MODERATE DOSES OF ALCOHOLIC BEVERAGES WITH DINNER AND POSTPRANDIAL HIGH DENSITY LIPOPROTEIN COMPOSITION

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Abstract — Moderate alcohol consumption is associated with a reduced risk of coronary heart disease. In this study, postprandial changes in plasma lipids, high-density lipoprotein (HDL) composition and cholesteryl ester transfer protein (CETP) and lecithin: cholesterol acyltransferase (LCAT) activity levels were investigated in response to moderate alcohol consumption. A dose of 40 g of alcohol was consumed as beer, wine or spirits by eight healthy middle-aged men before and during dinner thus simulating social drinking. Lipid parameters were studied before, and at 1, 3, 5, 9, and 13 h after dinner. An alcohol-induced elevation of plasma triacylglycerides was observed at 3 and 5 h after dinner, but total plasma cholesterol and apolipoprotein B were hardly affected. HDL lipids changed during the postprandial phase after alcohol consumption, HDL triglycerides were elevated at 5 and 9 h, HDL phospholipids were elevated at 9 and 13 h, and HDL cholesterol was elevated at 13 h. A 6% increase in the concentration of apolipoprotein A-I was observed at 13 h. Plasma LCAT activity was slightly increased 9 h after dinner, but CETP activity levels were not affected. The LCAT changes appeared similar for all three alcoholic beverages. It is concluded that moderate alcohol consumption with dinner affects plasma triglyceride concentration as well as HDL composition.

INTRODUCTION

Many epidemiological studies have shown that moderate alcohol consumption is associated with a reduced risk of coronary heart disease (CHD) (Renaud et al., 1993). The mechanisms underlying this reduced risk may involve both lipoprotein metabolism and haemostasis, including both platelet aggregation (Renaud et al., 1981) and fibrinolysis (Ridker et al., 1994; Hendriks et al., 1994).

Both epidemiological and experimental studies have demonstrated that moderate alcohol consumption increases high-density lipoprotein (HDL) concentration. HDL cholesterol is inversely related to myocardial infarction (Miller and Miller, 1975). This association may be HDL-mediated by stimulating reverse cholesterol transport, i.e. the transport of cholesterol from peripheral tissues to the liver for excretion (Fielding and Fielding, 1995). The HDL increase, induced by moderate alcohol consumption and involving the two subfractions HDL2 and HDL3, may explain up to half of the CHD risk reduction (Criqui et al., 1987; Gaziano et al., 1993).

Only a limited number of experimental studies have investigated the short-term effects of a moderate amount of alcohol on lipoprotein composition in normolipidaemic individuals (Goldberg et al., 1984; Franceschini et al., 1988; Superko, 1992; Hagiage et al., 1992; Van Tol et al., 1995). It has been suggested (Franceschini et al., 1988; Van Tol et al., 1995) that lipoprotein changes may occur in the normal postprandial phase, which facilitates improved removal of cholesterol from tissues. Franceschini et al. (1988), however, only investigated the effects of whisky either with or without a fat load provided early in the morning after an overnight fast. Van Tol et al. (1995) reported induction of net mass lipid transfer by wine consumption.

In the present study, the effects of moderate amounts of alcohol on HDL composition in the
normal postprandial phase were investigated. The experimental design reflects the way in which moderate amounts of alcohol are consumed in a social setting. Alcohol was provided either as beer, wine or spirits.

SUBJECTS AND METHODS

Subjects

Eight healthy non-smoking middle-aged men (45–55 years of age, body mass index 22.4–27.2) used to a Western lifestyle, diet, and an habitual moderate consumption of alcohol (1–4 drinks/day), but with no past history of alcohol abuse, participated in the study. Fasting plasma cholesterol, triglycerides, and HDL cholesterol were 5.6 ± 0.6 mmol/l, 1.7 ± 0.6 mmol/l, and 1.2 ± 0.2 mmol/l (mean ± SD), respectively. Informed consent was obtained from each subject; the research protocol, which complied with the Declaration of Helsinki, was approved by the Institute's external Medical Ethical Committee.

Study protocol

The study lasted 11 days, from Sunday evening to the second Wednesday morning, during which time each subject received a standard diet providing 10.7 MJ/day (overall composition: fat 34.5%; carbohydrates 52.7%; proteins 12.8% of total energy; polyunsaturated to saturated fat ratio 0.46). The 11 days included two experimental periods of 2 days, namely days 4 and 5 (period 1) and days 9 and 10 (period 2) of the study. During the experimental periods the men stayed in the Institute's metabolic ward. The men arrived in the evening before the experimental periods and stayed until the morning after. On the non-experimental days the men were allowed to live at home, but had to come to the metabolic ward to have dinner and to receive their food for the next 24 h. The subjects were not allowed to eat or drink anything but the food and drinks supplied.

