Alcohol consumption stimulates early steps in reverse cholesterol transport

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Abstract **Alcohol consumption is associated with increased HDL cholesterol levels, which may indicate stimulated reverse cholesterol transport. The mechanism is, however, not known. The aim of this study was to evaluate the effects of alcohol consumption on the first two steps of the reverse cholesterol pathway: cellular cholesterol efflux and plasma cholesterol esterification. Eleven healthy middle-aged men consumed four glasses (40 g of alcohol) of red wine, beer, spirits (Dutch gin), or carbonated mineral water (control) daily with evening dinner, for 3 weeks, according to a 4 × 4 Latin square design. After 3 weeks of alcohol consumption the plasma ex vivo cholesterol efflux capacity, measured with Fu5AH cells, was raised by 6.2% ($P < 0.0001$) and did not differ between the alcoholic beverages. Plasma cholesterol esterification was increased by 10.8% after alcohol ($P = 0.008$). Changes were statistically significant after beer and spirits, but not after red wine consumption ($P = 0.16$). HDL lipids changed after alcohol consumption; HDL total cholesterol, HDL cholesteryl ester, HDL free cholesterol, HDL phospholipids and plasma apolipoprotein A-I all increased ($P < 0.01$). In conclusion, alcohol consumption stimulates cellular cholesterol efflux and its esterification in plasma. These effects were mostly independent of the kind of alcoholic beverage.**—van der Gaag, M. S., A. van Tol, S. H. F. Vermunt, L. M. Scheek, G. Schaafsma, and H. F. J. Hendriks. **Alcohol consumption stimulates early steps in reverse cholesterol transport.** ***J. Lipid Res.*** **2001.** 42: **2077–2083.**

Supplementary key words beer • cholesterol efflux • cholesterol esterification • human • spirits • wine

Alcohol consumption is associated with a reduced risk for coronary heart disease (1–3). Epidemiological and physiological data indicate a causal relationship (4). Various mechanisms of action have been proposed for the protective action of alcohol consumption and more than 50% of the protective action of alcohol is associated with an increase in HDL cholesterol (5, 6). In addition, effects on hemostatic and vascular wall functioning may be involved (7, 8).

Previously we have shown that alcohol intake results in an increase in HDL cholesterol levels (9–11). The functional consequences of this HDL increase are not fully understood at present. One of the protective actions may involve an increased serum paraoxonase activity (11). Paraoxonase is an HDL-associated enzyme that may protect LDL against oxidation (12, 13).

HDL are important in reverse cholesterol transport (RCT) (14, 15). During the first step of RCT, free cholesterol is removed from peripheral cells (cholesterol efflux) by interaction between serum lipoproteins and cells. Free cholesterol released from the cell is esterified by lecithin: cholesterol acyltransferase and incorporated into the HDL particle (16). HDL cholesteryl esters can be transferred to apolipoprotein B (apoB)-containing lipoproteins in exchange for triacylglycerols by cholesteryl ester transfer protein (17). Finally, cholesteryl esters from lipoproteins are removed from plasma by the liver for catabolism via several pathways. Cholesteryl esters from LDL and VLDL (18) and from apoE-rich HDL (19) may be removed via the LDL receptor, whereas HDL cholesteryl esters may be removed by a nonendocytotic mechanism called selective uptake (20).

Literature on effects of alcohol consumption on the different steps of RCT is limited. We have therefore studied the effects of alcohol consumption on the first two steps of RCT: cellular cholesterol efflux and plasma cholesterol esterification (EST) in middle-aged men. Furthermore, changes in HDL lipids and apoA-I were examined.

MATERIALS AND METHODS

Subjects

Twelve middle-aged (45–60 years), nonsmoking men were recruited from a pool of volunteers at TNO Nutrition and Food

Abbreviations: apo, apolipoprotein; EST, cholesterol esterification; RCT, reverse cholesterol transport.

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Research (Zeist, The Netherlands) and through advertisement in a local newspaper. The protocol was carefully explained to the volunteers and their written informed consent was obtained. Subjects who fulfilled the following selection criteria were enrolled: used to consuming 7–28 glasses of alcohol-containing beverages per week, having a body mass index between 20 and 28 kg/m^2, having no family history of alcoholism, being healthy as indicated by a general medical questionnaire and a physical examination, and not taking medication on a regular basis. One subject withdrew because of treatment-related adverse effects. This Indonesian man, who usually consumed one to two glasses of alcohol per day dropped out because he suffered from flushing and palpitations (tachycardia) after consuming four glasses of spirits. Similar effects occur in aldehyde dehydrogenase-deficient people and therefore these adverse effects were classified as possibly treatment related. The remaining subjects, who finished the experiment successfully, were aged between 44 and 59 years (mean, 51.7 ± 5.4 years), had body mass indexes between 22.0 and 27.7 kg/m^2 (mean, 25.0 ± 1.8 kg/m^2), and their fasting total cholesterol concentrations were 4.0–7.6 mM (mean, 5.7 ± 1.2 mM). More baseline characteristics have been published previously (21).

Study protocol

The study was a 12-week, randomized, cross-over trial, according to a 4 × 4 Latin square design. Daily, all subjects consumed four glasses (40 g of alcohol) of red wine, beer, spirits (Dutch gin), or carbonated mineral water (control) with evening dinner, at the institute. Each beverage was consumed for 3 weeks. To avoid dietary confounding, total diet was controlled, and supplied by the institute. Subjects consumed all foods at home, except for evening dinner, which was served at 18:00 h at the institute, together with the four glasses of beverage. Subjects were not allowed to eat or drink anything but the foods supplied by the institute, except for tap water and coffee. Any food that had not been consumed was returned.

