Characteristics Associated with Apoprotein and Lipoprotein Lipid Levels in Middle-aged Women

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Recent evidence indicates that measurement of apoproteins may enhance evaluation of coronary heart disease risk. The purpose of the present study was to identify factors associated with individual variation in apoproteins (apo) A-I, A-II, and B and lipoprotein lipid levels in 541 healthy premenopausal women, a random sample ages 42 to 50 taken from driver's license lists. The results of multivariate analyses that included alcohol intake, obesity, smoking, exercise, and age as predictor variables showed alcohol consumption to be strongly, positively related to apo A-I and A-II and smoking and obesity to have modest lowering effects on apo A-I. Concentration of the high density lipoprotein subtraction, HDL-C, however, was highly negatively related to body mass index, with alcohol intake and smoking each contributing about 5% to the variation. HDL-C had a similar relationship to obesity, alcohol, and smoking, but the magnitude of effect was much smaller than that for HDL-C. Thus, the concentration of cholesterol relative to protein found in HDL, particularly HDL_c, was lower in overweight women and higher in women who reported alcohol intake. About 10% of variation in low density lipoprotein cholesterol (LDLc) was explained jointly by smoking, obesity, and alcohol intake compared with 15% of variation in apo B associated primarily with obesity (8%) and, to a lesser extent, with age and smoking. Physical activity was not independently associated with any of the lipoprotein lipid or apoprotein measures. In sum, results show that obese women exhibited reduced HDLc per mole of protein and that alcohol intake was linked to increased HDL particle number. In addition, the increase in apo B with age suggests that the rise in LDL observed in postmenopausal women may begin much earlier. (Arteriosclerosis 8:515-520, September/October 1988)
those who reported amenorrhea for 3 months or more at entry to the study.

At the baseline examination, information was collected on age, race, alcohol intake, cigarette smoking, parental history of heart disease, and leisure-time physical activity. Height and weight were measured and fasting blood samples were obtained.

Alcohol intake in grams per day was based on the self-reported frequency, amount, and type of alcoholic beverages normally consumed. The following equivalents were used to determine grams per day of intake: one drink of wine = 9.8 g, one drink of beer = 13 g, and one drink of liquor = 14 g. These values were multiplied by the number of drinks per day for each type of alcohol and summed for a measure of daily intake.

Parental history of coronary heart disease (CHD) was considered to be positive if a subject reported either parent ever having had heart disease or a heart attack or having died of heart disease before age 60.

The Paffenbarger questionnaire was used to estimate level of leisure-time physical activity measured in kilocalories per week.

Body mass index (BMI), an index of obesity, was calculated using weight in kilograms divided by height in meters squared.

Subjects fasted for 12 hours before venipuncture for determination of lipoprotein lipids, apoproteins, and triglycerides. Serum samples (5 ml) for apoprotein determinations were frozen at -70°C up to 3 months before overnight shipment in dry ice to the laboratory. Apo A-I and B were assayed by electroimmunoassay. The intra-assay coefficient of variation for apo A-I was 6.7% at a mean concentration of 139 mg/dl, and for apo B it was 9.9% (mean = 106 mg/dl). An enzyme-linked immunoabsorbant assay was used to determine apo A-II levels. The intra-assay coefficient of variation for apo A-II was 7.1% (mean = 31 mg/dl).

Analyses

Independent variables were categorized as follows: BMI (tertiles), cigarettes/day (0.1 to 19, ≥20), activity in kilocalories/week (<500, 500 to 999, 1000 to 1999, ≥2000), and alcohol intake in grams/day (<2.1, 2.1 to 8.0, >8.0). The youngest (42 to 45) and oldest (49, 50) women were grouped by age so that the five age categories contained comparable numbers of subjects in each stratum. One-way analysis of variance was used to test for significance of linear trend for differences in mean values of apoproteins and lipoprotein lipids among categories of each of the independent variables. Stepwise multiple linear regression models, with each apoprotein and lipoprotein lipid serving in turn as the dependent variable, were calculated. A stepwise method was selected because of the exploratory nature of the study. Avoiding the use of forced models minimizes, but does not eliminate, the problems of interpretation associated with multicollinearity among variables. Because the relationship of the lipoproteins with age in this sample was not clearly linear, regressions were performed that also included a polynomial term (age²) in addition to age in years.