During the four experimental days, the subjects received four different beverages at dinner, 400 ml of carbonated mineral water (control) or 40 g of alcohol in the form of red wine (Languedoc, France), beer (pilsner) or spirits (Dutch gin). Two glasses were served as an appetizer 1 h before dinner and two were taken during the meal. The caloric content of the meal including the drinks was 2342 kJ with mineral water, 3514 kJ with spirits, 3715 kJ with red wine, and 4183 kJ with beer. The treatments were allocated randomly to the eight subjects according to two Latin squares and were balanced for carry-over effects. Blood samples were taken 1 h before dinner (just before the appetizer) and at 1, 3, 5, 9, and 13 h after dinner. Dinner was served at 18:00 and was consumed within 30 min.

Analytical methods

Blood was collected from the intermedian cubital vein by a venoject system in tubes containing EDTA. Plasma was collected after a 10-min centrifugation at 1700 g at 4°C. Part of the plasma was immediately processed for ethanol analysis and precipitation of apolipoprotein B-containing lipoproteins; the remainder was snap frozen and stored at −80°C until analysis. Blood-alcohol concentrations were determined enzymatically in blood samples obtained before the appetizer and at 1 and 3 h after dinner by the method of Beutler and Michal (1977). HDL was separated from the other lipoproteins by precipitation of apolipoprotein B-containing lipoproteins with MgCl₂ and Na-phosphotungstate (Lopes-Virella et al., 1977). Total cholesterol, unesterified cholesterol, and triglycerides in plasma as well as in HDL, and phospholipids in HDL, were determined using enzymatic methods (Boehringer GmbH, Germany). Cholesteryl esters were calculated as the difference between total cholesterol and unesterified cholesterol. Apolipoproteins A-I, A-II, and B were determined immunoturbidimetrically in serum using anti-human apolipoprotein antisera (Boehringer GmbH, Germany). Lecithin: cholesterol acetyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) activity levels were analysed in blood samples obtained before the appetizer and at 1, 3, 5, and 9 h after dinner. Plasma LCAT activity level was determined as described previously (Dullaart et al., 1993) using exogenous substrate containing radiolabelled cholesterol. Plasma CETP activity level was determined using excess exogenous lipoprotein lipase (LPL) and HDL as previously described (Van Tol et al., 1997). Both LCAT and CETP activity levels determined using these methods are not affected by endogenous plasma lipoprotein concentration or composition. All CETP and LCAT activity assays were
Table 1. Mean blood-alcohol concentration in men 1 and 3 h after a dinner with beer, wine, spirits or mineral water

<table>
<thead>
<tr>
<th>Beverage</th>
<th>Blood-ethanol concentration (mmol/l)</th>
<th>1 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer</td>
<td>6.84 ± 1.02</td>
<td>1.95 ± 0.72</td>
<td></td>
</tr>
<tr>
<td>Wine</td>
<td>6.79 ± 1.09</td>
<td>1.98 ± 0.59</td>
<td></td>
</tr>
<tr>
<td>Spirits</td>
<td>8.25 ± 1.41*</td>
<td>2.76 ± 0.98</td>
<td></td>
</tr>
<tr>
<td>Mineral water</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. *P < 0.038 compared with beer and wine. Other details are as in the Subjects and methods section.

Table 2. Mean lecithin:cholesterol acyltransferase (LCAT) activity in men 1, 3, 5 and 9 h after a dinner with beer, wine, spirits or mineral water

<table>
<thead>
<tr>
<th>Beverage</th>
<th>LCAT activity (nmol/ml/h)</th>
<th>1 h</th>
<th>3 h</th>
<th>5 h</th>
<th>9 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer</td>
<td>48.6</td>
<td>48.0</td>
<td>50.2</td>
<td>51.7</td>
<td></td>
</tr>
<tr>
<td>Wine</td>
<td>51.4</td>
<td>49.0</td>
<td>52.9</td>
<td>52.2</td>
<td></td>
</tr>
<tr>
<td>Spirits</td>
<td>50.5</td>
<td>49.8</td>
<td>46.9</td>
<td>52.3</td>
<td></td>
</tr>
<tr>
<td>Mineral water</td>
<td>49.8</td>
<td>45.3</td>
<td>48.2</td>
<td>47.2</td>
<td></td>
</tr>
<tr>
<td>SED</td>
<td>3.57</td>
<td>3.17</td>
<td>2.86</td>
<td>2.36</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.898</td>
<td>0.172</td>
<td>0.457</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Values are means with SED. For other details, see the text.

performed in duplicate within a period of 4 days, using single batches of substrates. All samples obtained from two individuals (consuming four different drinks) were compared in the same assay run. The within-day coefficients of variation (CV) were 2.7% and 4.5% and between-day CV were 5.1% and 6.0% for CETP and LCAT, respectively.

