Beyond HDL-cholesterol increase: phospholipid enrichment and shift from HDL_3 to HDL_2 in alcohol consumers

Christian Schäfer,[1,*] Alexandr Parlesak,[†] Joachim Eckoldt,[†] Christiane Bode,[†] Johann Christian Bode,[*] Winfried März,[§] and Karl Winkler[**]

Division of Gastroenterology,* Robert-Bosch-Hospital Stuttgart, Stuttgart, Germany; Department of Physiology of Nutrition,† Hohenheim University, Stuttgart, Germany; Synlab Center of Laboratory Diagnostics,§ Heidelberg, Germany; and Department of Medicine,** University of Freiburg, Freiburg, Germany

Abstract The reduction of cardiovascular mortality associated with moderate alcohol consumption is chiefly thought to be mediated by an increase of high density lipoprotein cholesterol (HDL-CH). This study highlights additional qualitative changes of HDL that might augment this antiatherogenic effect. In 279 healthy men, alcohol and nutrient consumption were evaluated. Groups 1 (n = 62), 2 (n = 172), and 3 (n = 45) comprised subjects with alcohol consumption of 0–5.0, 5.1–30.0, and 30.1–75 g/day, respectively. Lipid analysis was performed in nonfractionated and fractionated plasma, including subfractions HDL_{2a}, HDL_{2b}, and HDL_3. No difference in LDL-cholesterol was observed. Compared with group 1, groups 2 and 3 exhibited significant increases of HDL-CH (group 1, 44 ± 10 mg/dl; group 2, 51 ± 11 mg/dl; group 3, 55 ± 11 mg/dl; mean ± SD, $P < 0.0005$), accompanied by enhanced lipidation of HDL (increase of the HDL_2-CH/HDL_3-CH ratio). Moreover, phospholipid enrichment of HDL occurred in alcohol consumers, whereas the ratios between other HDL components remained constant. Multivariate analysis revealed alcohol to have the foremost statistical influence on changes of the HDL fraction, followed by body mass index and physical activity level. The increased lipidation of HDL found in alcohol consumers might augment the antiatherogenic effect of HDL-CH increase. In addition, the phospholipid enrichment of HDL might reduce the inflammatory response of atherogenesis.—Schäfer, C., A. Parlesak, J. Eckoldt, C. Bode, J. C. Bode, W. März, and K. Winkler. **Beyond HDL-cholesterol increase: phospholipid enrichment and shift from HDL_3 to HDL_2 in alcohol consumers.** ***J. Lipid Res.*** **2007.** 48: **1550–1558.**

Supplementary key words low density lipoprotein • very low density lipoprotein • high density lipoprotein 2 • high density lipoprotein 3 • triglycerides • beer • wine • body mass index • apolipoprotein

Moderate regular alcohol consumption (5–30 g/day in men, 5–20 g/day in women) has been associated with a reduction of overall mortality attributable to a decreased incidence of ischemic heart disease and stroke (1–4). Among the antiatherosclerotic mechanisms discussed, the alcohol-induced increase of HDLs is thought to play a pivotal role in reducing atherosclerosis by increasing reverse cholesterol transport from the vessel walls to the liver (5).

This cross-sectional study in healthy working men was performed to further examine the effect of various alcohol consumption patterns on the lipid profile. Our working hypothesis was that moderate alcohol consumption induces not only quantitative, but also qualitative, changes of the lipid fractions, in particular HDL. Using extensive fractionation of plasma lipids and measurement of lipid and apolipoprotein components, we show that alcohol consumption is associated with increases of cholesterol and other components of the HDL fraction. In addition, two significant qualitative changes were identified that might affect atherogenicity: a shift from small, dense HDL_3 to lipid-rich HDL_2 (mainly HDL_{2a}), and a significant phospholipid (PL) enrichment of HDL in all subfractions.

Because not only alcohol consumption but also lifestyle factors, such as nutrition, physical activity, smoking, age, and obesity, may influence lipid metabolism, we performed a thorough interview to characterize the influence of these factors on HDL. Furthermore, it was examined whether consumption patterns (i.e., preference for beer or

Manuscript received 3 May 2006 and in revised form 4 December 2006 and in re-revised form 28 February 2007.

Published, JLR Papers in Press, April 12, 2007.
DOI 10.1194/jlr.M600513-JLR200

Abbreviations: apoA-I, apolipoprotein A-I; BMI, body mass index; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FC, free cholesterol; HDL-CH, high density lipoprotein-cholesterol; LDL-CH, low density lipoprotein-cholesterol; PAL, physical activity level; PL, phospholipid; PLTP, phospholipid transfer protein; SM, sphingomyelin; TG, triglyceride.

[1] To whom correspondence should be addressed.
e-mail: christian.schaefer@rbk.de

Copyright © 2007 by the American Society for Biochemistry and Molecular Biology, Inc.

This article is available online at http://www.jlr.org

Downloaded from www.jlr.org by guest, on June 14, 2012

wine) had differential effects on high density lipoprotein-cholesterol (HDL-CH), because the data from several epidemiological studies had been inconclusive (reviewed in Refs. 6, 7).

