Alcohol consumption and its relation to lipid-based cardiovascular risk factors among middle-aged women: the role of HDL$_3$ cholesterol

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Abstract

To study the association of alcohol consumption and lipid-based cardiovascular risk factors among middle-age women, cross-sectional analysis among 274 middle-aged healthy women with different drinking habits and a follow-up analysis of alcoholic women during abstinence was performed. Serum total cholesterol, low and high-density lipoprotein cholesterol (LDL and HDL cholesterol), triglycerides (TG), apolipoproteins A1 (Apo A1) and B (Apo B), and HDL-cholesterol subfractions 2 (HDL$_2$) and 3 (HDL$_3$) were measured. All lipid values except LDL cholesterol positively correlated with self-reported alcohol consumption. When alcoholics were excluded the correlation was significant only for HDL cholesterol, HDL$_3$, and Apo A1. The increasing trend of HDL cholesterol, HDL$_3$ and Apo A1 were clearly seen first in women consuming \( \geq 20–40 \) g/day of absolute alcohol. Alcohol consumption \( > 40 \) g/day increased all lipid values except LDL cholesterol. Abstinence for 2 weeks caused a significant decrease in HDL$_3$ cholesterol, and an increase in LDL cholesterol and Apo B. The results indicate that among middle-aged women the Apo A1 and HDL cholesterol via its HDL$_3$ but not HDL$_2$ subfraction might play a role in the beneficial coronary consequences associated with moderate alcohol consumption. However, the increasing beneficial trend first appears when daily drinking exceeds 20 g/day. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Alcohol consumption; Apo A1; Apo B; Cardiovascular risk; Cholesterol; HDL$_3$ cholesterol; HDL$_2$ cholesterol

1. Introduction

Epidemiological studies have shown a negative association between alcohol consumption and the risk of developing coronary artery disease (CAD) [1–4]. Over a broad range of alcohol consumption the relationship to all-cause mortality seems to be J-shaped [5–7]. This issue is not only due to individuals at high risk becoming nondrinkers [8,9] but because studies have suggested a beneficial association between moderate alcohol consumption and atherosclerosis progression and incidence events [10–13]. Furthermore, it has been shown that alcohol consumption may protect against severe coronary atherosclerosis [14]. Excessive alcohol use, however, has obvious detrimental effects on the cardiovascular system [12,13,15,16].

The mechanism through which alcohol might exert its protective effect remains unclear. Population studies have shown that moderate alcohol consumption usually does not change proatherogenic low-density lipoprotein (LDL) levels but high consumption may even decrease the level [17]. High-density lipoproteins (HDL) cholesterol, however, unlike other lipids show a dose-dependent relationship to alcohol intake [18–20]. Because HDL cholesterol is thought to play an important role in preventing atherosclerosis [21–23], the main hypoth-
2.1. Analysis of alcohol consumption

The effect of alcohol consumption on HDL subfractions among males is not clear. Some studies show the effect of alcohol on HDL_1 [29,30] and some also on HDL_2 [31,32]. It has also been hypothesized that heavy drinking preferentially increases HDL_2 and moderate drinking augments HDL_3 [33]. Results also show that Apo A1 levels rise with alcohol consumption [34] and that they may protect against atherectomy even better than HDL cholesterol does [35].

Little is known about alcohol consumption and its relation to lipid-based cardiovascular risk factors among women. In large cohorts of women, HDL cholesterol is the lipoprotein most strongly associated with CAD [36,37]. However, women answering surveys are particularly likely to underestimate their alcohol intake [38], and few studies have included either alcoholics or methods for measuring alcohol use that are adequate for obtaining reliable data. The present population based cross sectional study focuses on lipid values among women, employs a detailed history of alcohol use and includes also women with high alcohol consumption. The effect of 2 weeks cessation of alcohol consumption on lipids was studied, too.

2. Subjects and methods

The study protocol was approved by the Ethics Committees of Tampere University Hospital, the A-Clinic Foundation, and the Tampere Health Center. The study was performed according to the Helsinki Declaration on human experimentation. The sample size was powerful enough to give significance ($P < 0.05$) if the difference in HDL cholesterol was 0.2 mmol/l.

