

# Effects of Alcohol Consumption on Lipoproteins of Premenopausal Women

## A Controlled Diet Study

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**Abstract** A substantial portion of American women consume alcohol, but controlled studies of alcohol-induced changes in lipoproteins of women are rare. In this study, the effects of alcohol consumption (equivalent to two drinks per day) on the lipoprotein profiles of 34 premenopausal women were measured while controlling subjects' diet and various other potentially confounding variables including phase of the menstrual cycle. Alcohol and no-alcohol treatments were administered in a crossover design, and blood samples were obtained during the early follicular phase of the third month of treatment. With alcohol, HDL cholesterol levels increased 10%, LDL levels decreased 8%, and levels of lipoprotein(a)

were unchanged. The increase in HDL cholesterol was due to an increase in both HDL<sub>2</sub> and HDL<sub>3</sub>, and the overall size of HDL particles was increased. HDL particles containing apolipoprotein (apo) A-I and apoA-II as well as those containing apoA-I but no apoA-II were elevated in response to alcohol. Although these observations are limited to a single phase of the menstrual cycle, the alcohol-induced changes in lipoproteins are consistent with changes that are thought to confer protection against coronary heart disease. (*Arterioscler Thromb Vasc Biol.* 1995;15:179-184.)

**Key Words** • alcohol • plasma lipids • women • lipoproteins • apolipoproteins

Approximately 44% of American women aged 12 years and older drink alcoholic beverages,<sup>1</sup> ie, they consume alcohol at least once a month. Although adverse effects of alcohol are well documented,<sup>2</sup> alcohol consumption appears to have at least one beneficial effect: it decreases the risk of coronary heart disease (CHD).<sup>3-6</sup> Alcohol is thought to decrease the risk of CHD, at least in part, by increasing plasma levels of HDL.<sup>3,6</sup> This may have particular relevance for women, because HDL cholesterol (HDL-C) appears to be the most powerful lipid predictor of CHD in women, whereas LDL cholesterol (LDL-C) as a predictor is weak.<sup>7,8</sup> In contrast, both LDL-C and HDL-C have consistently been associated with CHD in men.<sup>9-11</sup>

Although heart disease is the leading cause of death in women,<sup>12</sup> current knowledge of how lifestyle habits affect blood lipids has been primarily derived from studies of men. To what extent these findings can be

extrapolated to women is unknown. Assessing lipoprotein profiles of premenopausal women is complicated because lipoproteins fluctuate in response to hormone changes across the menstrual cycle.<sup>13,14</sup> It is likely that this biological fluctuation and its contribution to experimental error has led to the exclusion of women from many controlled studies including those designed to assess the effects of alcohol consumption on plasma lipoproteins.

The purpose of this study was to assess the effects of alcohol consumption on lipoprotein metabolism in premenopausal women while controlling for confounding factors such as nutrient intake and time of blood sampling across the menstrual cycle.

## Methods

### Subjects

Premenopausal women aged 21 to 40 years were admitted into the study after passing a screening test designed to eliminate those with metabolic disturbances, conditions that perturb lipoprotein metabolism, and conditions not consistent with a typical menstrual cycle. Conditions or circumstances for exclusion included history of thyroid disease, regular use of prescription or over-the-counter medications, use of oral contraceptives, irregular menstrual cycles, pregnancy or breast feeding within the last year, dietary patterns incompatible with the study (eg, vegetarian diet), cigarette smoking, and body weight <80% or >130% of the 1983 Metropolitan Life Insurance table of desirable weights.<sup>15</sup> Also excluded were applicants with a history of drinking problems or a family history of alcoholism and those who identified themselves as nondrinkers. Five women withdrew from the study for personal reasons and one was dropped due to noncompliance with the study protocol. Of the 37 women who completed the study, three were dropped due to incomplete data for one or more lipid param-

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eters. All procedures were approved by the Institutional Review Boards of the National Cancer Institute, National Institutes of Health, and Georgetown University College of Medicine. Informed consent was obtained from all subjects in accordance with institutional guidelines. Subjects, paired by age and body mass index (BMI; weight in kilograms/height in meters squared), were randomly assigned to the crossover groups.