Statistical methods

The significance of all beverages combined as well as the effects of single beverages were determined by analysis of variance using Genstat or SAS statistical software. Values obtained after dinner were corrected for differences in values at 1 h before dinner just before the appetizer. Variables that showed a skewed distribution were transformed by their logarithms. The means are presented as geometrical means ± SD in Table 1 and in the text. Differences between mineral water and all alcohol beverages are expressed as means ± standard error of differences of means in Tables 2 and 3. The hyperlipidaemic response to the different treatments was quantified by calculating the area under the curve (AUC) for the period from 1 h before, until 9 h after, the meal. This AUC, defined by the line connecting individual triglyceride values and a line at the 9-h level parallel to the abscissa, was calculated by the trapezoidal rule. The null hypothesis was rejected at the 0.05 level of probability (P) in all statistical analyses.

RESULTS

Blood-alcohol concentration

Blood-alcohol concentrations ranged between 6.79 and 8.25 mmol/l at 1 h and between 1.95 and 2.76 mmol/l at 3 h after dinner with alcohol consumption (Table 1). No alcohol was detected in the blood after ingestion of the dinner with mineral water. All concentrations were below the Dutch legal limit for drinking and driving, i.e. 10.85 mmol/l (50mg/dl). Blood-alcohol concentrations were slightly higher at 1 h after drinking spirits than after drinking beer or wine (Table 1).

Plasma triglycerides and total cholesterol

Plasma triglycerides peaked, after all four beverages, 1 h after the meal and decreased thereafter. Plasma triglycerides were higher at 3 h after wine and spirits and at 5 h after beer and wine as compared to mineral water consumption (Fig. 1A). Plasma triglycerides were also higher at 3 h and 5 h after the meal when all alcoholic beverages were combined (Fig. 1B). The triglyceridaemic response, expressed as the AUC, was significantly greater for spirits (9.42 ± 5.50 mmol/l × 10 h; P = 0.021) but from mineral water (6.33 ± 2.96) the differences for beer (7.32 ± 1.94) and wine (8.73 ± 4.50) were not significant. The AUC for triglycerides was significantly greater (8.49 ± 4.16; P = 0.043) for all alcoholic drinks combined when compared to water (6.33 ± 2.96).

At 1 h after the meal, compared to mineral water (5.0 ± 0.7 mmol/l), however, plasma-cholesterol was higher for wine (5.4 ± 0.7 mmol/l) and spirits (5.3 ± 0.8 mmol/l) and for all alcoholic beverages combined (5.3 ± 0.7 mmol/l), but not for beer (5.1 ± 0.7 mmol/l). Plasma-cholesterol concentrations did not differ significantly between
Table 3. Mean cholesteryl ester transfer protein (CETP) activity in men 1, 3, 5 and 9 h after a dinner with beer, wine, spirits or mineral water

<table>
<thead>
<tr>
<th>Beverage</th>
<th>CETP activity (nmol/ml/h)</th>
<th>1 h</th>
<th>3 h</th>
<th>5 h</th>
<th>9 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer</td>
<td></td>
<td>105.8</td>
<td>106.5</td>
<td>107.0</td>
<td>101.1</td>
</tr>
<tr>
<td>Wine</td>
<td></td>
<td>106.7</td>
<td>106.5</td>
<td>105.4</td>
<td>99.4</td>
</tr>
<tr>
<td>Spirits</td>
<td></td>
<td>108.9</td>
<td>109.0</td>
<td>108.0</td>
<td>101.6</td>
</tr>
<tr>
<td>Mineral water</td>
<td></td>
<td>103.9</td>
<td>105.9</td>
<td>105.3</td>
<td>97.3</td>
</tr>
<tr>
<td>SED</td>
<td></td>
<td>2.86</td>
<td>3.48</td>
<td>2.57</td>
<td>2.62</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.180</td>
<td>0.627</td>
<td>0.474</td>
<td>0.133</td>
</tr>
</tbody>
</table>

Values are means with SED. For other details, see the text.

mineral water and the alcoholic drinks at any of the other time points after the meal (data not shown).

HDL triglycerides

HDL triglycerides decreased after dinner with each of the four beverages. HDL triglycerides were, however, higher at 3, 5 and 9 h after alcohol consumption as compared to mineral water consumption. The differences were significant at 3 h for wine and spirits and at 5 and 9 h for each of the three alcoholic beverages as well as for all alcoholic beverages combined (Fig. 1C).