2.1. Subjects

The study was based on 274 consecutive representative non-menopausal women participating in a voluntary health screening aimed at all women in the population between 40 and 45 years ($n = 3116$). The participation rate was 84.5%. Experienced nurses recorded the alcohol consumption by asking the type and amount of alcohol drunk during the average week. The alcohol amounts were calculated from the self-reported number of standard drinks (14 g), equating one bottle of beer (33 cl), one glass of red or white wine (12 cl), one small glass of strong wine (8 cl) or one shot (4 cl) of spirits. The women also filled in two structured alcohol questionnaires: the Malmö Modified Michigan Alcoholism Screening Test (Mm-MAST) [39] and the CAGE [40]. According to the results the women were divided in three groups [41].

2.1.1. Moderate drinkers

Moderate drinkers ($n = 139$) if they had less than two positive answers in the CAGE, less than four positive answers in the Mm-MAST, and a self-reported weekly consumption of less than 140 g absolute alcohol during last 2 months. Thus, this group included also light drinkers and abstainers. Two women in the social drinker group had diabetes but no other chronic diseases were found in this group.

2.1.2. Heavy drinkers

Heavy drinkers ($n = 62$), if they had at least two positive answers in the CAGE, or at least four positive answers in the Mm-MAST or their self-reported weekly alcohol consumption was at least 140 g of absolute alcohol. No alcoholics were found to be included in the heavy drinker group.

2.1.3. Alcoholics

Alcoholics ($n = 73$) were consecutive, actively drinking women who were admitted for treatment at the detoxification clinic of A-Clinic Foundation in Tampere. All had a well-documented history of chronic alcoholism. None of them had previous history of liver disease or showed clinical signs of liver diseases or other organic complications of alcohol abuse at the time of the interview. None of them had diabetes. The effect of abstinence on lipid values of 12 female alcoholics was analyzed after 1 and 2 weeks without drinking.

The characteristics of the women in the different groups are presented in Table 1. Body mass index (BMI) was calculated as weight/height$^2$. Information about regular medication was recorded. Women were classified as smokers if they regularly smoked at least five cigarettes per day. The lipid values of the heavy drinking women who smoked ($n = 29$) and those who did not ($n = 33$) were compared. These groups did not differ in self-reported alcohol consumption ($P > 0.05$). No statistically significant differences between the groups were found regarding the mean values for total cholesterol, HDL cholesterol, HDL_2, and HDL_3.
2.2. Analysis

The sera were obtained after a 12-h fast. Serum total cholesterol levels were measured by the enzymatic method [42]. The total concentration of HDL cholesterol and the cholesterol subfractions were measured by the dextran sulfate method [43] as previously described [44].

The precipitation reagent for total HDL cholesterol contained 10 g/l Dextralipid 50 (Sochibo, Boulogne, France) and 101.6 g/l MgCl2–6H2O and for HDL3 19.1 g/l Dextralipid 50 and 397.4 g/l MgCl2–6H2O. Then 250 μl serum was added to 25 μl of the appropriate reagent and after a 15-min incubation at ambient temperature, supernatants were separated by centrifugation for 20 min at 12 000 × g (Heraeus Biofuge 15, osterodeamtlarz, Germany). The HDL cholesterol contents of the supernatants were analyzed enzymatically on a Monarch 2000 Analyzer (Instrumentation Laboratory, Lexington, USA) using Chod-Pap cholesterol reagents (Cat No 237574); Boehringer Mannheim, Germany), and a primary cholesterol standard (Cat No 530, Orion, Espoo, Finland). The HDL3 subfraction was calculated by subtraction of HDL1 from total HDL. Triglyceride levels were measured by the enzymatic, kinetic method. Apo A1 and B levels were determined immunoturbidimetrically [45]. Serum LDL levels were calculated as (cholesterol–HDL cholesterol–0.45 × triglycerides) [46]. None of the women had triglyceride values > 4.0 mmol/l and thus none of them were excluded from this calculation. The percentages of HDL2 and HDL3 of total cholesterol and HDL cholesterol were calculated, and the values were compared between the groups.