Daily energy requirement was estimated for each subject according to Schofield (22). Mean energy intake was 11.4 MJ (range, 10.5–12.2 MJ), and did not differ between the four treatments. We calculated that the energy provided by alcohol as being 19.6 kJ/g, assuming the net utilizable energy content of alcohol to be 70% of the theoretically present 28 kJ/g (23). Thus, 40 g/day provided on average 7 energy%. The nonalcohol energy of the diet consisted of 11% protein, 36% fat, and 53% carbohydrates. Treatment effects of the alcoholic beverages might be caused by beverage-specific components other than alcohol. Therefore, the levels of these nonmacronutrients were not compensated for in the diet. Evening dinner consisted of a standard amount of soup, potatoes, vegetables (peas and carrots, runner beans, string beans; alternately), minced meat and gravy, and fruit plus yogurt. Compliance with the protocol was checked by a daily questionnaire. Body weight was determined twice weekly, and energy supply was adjusted without changing macronutrient composition, in order to maintain body weight. Medication use was checked by a weekly questionnaire. There were no reports of medication that could have affected the outcome.

The study was performed according to the ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines for good clinical practice, and was approved by an external medical ethics committee.

Analytical methods

At the end of each 3-week period of each treatment, blood samples were collected just before dinner ($t = -1$) and 3, 5, 9, 13, and 15 h after starting dinner. The t = 13-h sample represents the values indicated in this article as "fasting." Pre- and postprandial samples were collected in order to detect acute, transient effects of alcohol intake. Blood was taken from the antecubital vein and collected in tubes containing EDTA or clot activator. To obtain serum and EDTA plasma, blood was centrifuged for 20 min at 2,000 *g* and 4°C. Serum or plasma was separated, snap frozen, and stored at −80°C until analysis.

HDL was separated from the apoB fraction by precipitation of the latter with polyethylene glycol. Serum total and HDL cholesterol (cholesterol oxidase/peroxidase-amidopyrine method) and triacylglycerols (glycerol phosphate oxidase/peroxidase-amidopyrine method), as well as unesterified cholesterol, triacylglycerol, and phospholipids in HDL were analyzed enzymatically. The coefficients of variation within runs were 0.8% for total cholesterol, 1.5% for triacylglycerol, and 1.3% for HDL cholesterol. Cholesteryl esters were calculated as the difference between HDL free cholesterol and total HDL cholesterol. LDL cholesterol was calculated by the equation of Friedewald, Levy, and Fredrickson (24). ApoA-I and apoB were analyzed in serum by an immunoturbidimetric assay (Tina-quant® kit apoA-I, Tina-quant® kit apoB; Boehringer, Mannheim, Germany) on a Hitachi 911 analyzer. The coefficients of variation within runs were 1.1% for apoA-I and 0.6% for apoB.

Cellular cholesterol efflux was measured from Fu5AH cells as described by de la Llera Moya et al. (25). In short, Fu5AH cells (kindly donated by V. Atger, Laboratoire de Biochimie, Hôpital Broussais, Paris, France) were grown to confluency in the presence of [^{3}H]cholesterol. After removal of medium containing the labeled cholesterol, the cells were allowed to equilibrate for 24 h. Subsequently cholesterol efflux was measured in triplicate over 4 h in the presence of 20-fold diluted plasma samples. Cholesterol efflux (radiolabel present in the culture medium after 4 h), is expressed as a percentage of the radioactivity initially present in the cells (fractional efflux). Data were corrected for blanks, that is, the amount of label in the medium after 4 h in the absence of plasma. The fractional cholesterol efflux is a measure of the capacity of a specific plasma sample to remove labeled cholesterol from the cell membrane. Cholesterol efflux was measured with either plasma or serum, and no differences between both measures were observed. Only plasma values are presented. The within-assay coefficient of variation of the cholesterol efflux assay is 5.5%.

Plasma EST rates were measured after addition of [^{3}H]cholesterol to total plasma. After 1 h of incubation at 37°C, lipids were extracted and [^{3}H]cholesteryl esters were isolated as described (26). The reaction is linear with time for >3 h. The within-assay coefficient of variation is 5.4%.

Statistical analyses

Data were tested for normality and compared by analysis of variance. Treatment effects were evaluated by comparing fasting values. Only if the overall F test yielded a significant ($\alpha = 0.05$) treatment effect were differences between treatments tested for significance. If the outcome measures of the beer, wine, and spirits treatments did not differ, the individual values for those three treatments were averaged, compared with water, and referred to as the effect of alcohol.

RESULTS

Blood alcohol concentrations (BAC)

Mean BAC were zero before alcohol intake. Mean BAC increased to 9.9 mM (range, 5.2 to 17.6 mM) 1 h after alcohol intake and decreased to 5.4 mM (range, 0.5 to

TABLE 1. Blood alcohol concentrations (BAC) before and after dinner, after 3 weeks of treatment

Hours after Beverage Intake	Type of Beverage							
	Water		Beer		Wine		Spirits	
	Mean BAC	Range	Mean BAC	Range	Mean BAC	Range	Mean BAC	Range
0	0.0	0.0–0.1	0.0	0.0–0.1	0.0	0.0–0.1	0.0	0.0–0.1
1	0.0	0.0–0.1	9.1	5.2–12.0	9.5	6.5–13.1	11.0[a]	6.7–17.6
3	0.0	0.0–0.1	4.4	0.5–7.1	5.6[a]	2.3–8.0	6.3[b]	3.8–9.3

BAC expressed in millimolar units.
[a] Denotes a significant difference from beer of $P < 0.05$.
[b] Denotes a significant difference from beer of $P < 0.01$.