HDL phospholipids

After dinner HDL phospholipids decreased following each of the four beverages. HDL phospholipid levels were, however, higher at 9 and 13 h after alcohol, as compared to mineral water, consumption (Fig. 1D). The differences were significant at 13 h for each of the three alcoholic beverages and at 9 and 13 h for all alcoholic beverages combined.

HDL free and total cholesterol

HDL free cholesterol (Fig. 1E) was significantly higher at 13 h after the meal for all alcoholic beverages combined. HDL total cholesterol was also increased by alcohol, as compared to mineral water, consumption only at 13 h after the meal (Fig. 1F). Differences were significant for beer and spirits and for all alcoholic beverages combined (Fig. 1F).

Apolipoproteins A-I, A-II, and B

Apolipoprotein A-I tended to be elevated for all three alcoholic beverages combined after alcohol consumption (108 ± 16 mg/dl) as compared to mineral water consumption (102 ± 15 mg/dl) at 13 h, though this difference was not significant (P = 0.10). Apolipoprotein A-II increased at 13 h for wine (38 ± 4 mg/dl; P = 0.05) and at 13 h for all three alcoholic beverages combined (37 ± 5 mg/dl; P = 0.03) as compared to mineral water consumption (35 ± 5 mg/dl). Apolipoprotein B was not affected by alcohol consumption at any of the time points tested (data not shown).

HDL total cholesterol/HDL triglycerides

The ratio HDL cholesterol/HDL triglycerides was reduced 5 h postprandially after consumption of each of the alcoholic beverages (beer, 0.87 ± 0.95; P = 0.014; wine, 0.45 ± 0.58; P < 0.001; spirits, 0.62 ± 0.42; P = 0.002) and alcoholic drinks combined (0.65 ± 0.68; P = 0.002), compared with mineral water (1.53 ± 1.07). At 9 h after the meal, the latter ratio still tended to be reduced (P = 0.052). The ratios HDL total cholesterol/HDL phospholipids, HDL total cholesterol/apolipoprotein A-I and HDL phospholipids/apolipoprotein A-I did not differ significantly between mineral water and the alcoholic drinks at any of the time points after the meal (data not shown).

LCAT and CETP activity

Plasma LCAT activity levels were higher at 9 h after the meal for wine and spirits and for all three alcoholic beverages combined, as compared to mineral water consumption (Table 2). Alcohol consumption had no effect on CETP activity levels (Table 3).

DISCUSSION

The present study shows that a moderate dose of alcohol with evening dinner increases postprandial triglyceride concentrations and postprandial hyperlipidaemic response, but has little effect on postprandial cholesterol concentrations. HDL composition changed after alcohol consumption in a time-dependent manner: HDL triglycerides increased initially, followed by an increase in HDL phospholipids. Serum LCAT activity was
MODERATE ALCOHOL CONSUMPTION AND HDL COMPOSITION

Fig. 1. Postprandial plasma triglyceride concentration and high-density lipoprotein (HDL) composition.

(A) Postprandial plasma concentrations of triglycerides (TG) (mmol/l) after dinner with mineral water, beer, wine or spirits. (B) Postprandial plasma concentrations of triglycerides (TG) (mmol/l) after dinner with mineral water or an alcoholic beverage. (C) Postprandial concentrations of HDL triglycerides (HDL-TG) (mmol/l) after dinner with mineral water or an alcoholic beverage. (D) Postprandial concentrations of HDL phospholipids (HDL-PL) (mmol/l) after dinner with mineral water or an alcoholic beverage. (E) Postprandial concentrations of HDL free cholesterol (HDL-FC) (mmol/l) after dinner with mineral water or an alcoholic beverage. (F) Postprandial concentrations of HDL total cholesterol (HDL-TC) (mmol/l) after dinner with mineral water or an alcoholic beverage.

* Value obtained after mineral water consumption was significantly \((P < 0.01)\) different from the value after wine and spirits, respectively. † Value obtained after mineral water consumption was significantly \((P < 0.01)\) different from the value after beer and wine, respectively. * Value obtained after mineral water consumption was significantly \((P < 0.05)\) different from the value obtained after moderate alcohol consumption. ** Value obtained after mineral water consumption was significantly \((P < 0.01)\) different from the value obtained after moderate alcohol consumption.
also increased, whereas CETP activity was not affected. Finally, increases in HDL total cholesterol and HDL free cholesterol were observed as late as the following morning. The latter increase coincided with an increase of apolipoprotein A-II and possibly also apolipoprotein A-I. The postprandial HDL compositional changes are consistent with those reported earlier (Franceschini et al., 1988).