2.3. Statistical methods

BMDP software programs were used in the statistical analyses of the material [47]. The correlations of self-reported alcohol consumption and lipid values were studied using bivariate plots for linear regression. The lipid values were compared between the groups, by using analyses of variance. When variances were different, the Welch approximation was used. To compare the results with earlier studies, the women were divided also into five groups according to self-reported alcohol consumption. In both cases the lipid values that were significantly different using $P_{\text{ANOVA}}$ were also compared using pairwise $t$-test between the groups; separate, when the variances between the groups differed and pooled when they did not differ. Nonnumeric parameters between the groups were compared using cross-tabulations.

3. Results

Among 274 women all lipid values except LDL cholesterol significantly correlated with self-reported alcohol consumption (Table 2). When alcoholics were excluded, only Apo A1, HDL cholesterol and HDL3 cholesterol correlated significantly with the self-reported alcohol consumption (Table 2). A strong significant positive correlation was found also between Apo A1 and HDL2 ($r = 0.62, P < 0.001$) and Apo A1 and HDL3 ($r = 0.71, P < 0.001$).

Lipid values were compared between different groups based on self-report and results from two questionnaires (Table 3). $P_{\text{ANOVA}}$ was significant for total cholesterol, HDL3 cholesterol, triglycerides, Apo A1 and Apo B. Differences in lipid values using $t$-tests were found between the moderated drinkers and the alcoholics and between heavy drinkers and alcoholics. No differences were found between the groups in the percentage of abnormally low HDL cholesterol/total cholesterol ($< 0.20$), ApoA1 ($< 0.86$ g/l), HDL3 cholesterol ($< 0.8$ mmol/l), and HDL2 cholesterol ($< 0.3$ mmol/l).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the women divided into different groups according to the self-reported alcohol consumption and the results from structured alcohol questionnaires</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol consumption (g/week)</td>
<td>Moderate drinkers ($n = 139$)</td>
</tr>
<tr>
<td>Alcohol consumption (g/week)</td>
<td>39.0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.3</td>
</tr>
<tr>
<td>BMIb (kg/m²)</td>
<td>24.3</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>33.3</td>
</tr>
<tr>
<td>Anxiolytes (%)</td>
<td>1</td>
</tr>
<tr>
<td>Gonadal hormones (%)</td>
<td>6</td>
</tr>
<tr>
<td>Other medication (%)</td>
<td>12</td>
</tr>
</tbody>
</table>

[a] ANOVA, analysis of variance.
[ b] BMI, body mass index.
Table 2
Correlations between the self-reported alcohol consumption and different lipid measures among women

<table>
<thead>
<tr>
<th>Serum lipids</th>
<th>All women (n = 274)</th>
<th>All except alcoholics (n = 201)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.215</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.206</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL₂ cholesterol</td>
<td>0.107</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL₃ cholesterol</td>
<td>0.284</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.060</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.392</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apo A₁</td>
<td>0.353</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apo B</td>
<td>0.240</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

To further confirm the above findings, we studied lipid values based only on the self-reported alcohol consumption (Table 4). Here $P_{\text{ANOVA}}$ was significant concerning total HDL, Apo A₁, HDL₃ cholesterol, HDL₂, HDL₂/total cholesterol, HDL₃/total cholesterol, HDL₂/HDL cholesterol, and HDL₃/HDL cholesterol. An increasing trend of HDL cholesterol ($P < 0.05$, $P$-value not shown in table), HDL₁/cholesterol and Apo A₁ ($P < 0.05$, $P$-value not shown in table) was first seen among those women drinking > 20–40 g/day as compared to those drinking > 10–20 g/day. HDL₂/cholesterol values were significantly lower among those drinking > 10–20 g/day as compared to those drinking > 40 g/day ($P < 0.01$, $P$-value not shown in table) and those drinking 0–10 g/day.

The relative changes of total HDL cholesterol, its density based subtractions and apolipoprotein A₁ in the group drinking > 10–20 g/day (compared to those drinking 0–10 g/day) indicate constant increasing trend for HDL₃ but significant reduction of HDL₂ resulting in decreasing or plateau trend in total HDL cholesterol and apolipoprotein A₁ (Fig. 1). In-groups with higher consumption the trend for both HDL subfractions was increasing, resulting in increase in total HDL cholesterol and apolipoprotein A₁.