Results

Table 1 shows the mean values of the lipoproteins and apoproteins for the entire sample and also by categories of the independent variables, along with the significance level for the test for linear trend.

The apoproteins, but not the lipoprotein lipids, were significantly (p<0.05) higher among older women; women aged 47 to 50 had a mean apo A-I level about 4 to 6 mg/dl higher than women aged 42 to 46. Apo B rose by 15 mg/dl across age categories. Women with a BMI of ≥27.3 had mean HDLc and apo A-I levels 10 to 15 mg/dl lower than women with a BMI <22.0 (p<0.001). Apo A-II and HDLc differed by only 2 to 4 mg/dl between these groups. Obese women had a 13 to 19 mg/dl higher LDLc and apo B than did thinner women (p<0.001). HDLc and HDLc were significantly (p<0.05) lower among smokers, but apo A-I and A-II did not vary by smoking status. Smokers had HDLc levels 4 to 5 mg/dl lower and LDLc and apo B levels 10 to 15 mg/dl higher than nonsmokers. Women who exercised very little exhibited a lower HDLc of about 4 mg/dl (p<0.001), no difference in HDLc, and a 10 mg/dl higher LDLc (p<0.01) than women who reported expending at least 2000 kilocalories per week. Apoprotein levels did not vary significantly by physical activity. The higher the reported daily alcohol consumption, the higher the alpha lipoprotein lipids and apoproteins (p<0.01); the increase in mean apo A-I was about 10 mg/dl over intake levels from none or occasional to about one drink per day, and the increase in apo A-II was about 5 mg/dl (p<0.01), with nearly all of the increase for both apo A-I and A-II seen in the highest intake category (≥8 g/day). LDLc and apo B, on the other hand, decreased with increasing alcohol consumption (p<0.01); mean apo B levels were about 8 mg/dl higher among abstainers and light drinkers than among those who reported intake of at least 2.1 g/day of alcohol. The higher the fasting triglycerides, the lower the level of HDLc and HDLc (<p<0.001), but no relationship was seen between apo A-I and triglyceride levels; apo A-II, LDLc, and apo B levels rose as triglycerides increased (<p<0.01). No significant differences for apoprotein levels or lipoprotein lipids were observed by parental history of heart disease or by race (not shown), although blacks (n = 48) had higher alpha and lower beta apoproteins and lipoprotein lipids than did whites.

The results of the multivariate analyses are shown in Tables 2 and 3. When the multiple linear regression equation included age in years, age in years², BMI, cigarettes per day, kilocalories per week, and alcohol
intake in grams per day, the significant predictors of HDLc, HDLc, and apo A-I included alcohol intake, BMI, and cigarettes/day (Table 2). These variables combined explained about 23% and 13% of the variation in HDLc and HDLc, respectively, and 15% of the variation in apo A-I. Obesity showed the strongest relationship to HDLc, whereas HDLc, apo A-I, and apo A-II varied the most according to alcohol intake.

Neither age nor kilocalories per week were significantly associated with level of HDLc or apo A-I after adjusting for BMI, smoking, and alcohol consumption. The transformed variable, age, did not contribute significantly to the prediction of any of the dependent variables. Thus, it was not included in Tables 2 and 3.

Consistent with the univariate results, only alcohol intake and age were significantly (p<0.01) positively related to apo A-II.

Table 3 contains the results of multivariate analyses that examined predictors of LDLc and apo B. Cigarettes smoked per day explained about 5% of the variation in LDLc, with BMI and alcohol intake each contributing little more than 2% to the cumulative $R^2$ of 10%. Age was of borderline significance and contributed less than 1% to the variation in LDLc. Physical activity was no longer
significantly associated with LDLc level in the multivariate model. The predictor variables explained more of the variation in apo B (15%) than in LDLc. BMI accounted for 8% of variation in apo B, but with age and cigarettes per day each contributing about 3%. Alcohol intake was not associated with apo B after adjustment for the other factors.

Discussion

Because reliable methods to measure apoproteins have only recently been developed, few population studies have included them. The present study offered a rare opportunity to examine the interrelationships between well-documented CHD risk factors and the measures of lipoprotein lipids (as well as their carriers, the apoproteins) in a healthy, community-based population of women.