During the first week of the study, subjects answered a self-administered medical history questionnaire that included questions about alcohol and food intake. From this questionnaire, subjects' habitual alcohol consumption was assessed.

### Diets and Alcohol Treatments

Subjects consumed a controlled diet that was typical of the American diet for six consecutive menstrual cycles. Subjects began eating this controlled diet on the same day, but the final day of feeding varied depending on the length of subjects' individual cycles. Nutrient composition of the diet was calculated by using US Department of Agriculture *Handbook 8*.<sup>16</sup> A 14-day menu cycle was used to provide variety; each menu met the recommended dietary allowances for known nutrients. Menus were prepared in 840-kJ (200-kcal) increments by proportionally scaling each food item. Calories from food were distributed as 53% from carbohydrate or carbohydrate plus alcohol, 14% from protein, and 36% from fat (13% saturated fatty acids, 12% oleic acid, and 7% linoleic acid). Diets contained 150 mg cholesterol/1000 kcal.

Meals were prepared in the Beltsville Human Study Facility. Food items were weighed and served in proportion to caloric requirements. Breakfast and dinner were consumed in the facility during the week; carryout lunches and snacks were provided. Weekend meals were packaged for home consumption. As a contingent to participation, subjects agreed to consume all food and beverages provided to them for the study and no food or alcoholic beverages other than those provided. Use of vitamin and mineral supplements was not allowed. Body weights were monitored Monday through Friday, and caloric intake was adjusted as needed to maintain body weight within a kilogram. Two experimental treatments (alcohol and no alcohol) were administered in a crossover pattern, with each treatment lasting for three consecutive menstrual cycles. Subjects consumed either 31.5 mL (about 30 g) per day of grain alcohol mixed with fruit juice or the fruit juice vehicle without alcohol. For the no-alcohol treatment, alcohol calories were replaced with carbohydrate calories from soft drinks. The alcohol treatment was not blinded; the effects of the alcohol were readily apparent to subjects. Subjects agreed to consume the drinks over a 30-minute period just before bedtime.

### Blood Collection

To avoid the phase of the menstrual cycle as a confounding variable,<sup>13,14</sup> blood drawing schedules were individualized for each subject so that blood lipids could be assessed from a single phase. The early follicular phase of the menstrual cycle was chosen, as this is the time when plasma levels of estrogen and progesterone as well as plasma cholesterol<sup>17</sup> are at their nadir. Thus, lipid levels were minimally affected by variability in hormone levels both within and among subjects. Blood was drawn on day 5 of the menstrual cycle during the third month of the treatment. Blood was drawn between 6:30 and 8:30 AM after an overnight fast into vacuum tubes containing K<sub>2</sub>-EDTA (final concentration, 1.5 mg/mL).

### Lipoprotein Analyses

Plasma lipids and lipoproteins were measured at the George Washington University Lipid Research Clinic, where standardization with the Centers for Disease Control and Prevention (CDC) was maintained throughout the study for analysis of triglycerides, cholesterol, and HDL-C levels. Cholesterol, tri-

**TABLE 1. Baseline Characteristics of 34 Premenopausal Women**

Age, y	31±1
Body mass index, kg/m <sup>2</sup>	24.4±0.7
Drinks per week*	1.7±0.2
Cholesterol, mmol/L	4.35±0.14
Triglycerides, mmol/L	0.86±0.06
LDL cholesterol, mmol/L	2.49±0.12
HDL cholesterol, mmol/L	1.54±0.05

Values are mean±SEM. Lipids and lipoproteins were analyzed from plasma collected on day 5 of the menstrual cycle. None of the subjects smoked or used oral contraceptives.