Because Apo A₁, HDL cholesterol and HDL₃ subfraction correlated significantly with self-reported alcohol consumption even when the alcoholics were excluded, we studied these values further. The mean HDL cholesterol value among alcoholics was 0.17 mmol/l higher than among moderated drinkers (95% confidence interval from 0.02 to 0.32 mmol/l), and Apo A₁ values 0.19 mmol/l higher (95% confidence interval from 0.12 to 0.26 mmol/l). HDL₃ cholesterol values were 0.11 mmol/l higher among alcoholics than in moderated drinkers (95% confidence interval from 0.05 to 0.17 mmol/l). The concentrations of HDL cholesterol, HDL₃ cholesterol, and Apo A₁ in alcoholic women were 12% ($P < 0.05$), 11% ($P < 0.001$), and 13% ($P < 0.001$) higher than in the moderated drinkers, respectively.

Among the 12 alcoholic females who were followed for 2 weeks during abstinence, HDL₃ cholesterol decreased ($P = 0.004$), whereas LDL cholesterol ($P = 0.02$), and Apo B ($P = 0.02$) significantly increased.

Table 3
Lipid values (mean ± SD) of the women divided into different groups according to the self-reported alcohol consumption and the results from two structured alcohol questionnaires

<table>
<thead>
<tr>
<th></th>
<th>Moderate drinkers (n = 139)</th>
<th>Heavy drinkers (n = 62)</th>
<th>Alcohols (n = 73)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>1/2/3/4</td>
</tr>
<tr>
<td>Chol</td>
<td>5.10 ± 0.87</td>
<td>5.10 ± 0.95</td>
<td>5.80 ± 2.18</td>
<td>&amp;/ #/ 0.983/ &amp;</td>
</tr>
<tr>
<td>HDL</td>
<td>1.55 ± 0.35</td>
<td>1.57 ± 0.45</td>
<td>1.72 ± 0.58</td>
<td>0.089</td>
</tr>
<tr>
<td>HDL₂</td>
<td>0.56 ± 0.26</td>
<td>0.54 ± 0.31</td>
<td>0.61 ± 0.46</td>
<td>0.610</td>
</tr>
<tr>
<td>HDL₃</td>
<td>1.00 ± 0.16</td>
<td>1.02 ± 0.19</td>
<td>1.11 ± 0.24</td>
<td>&amp;/ #/ 0.375/*</td>
</tr>
<tr>
<td>HDL₂/Chol</td>
<td>0.31 ± 0.08</td>
<td>0.31 ± 0.09</td>
<td>0.31 ± 0.12</td>
<td>0.959</td>
</tr>
<tr>
<td>HDL₃/Chol</td>
<td>0.11 ± 0.05</td>
<td>0.11 ± 0.06</td>
<td>0.11 ± 0.08</td>
<td>0.939</td>
</tr>
<tr>
<td>HDL₂/Chol</td>
<td>0.20 ± 0.04</td>
<td>0.21 ± 0.05</td>
<td>0.21 ± 0.06</td>
<td>0.615</td>
</tr>
<tr>
<td>HDL₃/Chol</td>
<td>0.34 ± 0.10</td>
<td>0.32 ± 0.11</td>
<td>0.32 ± 0.13</td>
<td>0.384</td>
</tr>
<tr>
<td>HDL₃/HDL</td>
<td>0.66 ± 0.10</td>
<td>0.68 ± 0.11</td>
<td>0.68 ± 0.14</td>
<td>0.399</td>
</tr>
<tr>
<td>LDL</td>
<td>3.10 ± 0.80</td>
<td>3.10 ± 0.88</td>
<td>3.30 ± 2.15</td>
<td>0.730</td>
</tr>
<tr>
<td>Tg</td>
<td>1.00 ± 0.51</td>
<td>1.00 ± 0.47</td>
<td>1.70 ± 0.93</td>
<td>#/ #/ 0.541/#</td>
</tr>
<tr>
<td>ApoA₁</td>
<td>1.40 ± 0.18</td>
<td>1.50 ± 0.23</td>
<td>1.60 ± 0.30</td>
<td>#/ #/ 0.070/ &amp;</td>
</tr>
<tr>
<td>ApoB</td>
<td>0.70 ± 0.18</td>
<td>0.70 ± 0.18</td>
<td>0.90 ± 0.32</td>
<td>&amp;/ #/ 0.496/ &amp;</td>
</tr>
</tbody>
</table>