9.3 mM) 3 h after alcohol intake. Only small differences in BAC were observed after consumption of red wine, beer, or spirits (**Table 1**); the higher the alcohol content of the beverage, the higher the BAC 1 and 3 h after intake.

In the following, the average values of the three alcoholic beverages are first described and referred to as the effect of alcohol consumption. Second, the effects of the separate alcoholic beverages beer, wine, and spirits are described.

Cellular cholesterol efflux

Alcohol consumption resulted in an increase in fasting cellular cholesterol efflux of 6.2% ($P < 0.0001$). Increases were evident for all individuals (**Fig. 1**). Red wine, beer, and spirits increased cellular cholesterol efflux by 5.0% ($P = 0.004$), 6.9% ($P = 0.0002$), and 6.0% ($P = 0.0009$), respectively, as compared with mineral water, and there were no significant differences on cellular cholesterol efflux between beverages.

HDL lipids and apoA-I

Alcohol consumption increased fasting HDL cholesterol by 13.6% ($P < 0.0001$), HDL cholesteryl ester by 14.6% ($P < 0.0001$), HDL free cholesterol by 11.7% ($P = 0.004$), HDL phospholipids by 13.0% ($P < 0.0001$), and apoA-I by 11.9% ($P < 0.0001$). These parameters were also increased when all three alcoholic beverages were compared with mineral water, separately (**Table 2**). These increases did not differ between red wine, beer, and spirits. Fasting HDL triacylglycerol concentrations did not change.

Fasting serum total and LDL cholesterol, apoB, and triacylglycerol concentrations were not affected by 3 weeks of alcohol consumption or by consumption of red wine, beer, and spirits separately (Table 2).

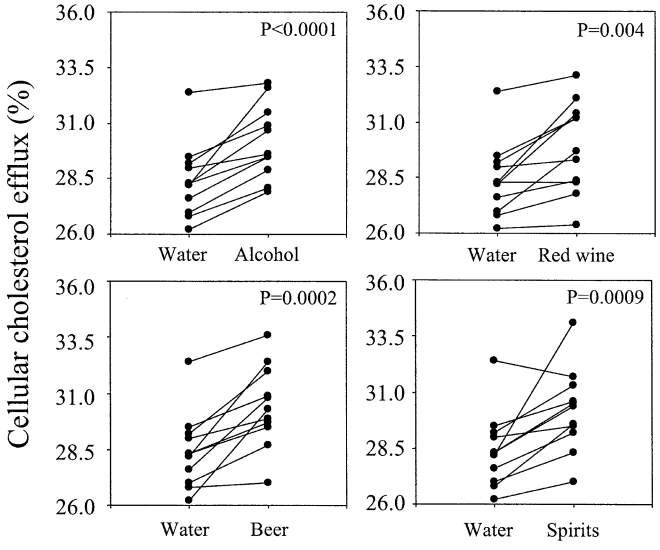

Fig. 1. Individual plasma ex vivo cellular cholesterol efflux capacity after consumption of alcohol for 3 weeks [(red wine + beer + spirits)/3], red wine, beer, or spirits (40 g of alcohol per day) as compared with mineral water.

EST

Alcohol consumption increased fasting plasma EST by 10.8% (86.2 ± 25.1 nmol/ml per h) as compared with water consumption (77.8 ± 28.5 nmol/ml per h; $P = 0.008$). EST was significantly increased by 12.3% ($P = 0.008$) and 13.4% ($P = 0.009$) after consumption of beer and spirits, respectively, as compared with mineral water, but this increase did not reach statistical significance after consumption of red wine (+6.6%; $P = 0.16$). No significant differences between alcoholic beverages were observed (data not shown).

Postprandial changes in serum HDL cholesterol, HDL cholesteryl ester, HDL free cholesterol, HDL phospholipids, HDL triacylglycerol, and plasma EST

Postprandial changes in HDL lipids and EST after consumption of alcoholic beverages are shown in **Fig. 2**. Overall, HDL cholesterol concentrations decreased until 9 h after evening dinner and increased thereafter. Alcohol consumption increased HDL cholesterol concentrations at all postprandial time points as compared with water consumption ($P < 0.0001$). Alcohol also consistently increased HDL cholesteryl ester ($P < 0.0001$) and HDL free cholesterol concentrations ($P < 0.05$) except at 15 h ($P = 0.14$) after dinner. HDL phospholipid concentrations decreased after dinner, and postprandial values were consistently higher after alcohol consumption as compared with water ($P < 0.0001$). Alcohol consumption increased plasma HDL triacylglycerol concentrations at 5 h ($P < 0.0001$) and 9 h ($P = 0.01$) after dinner as compared with water consumption.