\*From subjects' self-administered medical history questionnaire.

glycerides, and triglyceride blanks were analyzed enzymatically by using Abbott ABA-100 analyzers with reagents supplied by Abbott Diagnostics. Reagents were prepared with Na<sub>2</sub>-EDTA (final concentration, 4 mmol/L). VLDL was isolated by ultracentrifugation at  $d=1.006$ . Total HDL was fractionated by heparin-MnCl<sub>2</sub> precipitation.<sup>18</sup> LDL-C was calculated as the difference between the cholesterol measured in the LDL plus HDL infranant fraction ( $d>1.006$ ), and the HDL-C was measured by precipitation. Recoveries of cholesterol in the ultracentrifugal fractions were greater than 95% for each sample. Additionally, HDL<sub>3</sub> was isolated by ultracentrifugation at  $d=1.125$ , cholesterol in the  $d>1.125$  fraction was determined, and HDL<sub>2</sub> cholesterol was calculated as the difference between HDL-C and HDL<sub>3</sub> cholesterol.

Apolipoprotein (apo) A-I, apoA-II, and apoB were determined at the Diet and Human Performance Laboratory by the electroimmunoassay of Laurell.<sup>19</sup> Whole plasma samples from the alcohol and no-alcohol treatments were analyzed in triplicate using as a standard an in-house plasma pool calibrated with reference material from the CDC.<sup>20</sup>

Lipoprotein particles containing apoA-I but no apoA-II (LpA-I) were determined by differential electroimmunoassay using commercially available gels and standards (Sebia). This assay is a modification of the method of Laurell.<sup>19</sup> Lipoproteins containing both apoA-I and apoA-II (LpA-I:A-II) were determined as the difference between total apoA-I and LpA-I.

Lipoprotein(a) (Lp[a]) was determined at Tufts University Human Nutrition Research Center on Aging by enzyme-linked immunosorbent assay using commercially available plates (Terumo Medical Corp). The assay uses a combination of monoclonal antibody (anti-Lp[a] without cross-reactivity to plasminogen) and polyclonal antibody that is specific for the protein portion of Lp(a) (anti-apo[a]). The assay was standardized by using purified Lp(a), and results are expressed as grams of Lp(a) per liter.

The size of HDL particles was assessed by the method of Li et al<sup>21</sup> at Tufts University Human Nutrition Research Center on Aging. Briefly, the method consists of separating prestained HDL particles in polyacrylamide agarose gels with a 4% to 30% gradient, scanning the gels by densitometry, and determining the distribution of particles by size. An array of discrete HDL subfractions is separated by this technique. The HDL score reflects the size distribution of HDL particles and the relative concentration of each size. Smaller scores are associated with larger HDL particles.

### Statistical Analysis

The data were analyzed by paired *t* test using the programs of the SAS Institute.  $\alpha=.05$  was considered significant.<sup>22</sup> Correlation coefficients were determined by the Pearson product-moment method.

**TABLE 2. Lipid and Lipoprotein Levels of 34 Premenopausal Women Consuming a Controlled Diet With No-Alcohol or Alcohol Treatments**

	No Alcohol	Alcohol	P
Cholesterol	4.24±0.13	4.19±0.13	NS
Triglycerides	0.94±0.08	0.88±0.07	NS
LDL cholesterol	2.33±0.13	2.15±0.13	.001
HDL cholesterol	1.55±0.05	1.71±0.08	.0001
HDL cholesteryl ester	1.11±0.05	1.22±0.05	.003
HDL free cholesterol	0.44±0.03	0.49±0.03	.01
HDL phospholipids	1.62±0.04	1.74±0.04	.006
HDL <sub>2</sub> cholesterol	0.96±0.05	1.03±0.08	.03
HDL <sub>3</sub> cholesterol	0.59±0.01	0.65±0.03	.02
LDL-C/HDL-C	1.62±0.11	1.38±0.11	.0001
HDL size*	10.0±0.1	9.8±0.1	.0001
ApoB	0.79±0.03	0.77±0.03	NS
ApoA-I	1.32±0.03	1.42±0.04	.0001
ApoA-II	0.346±0.005	0.361±0.005	.0003
LpA-I	0.47±0.02	0.51±0.02	.001
LpA-I:A-II	0.86±0.02	0.91±0.03	.0001
Lp(a)	0.131±0.031	0.129±0.031	NS

