necessarily affect all subclasses of HDL in the same way. Previous case-control and angiographic studies suggest that CHD risk may be increased when HDL$_{3c}$ is reduced relative to HDL$_{3b}$ and HDL$_{3b}$, and we have postulated that increased HDL$_{2b}$ might be associated with increased CHD risk on the basis of its association with other lipoproteins, including small, dense LDL and LDL subclass phenotype B. Understanding the role of HDL in mitigating CHD risk may require more detailed analysis of HDL subclasses than previously appreciated.

Although on the basis of cross-sectional data, our results suggest that estrogen therapy does not simply reverse the increase in HDL$_{3b}$ during menopause. We found that 88% of the HDL increase with postmenopause estrogen therapy was a result of increases in HDL$_{3a}$ and HDL$_{2a}$ rather than HDL$_{2b}$ and that HDL$_{2b}$ levels were unaffected. Earlier studies by analytic ultra-
centrifugation suggest that women using postmenopausal estrogen therapy had higher HDL mass in F, 1.5 to 9 than nonusers, the differences being greater below F, 4.5 (HDL, and HDL,2a) than above (HDL,). The analytic ultracentrifuge also revealed that increases in plasma HDL mass were greater than HDL mass when oral contraceptive users (mostly non-estriadiol/estradiol combinations) were compared with nonusers. These results suggest that estrogen may promote greater increases in HDL, and HDL,2a than HDL,2b.

In both men and women, adjustment for HDL cholesterol eliminated the association between alcohol and HDL subclasses, suggesting that alcohol may influence HDL cholesterol levels, which in turn alter HDL subclasses in accordance with their relation to variations in HDL cholesterol levels. Whereas alcohol intake correlated with HDL, in both men and women, alcohol intake correlated with HDL, in men only. Fig 5 shows that this could reflect sex differences in the relation between HDL cholesterol and the HDL subclasses. Specifically, HDL,2a may be more strongly related to alcohol intake in men than women because HDL,2a is more strongly related to HDL cholesterol in men. Although a clinical trial comparing the effects of alcohol intake in men and women is lacking, we would predict on the basis of these results that the increase in HDL cholesterol in women is more likely to involve greater increases in HDL, than HDL,2a.

Other studies may not report increases in smaller HDL with age or adiposity because they combine HDL, with other HDL subclasses having dissimilar properties. Methods of classification have used density26 or differential precipitation with heparin and dextran27 to separate HDL into HDL, and HDL,2a. We have shown that HDL, cholesterol and total HDL mass reflect primarily levels of HDL, and HDL,2a and show no relation to HDL, whereas HDL, cholesterol reflects primarily variations in HDL, and HDL,2a. Another method classifies HDL into particles that contain both apo A-I and apo A-II (HDL[A-I with A-II]) with major components within HDL, and HDL,2a subclasses and particles that contain apo A-I and no apo A-II (HDL[A-I without A-II]) with major components within the HDL, and HDL,2a subclasses. We have shown that HDL, is reciprocally related to HDL, and HDL,2a. Therefore, effects involving HDL, could be diluted by dissimilar changes in HDL, or HDL, in measurements of HDL[A-I with A-II]. For example, previous studies report that male sexual maturation does not significantly alter HDL, cholesterol, apo A-II, and HDL[A-I with A-II] concentrations, whereas we found significantly higher HDL, levels in men versus boys (Fig 1) and a significant positive correlation between HDL, and alcohol intake reported by others appear consistent with the higher HDL, of Fig 4 and the observation we have made elsewhere that HDL, cholesterol and HDL, mass correlate most strongly with HDL,2b.

The effects we observed for HDL, generally coincide with changes in HDL, cholesterol or HDL[A-I without A-II] reported by others. The report that HDL cholesterol, HDL, cholesterol, apo A-I, and HDL[A-I without A-II] decrease during male sexual maturation is consistent with our own observation that HDL, as well as HDL,2a is lower in men than boys and decreases with age. Consistent with earlier reports based on HDL cholesterol measurements,33,34 we found that female sexual maturation does not alter HDL cholesterol levels and that the divergence between men’s and women’s HDL subclass distributions appears to occur during puberty. Others also report that overweight men and women have lower levels of HDL cholesterol, HDL, cholesterol, and apo A-I.31 Fig 5 shows that in both men and women, increasing levels of body mass index were associated with lower levels of HDL,2b. These cross-sectional observations are consistent with reported changes in HDL subclasses in men who have lost weight by dieting or by exercise.32

Caveats and Summary

Caution may be warranted in extrapolating our findings to other populations, given the moderate size of the sample and the selection of subjects principally among Mormons, who may differ in both lifestyle and socioeconomic status from other groups. Nevertheless, findings on HDL subclasses suggest several novel possibilities. The increased CHD risk associated with male sexual maturation and male or female adiposity could be mediated in part via elevated HDL, or reduced HDL,2b. Increased CHD risk in women after menopause could also be caused in part by elevated HDL,2b. Reduced CHD risk in moderate drinkers could result from increased HDL, or HDL, but presumably not reduced HDL,2b.

Acknowledgments

This study was supported in part by grants HL-02183, HL-24462, and HL-18574, First Independent Research and Transition Award HL-38760 from the National Heart, Lung, and Blood Institute, and a grant funded by the National Dairy Promotion and Research Board, administered in cooperation with the National Dairy Council, and was conducted at the Lawrence Berkeley Laboratory (Department of Energy DE-AC03-76SF00098 to the University of California). We wish to thank Laura Holl, Charlotte Brown, and Bahareh Sahami for laboratory analysis of gradient gel electrophoresis.

References

Williams et al

HDL Subclasses and Age, Adiposity, and Alcohol

1661


Associations of age, adiposity, alcohol intake, menstrual status, and estrogen therapy with high-density lipoprotein subclasses.

P T Williams, K M Vranizan, M A Austin and R M Krauss

doi: 10.1161/01.ATV.13.11.1654

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/13/11/1654