Postprandial changes in HDL lipids hardly differed between the alcoholic beverages (Fig. 2). Three hours after dinner, HDL free cholesterol was significantly increased after red wine only ($P = 0.007$). HDL triacylglycerol was

TABLE 2. Fasting plasma HDL lipids, apoA-I, total cholesterol, LDL cholesterol, apoB, and triacylglycerol after 3 weeks of consumption of red wine, beer, spirits (40 g of alcohol per day), and mineral water

	Red Wine		Beer		Spirits			
	Mean ± SD	% Change	Mean ± SD	% Change	Mean ± SD	% Change	Water Mean ± SD	P^a
HDL total cholesterol	1.25 ± 0.24[b]	12.6	1.24 ± 0.20[b]	11.7	1.28 ± 0.23[c]	15.3	1.11 ± 0.22	<0.0001
HDL cholesteryl ester	1.02 ± 0.20[b]	13.3	1.00 ± 0.16[b]	11.1	1.05 ± 0.18[c]	16.7	0.90 ± 0.17	<0.0001
HDL free cholesterol	0.24 ± 0.06[b]	14.3	0.23 ± 0.06[d]	9.5	0.23 ± 0.06[d]	9.5	0.21 ± 0.06	0.004
HDL triacylglycerol	0.15 ± 0.04	7.1	0.15 ± 0.04	7.1	0.14 ± 0.04	0.0	0.14 ± 0.04	0.44
HDL phospholipids	1.54 ± 0.22[c]	13.2	1.51 ± 0.16[e]	11.0	1.56 ± 0.15[c]	14.7	1.36 ± 0.13	<0.0001
ApoA-I	1.30 ± 0.11[c]	11.1	1.30 ± 0.09[c]	11.1	1.31 ± 0.10[c]	12.0	1.17 ± 0.08	<0.0001
Total cholesterol	4.73 ± 0.71	−0.2	4.88 ± 0.87	3.0	4.85 ± 0.94	2.3	4.74 ± 0.87	0.52
LDL cholesterol	2.93 ± 0.70	5.2	3.10 ± 0.85	0.3	3.05 ± 0.93	−1.3	3.09 ± 0.83	0.30
ApoB	0.99 ± 0.18	−1.0	1.03 ± 0.21	3.0	1.02 ± 0.24	2.0	1.00 ± 0.20	0.82
Triacylglycerol	1.30 ± 0.58	0.8	1.30 ± 0.66	0.8	1.26 ± 0.76	−2.3	1.29 ± 0.93	0.94

Lipids are given as millimoles per liter and apolipoproteins A-I and B as grams per liter.
[a] Probability for the comparison between alcohol [(red wine + beer + spirits)/3] and water.
[b] Denotes a significant difference from water of $P < 0.01$.
[c] Denotes a significant difference from water of $P < 0.0001$.
[d] Denotes a significant difference from water of $P < 0.05$.
[e] Denotes a significant difference from water of $P < 0.001$.

increased at 9 h after dinner with beer ($P = 0.01$) and red wine ($P = 0.003$) only, and at 15 h after dinner with red wine ($P = 0.04$).

EST decreased with time after dinner and alcohol consumption increased postprandial EST at 3, 9, and 13 h after dinner ($P < 0.05$) as compared with water consumption. Spirits increased EST before dinner ($P = 0.02$), 3 h after dinner ($P = 0.003$), and 13 h after dinner ($P = 0.009$). In addition, EST was increased 13 h after dinner with beer ($P = 0.008$) as compared with water.

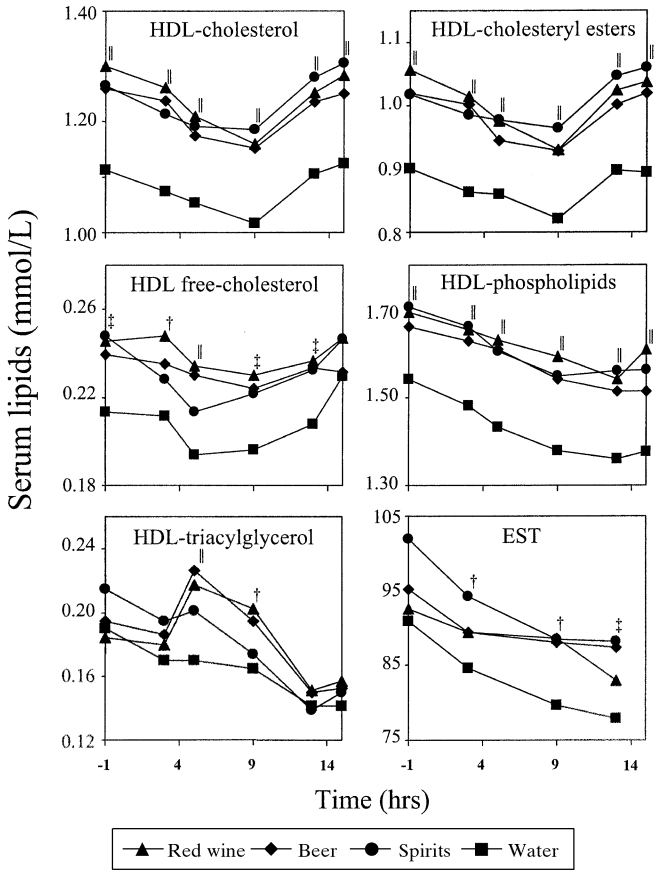

Fig. 2. Postprandial changes in HDL lipids and plasma EST rates after 3 weeks of consumption of red wine, beer, or spirits (40 g of alcohol per day) as compared with mineral water. † Significant difference between alcohol and water, $P < 0.05$; ‡ significant difference between alcohol and water, $P < 0.01$; ‖ significant difference between alcohol and water, $P < 0.0001$.