Of the characteristics examined, BMI and alcohol intake showed the greatest overall association with lipoprotein patterns, with obese nondrinkers exhibiting a more atherogenic lipoprotein profile than leaner women who reported moderate regular alcohol consumption.

Obesity was accompanied by elevated apo B and LDLc, markedly reduced HDLc, and a modest reduction in HDLc and apo A-I concentrations. Thus, HDLc cholesterol concentration per mole of protein was less in obese women compared with leaner women. The observed relationship between BMI and lipoprotein patterns may be partly mediated through triglyceride concentrations; the Pearson correlation coefficient between BMI and (log) triglycerides in this population was 0.43. Within this normotriglyceridemic population (range = 29 to 332 mg/dl), as triglycerides rose, HDLc and HDLc declined and apo A-II increased slightly, while little change was found in apo A-I (Table 1). Phillips et al.\(^\text{15}\) and Deckelbaum et al.\(^\text{16}\) have proposed that the decrease in HDLc relative to apo A-I with increasing triglycerides reflects an exchange of cholesteryl esters for triglycerides between HDL and VLDL where the core cholesteryl esters are replaced by triglycercide molecules in the HDL particle and the cholesteryl esters are transferred to VLDL. It is via the apoproteins that this transfer is thought to occur.\(^\text{17}\) As HDLc carries about twice as much cholesterol per mole of apo A-I than does HDLc, the HDLc level should increase when cholesteryl transport in HDL increases without a proportional increase in apo A-I synthesis,\(^\text{18}\) a pattern consistent with these results.

In addition, the increase in apo A-II relative to apo A-I observed with increasing triglyceride levels may influence the cholesterol-binding capacity of HDL. Cheung and Albers\(^\text{18}\) and Tyroier et al.\(^\text{16}\) found that the higher the ratio of apo A-I to apo A-II, the higher the HDLc level. Women have been found to have higher apo A-I levels than men,\(^\text{9,18-21}\) but similar apo A-II concentrations\(^\text{18}\) and estrogen administration resulted in a rise in HDLc and apo A-I, but not in apo A-II, as reported by Schafer et al.\(^\text{22}\) Thus the ratio of apo A-I/A-II in women with elevated triglycerides is likely to resemble the apo A-I/A-II ratio characteristic of men.

Although triglyceride levels and BMI appeared to be associated with HDLc composition, alcohol intake was linked to HDL particle number, as suggested by the increased concentration of both the alpha lipoprotein lipids and apoproteins among women who reported regular alcohol consumption, as compared with those who drank little or no alcohol. The results showed little increase in the mean levels of apo A-I and apo A-II until alcohol intake reached about 8 g/day (slightly less than one drink), possibly reflecting a threshold of alcohol consumption below which little effect on apoprotein levels is seen. Couzigou et al.\(^\text{23}\) proposed that alcohol intake raises HDL and apoprotein A-I and A-II levels by increased intestinal synthesis and secretion. The results of the present study were consistent with others that have linked alcohol consumption to increased apo A-I and HDLc.\(^\text{7,15}\) and to apo A-II.\(^\text{5}\) Specifically, both HDLc and, to a lesser extent, HDLc were elevated in women reporting alcohol consumption; a dose-response pattern was observed for increasing alcohol intake. Two small experimental studies\(^\text{4,24}\) have reported a change in HDLc, but not in HDLc, with a change in alcohol intake, while another experimental study found both subfractions to be posi-

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**Table 2. Multiple Linear Regression Coefficients for Independent Variables with HDLc, LDLc, Apo A-I, and Apo A-II**

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>HDLc</th>
<th>HDLc</th>
<th>Apo A-I</th>
<th>Apo A-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>0.038</td>
<td>0.315</td>
<td>0.725</td>
<td>0.700</td>
</tr>
<tr>
<td>Body mass index [weight (kg)/height(^2) (m)]</td>
<td>-0.734</td>
<td>-0.242</td>
<td>-0.400</td>
<td>-0.085</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>-0.223</td>
<td>0.088</td>
<td>-0.210</td>
<td>-0.053</td>
</tr>
<tr>
<td>Kilocalories/week</td>
<td>0.0002</td>
<td>-0.069</td>
<td>-0.0008</td>
<td>-0.0003</td>
</tr>
<tr>
<td>Alcohol intake (g/day)</td>
<td>0.186</td>
<td>0.156</td>
<td>0.609</td>
<td>0.294</td>
</tr>
<tr>
<td>Cumulative R(^2)</td>
<td>0.225</td>
<td>0.125</td>
<td>0.150</td>
<td>0.132</td>
</tr>
</tbody>
</table>

The number was 523. The values in parentheses are SE.