* $P_1$ (ANOVA), $P_2$ (C vs. A), $P_3$ (B vs. A), $P_4$ (C vs. B). Chol, Cholesterol; LDL, LDL-cholesterol; Tg, Triglycerides. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 
HDL3 subfraction correlated significantly with self-reported alcohol consumption among social and heavy drinking women, but not in alcoholics. Overall the level of consumption at which HDL cholesterol, HDL3 subfraction, and in Apo A1 first increased was higher in the present study than in earlier epidemiological studies. Possible bias was thus considered. Age, smoking, use of oral hormones and obesity are known to affect lipid values. The social and heavy drinker groups were age-matched, and the alcoholics were significantly younger than the other groups, which alone would have caused lower, not higher, lipid values in the alcoholics. Smoking is known to decrease HDL cholesterol values [50], and alcohol consumption correlates positively to smoking. This may partly explain the fact, that in the present study, where smoking also strongly correlated
with drinking, the alcohol-induced changes in HDL cholesterol values appeared with higher consumption amounts than in earlier studies. Additionally, no differences in BMI or in the use of hormones were observed between the groups.

The present study did not indicate any beneficial changes in total cholesterol, in LDL cholesterol, in triglycerides, or in triglycerides rich apolipoprotein B. In contrast, triglycerides, apo B, and total cholesterol were significantly elevated among women drinking >40 g/day as compared to women drinking 0–10 g/day. These results also indicate that although alcohol consumption increases HDL cholesterol, its ratio to total cholesterol (HDL/Chol) does not change among women. The fact that alcohol intake apparently did not change total cholesterol or its ratio to HDL cholesterol, LDL cholesterol or triglyceride values between those drinking 0–10 versus >10–20 g/day is in good agreement with an earlier corresponding study among Finnish men [20]. However, beneficial changes in total cholesterol levels (increased HDL/Chol ratio, and reduced total cholesterol and Apo B) earlier detected among male alcoholics [17,20] were not observed in the present study among female alcoholics, indicating possible gender difference.

It is known that alcohol consumption is underestimated, especially among heavily drinking women [38]. In our study well-experienced nurses interviewed all the subjects personally, and in addition, two questionnaires were used. Thus, these self-reports are likely to be more reliable than those obtained with less demanding techniques. Earlier epidemiological studies found that women reporting a consumption of 10 g/day might, actually drank 20 g/day. On the other hand, some differences in drinking pattern among Finnish women may cause some differences in lipid values when compared to studies made in other countries. For example, daily drinking is rare in Finland; most of the moderate and heavy drinkers only drink during weekends, and there is hitherto little evidence of the consequences of different drinking habits on lipid values.

The main hypothesis for the protective effect of alcohol on CAD, that it is mediated by HDL cholesterol, is based on the hypothesis that reverse cholesterol transport is the principal protective mechanism [51]. However, HDL may have other antiatherogenic properties, such as an antioxidant effect and the ability to reduce LDL uptake by endothelial cells, prevent LDL aggregates from forming, counteract the platelet-activating effect of LDL, increase the solubility of cholesterol in bile and promote fibrinolysis [52]. Other suggested mechanisms include changes in insulin resistance caused by alcohol [53–55], involvement of platelet aggregation [56], and finally, an effect not from alcohol at all but from the antioxidant in red wine [57].

In conclusion, the present data support the possibility that among moderately drinking women, the alcohol-induced changes in HDL₃ cholesterol and Apo A₁, have a role in the beneficial consequences of alcohol on CAD. The alcohol-caused increase in HDL₃ is emphasized by the fact that abstinence decreased this value. Clear positive changes in the level of HDL cholesterol metabolism based risk factors were seen primarily among women when daily drinking exceeds 40 g/day. Thus, taking into account the harm and risk associated with heavy drinking and alcoholism, alcohol cannot be recommended as a means to improve lipid values in order to avoid CAD for non-postmenopausal middle-aged women.

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