DISCUSSION

The present study shows that cellular cholesterol efflux is increased by 6.2% and EST by 10.8% after 3 weeks of alcohol consumption in healthy middle-aged men. **Figure 3** schematizes the effects of alcohol consumption on the first two steps of the RCT pathway and on HDL lipids. EST, apoA-I, HDL cholesterol, and HDL phospholipids are consistently higher after alcohol consumption as compared with water consumption. These increases are independent of the kind of alcoholic beverage (red wine, beer, or spirits), which suggests that effects were due to alcohol rather than to other compounds of alcoholic drinks.

Alcoholic beverages were supplied with evening dinner, representing the habitual pattern of alcohol intake in the Netherlands. The measurements in the fasting

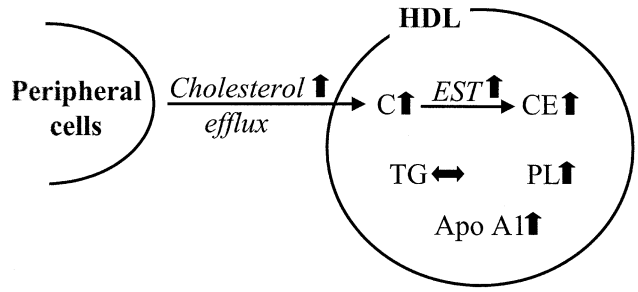

Fig. 3. Effects of alcohol consumption on early steps in RCT (cellular cholesterol efflux and plasma EST, and on plasma HDL lipids. C, Free cholesterol; CE, cholesteryl ester; TG, triacylglycerol; PL, phospholipids; up arrow, positive effects of alcohol; double arrow, no effect of alcohol.

state, which were performed after 3 weeks of beverage consumption, are indicative of the steady state effects of chronic consumption of red wine, beer, and spirits. The present study was fully diet controlled and had a balanced cross-over design. The observed elevated cellular cholesterol efflux and EST can therefore not be attributed to carry-over effects, variation between individuals or to dietary confounding.

The increased cellular cholesterol efflux after alcohol consumption was evident in almost all individuals and with all three alcoholic beverages. These findings do not agree with previous in vitro and animal studies. Chronic feeding of rats with high quantities of alcohol inhibits the capacity of HDL to induce cholesterol efflux from mouse macrophage (27). Also, high concentrations of alcohol in vitro inhibit cholesterol efflux from rat fibroblasts to human plasma HDL and to apoA-I complexed with phosphatidylcholine (28). However, Senault et al. (29) suggested an increased cholesterol efflux from Fu5AH cells to serum after red wine consumption.

Cellular cholesterol efflux is one of the first steps of the reverse cholesterol pathway. Two mechanisms have been proposed for cholesterol efflux (15). First, cholesterol is desorbed from the plasma membrane to phospholipid-containing acceptors such as HDL. Second, the ATP-binding cassette transporter protein 1 (ABC1) may mediate cellular cholesterol and phospholipid efflux, directing cholesterol and phospholipids to lipid-poor pre-β-HDL particles by direct interaction of ABC1 with apoA-I (30). In this study, we did not investigate ABC1, but we found increased levels of apoA-I after alcohol consumption. In addition, plasma levels of HDL phospholipids, which seem to be major determinants of cholesterol efflux (31), were found to be increased after alcohol consumption in this study. Similar increases were reported previously (10, 32, 33).

The mechanism whereby alcohol increases HDL lipid levels is not clear. Both hepatic lipase and CETP activity may be involved. Hepatic lipase activity is reported to decrease within several hours after alcohol consumption (34, 35). De Oliveira et al. (36) reported an 8% decrease in hepatic lipase, as well as a 23% increase in lipoprotein lipase, after consumption of vodka over 4 weeks. Others, however, did not find any changes in lipase activities after chronic alcohol consumption (37). Also, several studies (10, 38–41) have shown that alcohol consumption does not affect CETP activities, and therefore no changes in CETP activity were expected in the present study. Alternatively, one study showed that alcohol consumption increases the transport rates of both apoA-I and apoA-II, without affecting their fractional catabolic rate (36). An increased transport rate is most likely due to increased hepatic production of these apolipoproteins (36, 42, 43).

In the present study we did not investigate whether the increase in HDL cholesterol is due to effects on HDL_2 or HDL_3 subfractions. Gaziano et al. (44) showed that total HDL as well as its HDL_2 and HDL_3 subfractions were strongly associated with alcohol consumption and both subfractions were associated with decreased risk of myocardial infarction (44, 45).

After cholesterol efflux from cells to plasma, the free cholesterol from the cell is esterified by lecithin:cholesterol acyltransferase and incorporated into HDL particles (14). We found increased rates of plasma EST after 3 weeks of beer, spirits, and overall alcohol consumption, but not after red wine consumption. These findings suggest that, in addition to cellular cholesterol efflux, alcohol stimulates a second step in RCT as well. Earlier we reported a tendency for increased EST in the postprandial phase due to red wine consumption with dinner (40) and increased levels of LCAT activity 9 h after dinner with red wine or spirits (46). Here we report increases both in EST and in apoA-I. EST may be affected by apoA-I as well: Schuler-Lüttmann et al. (47) reported that apoA-I may determine esterification capacity.