Apo indicates apolipoprotein; LpA-I, HDL particles containing apoA-I but no apoA-II; LpA-I:A-II, HDL particles containing both apoA-I and apoA-II; and Lp(a), lipoprotein(a). Treatments (no alcohol or alcohol) were administered in a crossover design. Treatment order groups were combined since there was no effect due to period, ie, no carryover effect. Lipids and lipoproteins were analyzed from plasma collected on day 5 of the third menstrual cycle of the treatment. Values are mean±SEM. Values for lipids are in millimoles per liter; for apolipoproteins, grams per liter.

\*Smaller scores are associated with larger HDL particles.

## Results

The women in this study were healthy with mean baseline values for cholesterol and LDL-C below the 50th percentile for their age and sex<sup>23</sup> (Table 1). The frequency of alcohol intake of this group of women averaged 1.7 drinks per week (range: 1 to 8 drinks) as determined from self-reported data on questionnaires administered during the study.

The amount of alcohol consumed during the alcohol treatment period was the equivalent of approximately two drinks per day. The percent of total calories from alcohol ranged from 8% for women consuming 2800 kcal/d to 16% for those consuming 1400 kcal/d. The mean values for caloric intake and the percent of calories from alcohol were 2000 kcal and 11%, respectively. Subjects reported no adverse reactions to the alcohol treatment. A comparison of data by treatment order (no alcohol to alcohol versus alcohol to no alcohol) showed no difference for any of the lipid variables reported, and thus crossover groups were combined by treatment.

The alcohol treatment did not alter total cholesterol or triglyceride levels, yet both LDL-C and HDL-C levels were significantly changed (Table 2). LDL-C decreased with alcohol an average of 0.18 mmol/L (7 mg/dL), or about 8%, compared with the no-alcohol treatment. HDL-C increased by 0.16 mmol/L (6 mg/dL), or about 10%. HDL cholesteryl ester, free cholesterol, and phospholipids increased to a similar degree (7% to 11%) in response to the alcohol treatment.

The ratio of LDL-C to HDL-C was lowered as a result of the alcohol treatment in 30 of the 34 women. HDL-C was increased in 79% and LDL-C was decreased in 73% of the women after the alcohol treatment. Although

alcohol was an effective modulator of both LDL and HDL, Lp(a) levels were not changed (Table 2).

The increase in HDL-C associated with the alcohol treatment was due to an increase in both HDL<sub>2</sub> and HDL<sub>3</sub>. The two subfractions appeared to increase in parallel, with each increasing by about 8%. Yet the overall size of HDL particles was slightly but very consistently larger when subjects consumed alcohol (Table 2).

Alcohol increased plasma apoA-I levels 7%, whereas apoA-II increased by only 4%. Despite the significant decrease in LDL-C associated with the alcohol treatment, no change was found in levels of apoB, the major protein component of LDL. LpA-I increased by 8% due to alcohol consumption. LpA-I:A-II was increased, and the percent of total apoA-I as LpA-I was unchanged at 36%.

Lipid changes (alcohol minus no alcohol) as a function of BMI were assessed by rank correlation. These changes did not differ for any lipid variable regardless of whether the BMI comparisons were made between the highest half of BMI and the lowest or among BMI tertiles. To determine if initial levels of HDL-C influenced the response to alcohol, we compared the lipid changes observed for the 50% of subjects with the highest HDL-C values at baseline to those with the lowest HDL-C values. No differences were detected for any of the parameters measured.