Alcohol consumption induces marked differences in plasma triglycerides at 3 and 5 h after dinner. These effects on plasma triglyceride concentrations are mainly due to the triglyceride accumulation in very low density lipoproteins (Van Tol et al., 1995). HDL triglycerides decrease in concentration during the postprandial phase. This decrease is mitigated at 5 and 9 h after moderate alcohol consumption. The relatively high HDL triglyceride content may result from postprandial very low-density lipoprotein (VLDL) triglycerides being exchanged for HDL cholesteryl esters via CETP. It is well known that this neutral lipid transfer is dependent on the concentration of VLDL triglycerides (Eisenberg, 1984). This exchange does not coincide with increased plasma CETP activity levels as measured with exogenous substrates. The latter activity correlates well with plasma CETP mass measured by an immunoassay (Hannuksela et al., 1992). It was reported that CETP activity (measured with exogenous substrates) is decreased in alcohol abusers (Savolainen et al., 1990; Hannuksela et al., 1992), the changes being reversible by abstinence. However, these observations were not confirmed by Nishiwaki et al. (1994) nor by Riemen et al. (1997) in moderate alcohol consumers. We (Van Tol et al., 1995), have shown that net mass transfer of cholesteryl esters out of HDL and net mass transfer of triglycerides into HDL is indeed stimulated by moderate consumption of wine with dinner. Net mass transfer was found to be stimulated at 3 h after alcohol consumption without an increase in CETP activity level (Van Tol et al., 1995). Postprandially, plasma triglyceride-rich lipoproteins therefore appear to be a more important determinant of cholesteryl ester transfer from CETP mass.

Turnover of triglyceride-rich particles in plasma may also lead to an increased number of surface fragments and HDL particles. Formation of HDL particles may therefore depend on LPL activity. Several studies have shown that LPL activity is increased both in alcohol abusers (Ekman et al., 1981; Taskinen et al., 1982) as well as in moderate alcohol consumers (Schneider et al., 1985; Nishiwaki et al., 1994).

Newly synthesized HDL particles from surface fragments of triglyceride-rich lipoproteins may contribute to an increased phospholipid concentration in postprandial HDL and facilitate the transfer of free cholesterol from tissues into HDL (Sakr et al., 1966). An alcohol-induced increase in HDL free cholesterol was observed in the present study at 13 h after dinner following the alcohol-induced increase in HDL phospholipids.

HDL free cholesterol needs to be esterified by LCAT before uptake by the liver can occur in the form of cholesteryl esters (Steinberg, 1996). In the present study, alcohol consumption increased LCAT activity 9 h after the meal. This observation is in keeping with data reported elsewhere (Van Tol et al., 1995), which showed no increase in LCAT activity 3 h after drinking wine with dinner. Previous studies reported that LCAT activity, measured after an overnight fast, is not affected by alcohol consumption both in alcoholics (Albers et al., 1982; Haffner et al., 1985) or after moderate alcohol consumption (Nishiwaki et al., 1994). This suggests that the increase in LCAT activity may be transient, mainly occurring in the late postprandial phase.

HDL cholesterol concentration was elevated by alcohol consumption only 13 h after the meal. Differences were significant for beer and spirits and for all alcoholic beverages combined. Subfractions of HDL were not analysed in the present study, but previous reports have indicated that either HDL₂ (Contaldo et al., 1989) or HDL₃ (Haskell et al., 1984) or both HDL₂ and HDL₃ (Ekman et al., 1981; Taskinen et al., 1982) may be increased after alcohol consumption. Epidemiological data indicate that, independent of the type of subfraction, an increase in HDL cholesterol is correlated with a reduced CHD risk (Gaziano et al., 1993).

The present study reports on postprandial changes in HDL composition after moderate alcohol consumption with an evening meal. This setting may reflect alcohol consumption in a social setting. Mineral water was used as an alcohol-free control representing a calorie-free drink commonly consumed with dinner. Consequently,
caloric intakes during dinner with and without an alcoholic beverage were different. The present study suggests that no consistent differences exist between the effects of the three alcoholic beverages tested. Several other studies have reported similar effects of spirits (Goldberg et al., 1984; Franceschini et al., 1988; Superko, 1992) and wine (Hagiage et al., 1992) on lipoprotein metabolism. To our knowledge, beer, wine, spirits, and water have never been studied in one controlled experiment. The results of the present study suggest that the various alcoholic beverages may have similar effects implying that the physiological changes described are mediated by alcohol, rather than by other components in alcoholic beverages.

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