Others have shown that disease state and diet affect RCT. Cellular cholesterol efflux is lower in obese subjects (by 34%) (48), in subjects with diabetes (by 7%) and coronary artery disease (by 7%) (49), and in patients with primary hypertriglyceridemia and low HDL cholesterol levels (by about 30%) (50) as compared with healthy subjects. Studies investigating effects of diets on cholesterol efflux are rather scarce. Blanco-Molina et al. (51) reported that a high cholesterol diet increased the cholesterol efflux by 8%. In addition, dietary oleic acid increased cellular cholesterol efflux to postprandial serum (52). In the present study, cholesterol efflux to plasma or serum was found to be increased by 6.2% after alcohol consumption ($P < 0.0001$).

To summarize, we found increased capacity of plasma to induce cellular cholesterol efflux and increased plasma EST rates, as well as increased concentrations of apoA-I, HDL cholesteryl ester, HDL free cholesterol, and HDL phospholipid after alcohol intake for 3 weeks in healthy men. In addition, postprandial HDL cholesterol and HDL phospholipid levels, and EST rates, were consistently higher after alcohol consumption as compared with water consumption. These findings suggest that alcohol consumption may increase early steps in the RCT pathway (cellular cholesterol efflux and plasma EST), independent of the type of alcoholic beverage (red wine, beer, or spirits) consumed.

Dr. Natalie Fournier (Laboratoire de Biochimie, Hôpital Broussais, Paris, France) is thanked for advice during the cholesterol efflux experiments. We thank Cora Groffen and Inge Lankhuizen for technical assistance.

Manuscript received 9 March 2001, in revised form 6 July 2001, and in re-revised form 10 September 2001.

REFERENCES

1. Colditz, G. A., L. G. Branch, R. J. Lipnick, W. C. Willett, B. Rosner, B. Posner, and C. H. Hennekens. 1985. Moderate alcohol and decreased cardiovascular mortality in an elderly cohort. *Am. Heart J.* **109:** 886–889.
2. Stampfer, M. J., G. A. Colditz, W. C. Willett, F. E. Speizer, and C. H. Hennekens. 1988. A prospective study of moderate alcohol consumption and the risk of coronary disease and stroke in women. *N. Engl. J. Med.* **319:** 267–273.
3. Grobbee, D. E., E. B. Rimm, U. Keil, and S. Renaud. 1999. Alcohol