## Discussion

It is widely believed that environmental factors have a large effect on risk of CHD. Hegsted and Ausman<sup>4</sup> found that three dietary variables, polyunsaturated fat, saturated fat, and alcohol consumption, explained 85% of the variation in CHD in men. In that study, a strong negative correlation ( $r = -.58$ ) was found for the relation between alcohol consumption and CHD.

Women who drink 3 to 9 drinks a week have a relative risk of CHD of 0.6 compared with nondrinkers.<sup>3</sup> This protective effect is commonly thought to be mediated through altered lipoprotein levels, specifically by increasing levels of HDL. Yet it should be noted that alcohol also causes changes in various hemostatic factors involved in coagulation and fibrinolysis.<sup>24,25</sup> For women, it has been estimated that an increment in HDL-C of 0.26 mmol/L (10 mg/mL) is associated with a 32%<sup>26</sup> to 42%<sup>7,27</sup> decrement in CHD risk. According to these estimates, the 0.16 mmol/L (6 mg/dL) change observed in the present study would translate into a rather impressive reduction in risk of CHD ranging from 19% to 25%. From these estimates, it appears that alcohol consumption could confer a considerable degree of protection against CHD in women by elevating levels of HDL.

Experimental studies of men have documented alcohol-induced changes in HDL-C levels.<sup>28,29</sup> However, we know of only one report of alcohol-induced changes in lipoproteins of premenopausal women in which alcohol intake was controlled.<sup>30</sup> In that study, no changes in plasma lipoproteins were detected after women consumed 35 g alcohol/d for 3 weeks. The authors suggested that this might be due to the initially high levels of HDL in women compared with men. However, their study did not control the subjects' diets, nor did it synchronize blood collection with a specific phase of the menstrual cycle.

Observational studies have consistently shown that women who drink alcohol have higher levels of HDL-C than do those who do not.<sup>31-33</sup> This association of alcohol consumption with increased levels of HDL-C may account for the reduced incidence of myocardial infarction,<sup>5,34</sup> CHD, and ischemic stroke<sup>3</sup> observed in women who were moderate drinkers compared with those who were nondrinkers.

It is widely believed that HDL<sub>2</sub> is the component of HDL that is critical for protection against CHD.<sup>35,36</sup> However, the relative benefits of HDL<sub>2</sub> and HDL<sub>3</sub> have not been well delineated, and there is recent evidence that HDL<sub>3</sub> is at least, if not more, protective.<sup>37,38</sup> In the present study both HDL<sub>2</sub> and HDL<sub>3</sub> cholesterol levels were increased to about the same degree. The mean HDL particle size, however, increased slightly, suggesting an increase in the size of both HDL<sub>2</sub> and HDL<sub>3</sub>.

In a study of male subjects, increases in HDL<sub>2</sub> or HDL<sub>3</sub> were a function of the amount of alcohol consumed.<sup>28</sup> Only HDL<sub>3</sub> cholesterol was increased when men drank 30 g alcohol/d, whereas both HDL<sub>2</sub> and HDL<sub>3</sub> cholesterol were increased when they drank 60 g alcohol/d. It could be argued that in female subjects, 30 g alcohol was an effectively higher dose than it would have been in men, thus explaining the concomitant increase in HDL<sub>3</sub> and HDL<sub>2</sub>. However, this may not be the case, since the responses of HDL<sub>2</sub> and HDL<sub>3</sub> to alcohol did not differ between small and large women.