*p<0.01.

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**Table 3. Multiple Linear Regression Coefficients for Independent Variables with LDLc and Apo B**

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>LDLc</th>
<th>Apo B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>1.425</td>
<td>3.098</td>
</tr>
<tr>
<td>Body mass index [weight (kg)/height(^2) (m)]</td>
<td>0.772</td>
<td>1.457</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>0.638</td>
<td>0.427</td>
</tr>
<tr>
<td>Kilocalories/week</td>
<td>-0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>Alcohol intake (g/day)</td>
<td>-0.407</td>
<td>-0.147</td>
</tr>
<tr>
<td>Cumulative R(^2)</td>
<td>0.102</td>
<td>0.148</td>
</tr>
</tbody>
</table>

The number was 523. The values in parentheses are SE.

*p<0.01.
tively related to alcohol intake. A recent British cross-sectional study of 366 healthy men also found both HDL2 and HDL3 to be related to alcohol intake, although the effect on HDL2 was greater than on HDL3. In studies of chronic alcoholics, both HDL2 and HDL3 decreased with abstinence from alcohol. The results of the present study support the hypothesis that moderate alcohol consumption may be linked to reduced CHD risk via an effect on HDLc.

Independent of BMI and alcohol intake, smokers exhibited a lower mean concentration of HDLc and HDLc per mole of protein, as well as a higher mean of LDLc and apo B, than nonsmokers. Thus, cigarette smoking was associated with a lipoprotein profile that suggests a reduced cholesterol carrying capacity for the alpha apoproteins and an increased plasma concentration of LDL particles. This is the first investigation to find a positive relationship between smoking and apo B; the one other study that examined this relationship sampled a much younger population of women. That study and one other also examined the association of cigarette smoking with apo A-I and apo A-II in much smaller groups of women with results similar to those reported here.

Despite the narrow age range of the study sample (42 to 50 years), mean apoprotein levels were higher in the older than in the younger age groups up to age 49 or 50. While one cross-sectional study has found an increase in apo A-I with age among women, others have not. Older women were found by Cheung and Albers to have a higher mean apo A-II than younger women. It is possible that the nature of the sample selection for the current study may have influenced the association of age and the apoprotein levels. Subjects were all premenopausal because postmenopausal women were excluded. Older premenopausal women may have higher steroid hormone levels than do women of the same age who become menopausal. Evidence exists for a positive influence of endogenous and exogenous estrogen levels on apo A-I, A-II, and HDLc. Consistent with this effect, HDLc, but not HDLc, in the present study population rose with age until age 49 although the trend was not statistically significant. Also of interest is the increase in apo B and LDLc observed with increasing age in the study group. The rise in LDLc noted among older women may begin before cessation of menses and may be accompanied by a marked increase in apo B.

In a study comparing women runners to sedentary women, physical activity has been shown to be positively linked to HDLc but not to HDLc. Others have found HDLc, but not HDLc, to be higher among women who exercise compared with those who do not. The results of the present study suggest that activity level in a community sample of middle-aged women is not an independent determinant of lipoprotein lipid or apoprotein concentration after controlling for BMI and cigarette smoking. The level of physical activity necessary to influence HDLc independently of alcohol, smoking, and obesity may be greater than that found among a community-based sample of women.

In conclusion, the results of this study show that individual behaviors influence both apoprotein and lipoprotein lipid concentrations, resulting in differences in interindividual lipoprotein composition and mass that cannot be measured by lipids alone, particularly in individuals with elevated triglycerides. An enhanced understanding of factors that affect levels of apoproteins relative to lipoprotein levels may improve prediction of CHD risk among women as they enter the higher risk postmenopausal years.

Acknowledgment
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