and cardiovascular system. *In* Health Issues Related to Alcohol Consumption. E. I. MacDonald, editor. ILSI Europe, Blackwell Science, Philadelphia, PA. 125–179.
4. Rimm, E. B., P. Williams, K. Fosher, M. Criqui, and M. Stampfer. 1999. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. *Br. Med. J.* **319:** 1523–1528.
5. Criqui, M. H., L. D. Cowan, H. A. Tyroler, S. Bangdiwala, G. Heiss, R. B. Wallace, and R. Cohn. 1987. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking in cardiovascular mortality: results from the Lipids Research Clinics Follow-up Study. *Am. J. Epidemiol.* **126:** 629–637.
6. Castelli, W. P., T. Gordon, M. C. Hjortland, A. Kagan, J. T. Doyle, C. G. Hames, S. B. Hulley, and W. J. Zukel. 1977. Alcohol and blood lipids. The Cooperative Lipoprotein Phenotyping Study. *Lancet.* **2:** 153–155.
7. Van der Gaag, M. S., J. B. Ubbink, P. Sillanaukee, S. Nikkari, and H. F. J. Hendriks. 2000. Effect of consumption of red wine, spirits, and beer on serum homocysteine. *Lancet.* **355:** 1522.
8. Van Tol, A., and H. F. J. Hendriks. 2001. Moderate alcohol consumption: effects on lipids and cardiovascular disease risk. *Curr. Opin. Lipidol.* **12:** 19–23.
9. Van Tol, A., M. S. van der Gaag, L. M. Scheek, T. van Gent, and H. F. J. Hendriks. 1998. Changes in postprandial lipoproteins of low and high density caused by moderate alcohol consumption with dinner. *Atherosclerosis.* **141:** S101–S103.
10. Hendriks, H. F. J., J. Veenstra, A. van Tol, J. E. M. Groener, and G. Schaafsma. 1998. Moderate doses of alcoholic beverages with dinner and postprandial high density lipoprotein composition. *Alcohol Alcohol.* **33:** 403–410.
11. Van der Gaag, M. S., A. van Tol, L. M. Scheek, R. W. James, R. Urgert, G. Schaafsma, and H. F. J. Hendriks. 1999. Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomised intervention study in middle-aged men. *Atherosclerosis.* **147:** 405–410.
12. Mackness, M. I., A. Arrol, B. Mackness, and P. N. Durrington. 1997. Alloenzymes of paraoxonase and effectiveness of high-density lipoproteins in protecting low-density lipoprotein against lipid peroxidation. *Lancet.* **349:** 851–852.
13. Mackness, B., M. I. Mackness, S. Arrol, W. Turkie, and P. N. Durrington. 1998. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. *FEBS Lett.* **423:** 57–60.
14. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
15. Rothblat, G. H., M. de la Llera-Moya, V. Atger, G. Kellner-Weibel, D. L. Williams, and M. C. Phillips. 1999. Cell cholesterol efflux: integration of old and new observations provides new insights. *J. Lipid Res.* **40:** 781–796.
16. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
17. Tall, A. R. 1993. Plasma cholesteryl ester transfer protein. *J. Lipid Res.* **34:** 1255–1274.
18. Schneider, W. J. 1991. Removal of lipoproteins from plasma. *In* Biochemistry of Lipids, Lipoproteins and Membranes. D. E. Vance and J. Vance, editors. Elsevier, Amsterdam. 461–487.
19. Pitas, R. E., T. L. Innerarity, K. S. Arnold, and R. W. Mahley. 1979. Rate and equilibrium constants for binding of apoE HDL_c (a cholesterol-induced lipoprotein) and low density lipoproteins to human fibroblasts: evidence for multiple receptor binding of apoE HDL_c. *Proc. Natl. Acad. Sci. USA.* **76:** 2311–2315.
20. Pittmann, R. C., T. P. Knecht, M. S. Rosenbaum, and C. A. Taylor. 1987. A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters. *J. Biol. Chem.* **262:** 2443–2450.
21. Van der Gaag, M. S., R. van de Berg, H. van den Berg, G. Schaafsma, and H. F. J. Hendriks. 2000. Moderate consumption of beer, red wine and spirits has counteracting effects on plasma antioxidants in middle-aged men. *Eur. J. Clin. Nutr.* **54:** 586–591.
22. Schofield, W. N. 1985. Predicting basal metabolic rate, new standards and review of previous work. *Hum. Nutr. Clin. Nutr. C.* **39:** 5–41.
23. Rumpler, W. V., D. C. Rhodes, D. J. Baer, J. M. Conway, and J. L. Seale. 1996. Energy value of moderate alcohol consumption by humans. *Am. J. Clin. Nutr.* **64:** 108–114.
24. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
25. de la Llera Moya, M., V. Atger, J. L. Paul, N. Fournier, N. Moatti, P. Giral, K. E. Friday, and G. Rothblat. 1994. A cell culture system for screening human serum for ability to promote cellular cholesterol efflux. *Arterioscler. Thromb.* **14:** 1056–1065.
26. Dullaart, R. P., S. C. Riemens, L. M. Scheek, and A. van Tol. 1999. Insulin decreases plasma cholesteryl ester transfer but not cholesterol esterification in healthy subjects as well as in normoglyceridaemic patients with type 2 diabetes. *Eur. J. Clin. Invest.* **29:** 663–671.
27. Marmillot, P., M. N. Rao, Q-H. Liu, S. J. Chirtel, and M. R. Lakshman. 2000. Effect of dietary omega-3 fatty acids and chronic ethanol consumption on reverse cholesterol transport in rats. *Metabolism.* **49:** 505–512.
28. Avdulov, N. A., S. V. Chochina, U. Igbavboa, and W. G. Wood. 2000. Cholesterol efflux to high density lipoproteins and apolipoprotein A-1 phosphatidylcholine complexes is inhibited by ethanol: role of apolipoprotein structure and cooperative interaction of phosphatidylcholine and cholesterol. *Biochemistry.* **39:** 10599–10606.
29. Senault, C., D. Betoulle, G. Luc, P. Hauw, D. Rigaud, and F. Fumeron. 2000. Beneficial effects of a moderate consumption of red wine on cellular cholesterol efflux in young men. *Nutr. Metab. Cardiovasc. Dis.* **10:** 63–69.
30. Wang, N., D. L. Silver, P. Costet, and A. R. Tall. 2000. Specific binding of apoA-I, enhanced cholesterol efflux and altered plasma membrane morphology in cells expressing ABC1. *J. Biol. Chem.* **275:** 33053–33058.
31. Fournier, N., J. L. Paul, V. Atger, A. Cogny, T. Soni, M. de la Llera-Moya, G. H. Rothblat, and N. Moatti. 1997. HDL phospholipid content and composition as a major factor determining cholesterol efflux capacity from Fu5AH cells to human serum. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2685–2691.
32. Van der Gaag, M. S., A. Sierksma, G. Schaafsma, A. van Tol, T. Geelhoed-Mieras, M. Bakker, and H. F. J. Hendriks. 2000. Moderate alcohol consumption and changes in postprandial lipoproteins of premenopausal and postmenopausal women: a diet-controlled, randomized intervention study. *J. Women's Health Gender-Based Med.* **9:** 607–616.
33. Hendriks, H. F. J., M. R. T. van Haaren, R. Leenen, and G. Schaafsma. 2001. Moderate alcohol consumption and postprandial plasma lipids in men with different risks for coronary heart disease. *Alcohol Clin. Exp. Res.* **25:** 563–570.
34. Goldberg, C. S., A. R. Tall, and S. Krumholz. 1984. Acute inhibition of hepatic lipase and increase in plasma lipoproteins after alcohol intake. *J. Lipid Res.* **25:** 714–720.
35. Taskinen, M-R., M. Välimäki, E. A. Nikkilä, T. Kuusi, and R. Ylikahri. 1985. Sequence of alcohol-induced initial changes in plasma lipoproteins (VLDL and HDL) and lipolytic enzymes in humans. *Metabolism.* **34:** 112–119.
36. De Oliveira e Silva, E. R., D. Foster, M. McGee Harper, C. E. Seidman, J. D. Smith, J. L. Breslow, and E. A. Brinton. 2000. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apolipoproteins A-I and A-II. *Circulation.* **102:** 2347–2352.
37. Välimäki, M., M-R. Taskinen, R. Ylikahri, R. Roine, T. Kuusi, and T. Nikkilä. 1988. Comparison of the effects of two different doses of alcohol on serum lipoproteins, HDL-subfractions and apolipoproteins A-I and A-II: a controlled study. *Eur. J. Clin. Invest.* **18:** 472–480.
38. Nishiwaki, M., T. Ishikawa, T. Ito, H. Shige, K. Tomiyasu, K. Nakajima, K. Kondo, H. Hashimoto, K. Saitoh, M. Manabe, E. Miyajima, and H. Nakamura. 1994. Effects of alcohol on lipoprotein lipase, hepatic lipase, cholesteryl ester transfer protein, and lecithin: cholesterol acyltransferase in high-density lipoprotein cholesterol elevation. *Atherosclerosis.* **111:** 99–109.
39. Riemens, S. C., A. van Tol, K. Hoogenberg, T. van Gent, L. M. Scheek, W. J. Sluiter, and R. P. F. Dullaart. 1997. Higher high density lipoprotein cholesterol associated with moderate alcohol consumption is not related to altered plasma lecithin:cholesterol acyltransferase and lipid transfer protein activity levels. *Clin. Chim. Acta.* **258:** 105–115.
40. Van Tol, A., J. E. M. Groener, L. M. Scheek, T. van Gent, J. Veenstra, H. van de Pol, H. F. J. Hendriks, and G. Schaafsma. 1995. Induction of net mass lipid transfer reactions in plasma by wine consumption with dinner. *Eur. J. Clin. Invest.* **25:** 390–395.