A more recent approach is to differentiate HDL particles by apoprotein content. HDL particles that contain LpA-I have been suggested as being the anti-atherogenic subfraction; particles that contain LpA-I:A-II were not thought to mediate reverse cholesterol transport.<sup>39-41</sup> This concept has recently been contradicted, and these two apo-specific subfractions have been reported to be similar in ability to promote cholesterol efflux.<sup>42-44</sup> A study of men with varied levels of alcohol intake found decreasing concentrations of LpA-I and increasing concentrations of LpA-I:A-II as the amount of alcohol intake increased.<sup>45</sup> In contrast, we found that alcohol induced an increase in both LpA-I and LpA-I:A-II; thus, regardless of whether one or both subfractions promote cholesterol efflux, the alcohol-induced changes in these apo-specific particles should be beneficial.

We observed a reduction in LDL-C when the subjects consumed alcohol, a finding consistent with observations from the Cooperative Lipoprotein Phenotyping Study<sup>46</sup> and the Honolulu Heart Program,<sup>47</sup> in which LDL-C was consistently negatively correlated with reported alcohol consumption. With the exception of four women, LDL-C/HDL-C ratios were improved by the alcohol treatment. Despite the significant change in LDL-C in the present study, apoB concentrations were not changed. Since each LDL particle is thought to carry a single copy of apoB, this would suggest that the number of LDL particles was not significantly changed by alcohol consumption but that LDL particles transported less cholesterol. Alcohol increased the components of HDL; HDL-C (both free cholesterol and cholesteryl ester), phospholipids, and apoA-I levels increased to approximately the same degree, whereas apoA-II increased to a lesser degree. Thus, any changes in the composition of HDL appeared to be minor.

We investigated the effects of alcohol on Lp(a) because this lipoprotein is positively associated with CHD and because in one study<sup>48</sup> Lp(a) levels increased in alcoholic men after withdrawal from alcohol. Under the conditions of our study, Lp(a) was not altered by alcohol consumption, a finding consistent with the concept that this lipoprotein is controlled, to a marked degree, by genetics<sup>49</sup> and is resistant to dietary manipulation.<sup>50</sup> In accordance with previous studies,<sup>28,51-53</sup> we found that alcohol does not appreciably elevate fasting levels of plasma triglycerides in normolipemic women consuming moderate levels of alcohol. However, it should be noted that certain conditions, notably preexisting hypertriglyceridemia<sup>53</sup> and obesity,<sup>54</sup> may predispose individuals to alcohol-induced increases in fasting triglyceride levels.

Length of fasting prior to blood drawing can also influence plasma triglyceride levels. In this study, subjects were instructed to consume the alcohol-containing cocktails or fruit juice control beverages just prior to bedtime, with bedtime purposely undefined. Although this did not allow for a standard 12-hour fast prior to blood drawing, this consideration was secondary to the safety of the subjects. It was of utmost importance to schedule the drinking time in a manner that would not tempt subjects to drive after consuming alcohol.

Our observations are limited to a specific phase of the menstrual cycle and may not be generalizable to periovulatory or luteal phases of the cycle when hormone levels are elevated. The potential interaction of alcohol and hormones across the menstrual cycle has not been assessed. Additionally, any beneficial effects of alcohol consumption on plasma lipoprotein patterns must be considered in context with other risks of cardiovascular disease and also in relation to overall health. Alcohol intake is related to hypertension,<sup>55-57</sup> a major risk factor for cardiovascular disease, and may promote subarachnoid hemorrhage in women.<sup>3</sup> Alcohol is also associated with a number of noncardiovascular health problems; and for women, a major concern in this category is the recent association of alcohol consumption with breast cancer.<sup>58-60</sup>

This study is one of a limited number of studies assessing lipoprotein response to alcohol consumption by women. We controlled for a number of potentially confounding variables including dietary pattern, phase of menstrual cycle, oral contraceptive use, obesity, smoking status, use of medications, and ability to measure alcohol intake. We concluded that alcohol equivalent to two drinks per day produced a beneficial effect on the lipoprotein profiles of premenopausal women as measured at the follicular phase of the menstrual cycle. To determine whether these effects can be generalized, studies of alcohol-induced changes in lipoproteins are needed that encompass all phases of the cycle.

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