41. Ito, T., M. Nishiwaki, T. Ishikawa, and H. Nakamura. 1995. CETP and LCAT activities are unrelated to smoking and moderate alcohol consumption in healthy normolipidemic men. *Jpn. Circ. J.* **59:** 541–546.
42. Amarasuiya, R. N., A. K. Gupta, M. Civen, Y. C. Horng, T. Maeda, and M. L. Kashyap. 1992. Ethanol stimulates apolipoprotein A-I secretion by human hepatocytes: implications for a mechanism for atherosclerosis protection. *Metabolism.* **41:** 827–832.
43. Tam, S-P. 1992. Effect of ethanol on lipoprotein secretion in two human hepatoma cell lines, HepG2 and Hep3B. *Alcohol Clin. Exp. Res.* **16:** 1021–1028.
44. Gaziano, J. M., J. E. Buring, J. L. Breslow, S. Z. Goldhaber, B. Rosner, M. Vandenburgh, W. Willett, and C. H. Hennekens. 1993. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. *N. Engl. J. Med.* **329:** 1829–1834.
45. Buring, J. E., G. T. O'Connor, S. Z. Goldhaber, B. Rosner, P. N. Herber, C. B. Blum, J. L. Breslow, and C. H. Hennekens. 1992. Decreased HDL_2 and HDL_3 cholesterol, apoAI and apoAII, and increased risk of myocardial infarction. *Circulation.* **85:** 22–29.
46. Hendriks, H. F. J., J. Veenstra, A. van Tol, J. E. M. Groener, and G. Schaafsma. 1998. Moderate doses of alcoholic beverages with dinner and postprandial high density lipoprotein composition. *Alcohol Alcohol.* **33:** 403–410.
47. Schuler-Lüttmann, S., Y. Zhu, M. Hoffmann, W. März, G. Feussner, H. Wieland, G. Assmann, and A. von Eckardstein. 2000. Cholesterol efflux from normal and Tangier disease fibroblasts into normal, high-density lipoprotein-deficient, and apolipoprotein E-deficient plasmas. *Metabolism.* **49:** 770–777.
48. Sasahara, T., P. Nestel, N. Fidge, and D. Sviridov. 1998. Cholesterol transport between cells and high density lipoprotein subfractions from obese and lean subjects. *J. Lipid Res.* **39:** 544–554.
49. Syvänne, M., G. Castro, C. Dengremont, C. De Geitere, M. Jauhiainen, C. Ehnholm, S. Michelagnoli, G. Franceschini, J. Kahri, and M-R. Taskinen. 1996. Cholesterol efflux from Fu5AH hepatoma cells induced by plasma of subjects with or without coronary artery disease and non-insulin-dependent diabetes: importance of LpA-I: A-II particles and phospholipid transfer protein. *Atheroclerosis.* **127:** 245–253.
50. Brites, F. D., C. D. Bonavita, C. De Geitere, M. Cloës, B. Delfly, M. J. Yael, J-C. Fruchart, R. W. Wikinski, and G. R. Castro. 2000. Alterations in the main steps of reverse cholesterol transport in male patients with primary hypertriglyceridemia and low HDL-cholesterol levels. *Atherosclerosis.* **152:** 181–192.
51. Blanco-Molina, A., G. Castro, D. Martín-Escalante, D. Bravo, J. López-Miranda, P. Castro, F. López-Segura, J. C. Fruchart, J. M. Ordovás, and F. Pérez-Jimenéz. 1998. Effects of different dietary cholesterol concentrations on lipoprotein plasma concentrations and on cholesterol efflux from Fu5AH cells. *Am. J. Clin. Nutr.* **68:** 1028–1033.
52. Sakr, S. W., C. Senault, S. Vacher, N. Fournier, and A. Girard-Globa. 1996. Oleic acid-rich fats increase the capacity of postprandial serum to promote cholesterol efflux from Fu5AH cells. *Biochim. Biophys. Acta.* **1300:** 49–55.