SUBJECTS AND METHODS

Study subjects

This study, which followed the guidelines of the Declaration of Helsinki of 1975, as revised in 1983, was approved by the Ethical Review Board of the University of Tübingen. After providing informed consent, 290 healthy male working volunteers, aged 20–70 years, were recruited at their work place (63% automobile industry, 12% wine factory, 18% brewery, 7% health care staff and others). At the first date of examination, blood was obtained after an overnight fast for biochemical testing. Soon thereafter, a 1 hour personal computer-based interview was performed by an experienced nutritional scientist to determine the nutritional profile, including alcohol consumption and energy expenditure.

Of the 290 recruited persons, 11 were excluded for various reasons: self-reported previous myocardial infarction or ischemic stroke (n = 2), heavy (⩾75 g/day) or highly irregular alcohol consumption (n = 6), unreliable diet history (n = 1), and anti-hyperlipidemic medication (n = 2).

The remaining 279 individuals were grouped according to their amount of daily alcohol consumption. Group 1 comprised 62 persons with an alcohol consumption of ⩽5 g/day, 172 persons with moderate alcohol consumption of >5 and ⩽30 g/day were assigned to group 2, and 45 males with increased alcohol consumption of >30 and <75 g/day were assigned to group 3. Of group 1, 14 persons were teetotalers. To characterize the consumption patterns of groups 2 and 3, we defined a preference for beer as >80% alcohol calories consumed as beer and, likewise, a preference for wine versus mixed consumption (not meeting any of these criteria).

Evaluation of nutrition and physical activity

To evaluate the nutritional profile of each subject, an experienced interviewer carefully reviewed the intake of food and beverages (including alcoholic beverages) during the past 7 days using a computer-based system, which provided photographs for the assessment of portion sizes. The accuracy of the interview data compared with the actual food intake had been validated previously (8). Nutrient intake was analyzed using the German Nutrient Data Base (Bundeslebensmittelschlüssel), comprising 150 nutritional variables for 11,000 food items. In addition, the physical activity level (PAL) and other lifestyle features were evaluated based on interview data. The plausibility of the total caloric intake was controlled for by calculating the energy demand (ED) for each individual depending on the resting energy level (REE) (9) and PAL: ED = (REE + PAL) × 1.06.

Laboratory analyses

The obtained blood was quickly transported to the laboratory of the Robert-Bosch-Hospital, where analyses of routine parameters were performed using standard equipment. EDTA plasma samples were shipped at ambient temperature to the Department of Clinical Chemistry, University of Freiburg, where lipoprotein metabolism and lipoprotein subfractions were analyzed by sequential preparative ultracentrifugation (10).

Briefly, VLDL (d < 1.006 g/ml), LDL (d = 1.019–1.063 g/ml), and HDL (d = 1.063–1.21 g/ml) were isolated by sequential preparative ultracentrifugation from 5 ml of plasma (50.3 Ti rotor, L8-55M centrifuge; Beckman Instruments). Analogously, HDL subfractions were obtained: HDL_{2b} (d = 1.063–1.100 g/ml), HDL_{2a} (d = 1.100–1.150 g/ml), and HDL_3 (d = 1.150–1.210 g/ml). Lipids in each (sub)fraction were determined as follows (10). Cholesterol and triglycerides (TGs) were determined enzymatically with the cholesterol oxidase-peroxidase amino phenazone phenol and the glycerol-3-phosphate oxidase-peroxidase amino phenazone phenol methods (Roche Diagnostics, Mannheim, Germany), respectively. Free cholesterol (FC) and PLs were determined enzymatically with the cholesterol oxidase-peroxidase amino phenazone phenol method and by phospholipase D, cholinoxidase, and peroxidase, respectively, with commercially available reagents (Wako Chemicals, Osaka, Japan). The concentration of esterified cholesterol was calculated from the difference of total cholesterol and FC. In addition, apolipoproteins A-I and A-II (apoA-I and apoA-II) were determined immunoturbidimetrically [anti-apoA-I from Greiner (Flacht, Germany) and anti-apoA-II from Kamiya Biomedical (Seattle, WA)]. This comprehensive analysis was performed on each individual sample.

Statistics

Unless indicated otherwise, results are reported as means ± SD. The levels of significance between groups 1, 2, and 3 were calculated using ANOVA with Tukey's honestly significant difference test for post hoc analysis. Prerequisites for ANOVA were normal distribution and homogeneity of variances; otherwise, a log transformation of the data was performed. If the conditions were not met, a Kruskal-Wallis test, followed by a Mann-Whitney *U*-test with Bonferroni correction for post hoc evaluation, was applied.

We also conducted a multivariate analysis of the influence of various factors on HDL components. The multiple linear regression model was calculated according to the formula $y = c_1x_1 + c_2x_2 + \ldots c_nx_n + a$ (where y is the dependent variable, $c_1 \ldots c_n$ are calculated coefficients, $x_1 \ldots x_n$ are independent variables, and a is the intercept). As independent variables, alcohol consumption, the influence of beer versus wine, anthropometric characteristics, calorie intake, and energy output were chosen. To compare beer versus wine, we determined the percentage of daily beer consumption [$RC_{beer\ vs.\ wine}(\%)$] in relation to the sum of beer and wine consumption, based on alcohol (EtOH) content [$EtOH_{beer} \times 100/(EtOH_{beer} + EtOH_{wine})$]. Drinks other than beer or wine were disregarded, because they were quantitatively unimportant (**Table 1**).

RESULTS

Characteristics of the study population

The basic anthropometric data, alcohol consumption, nutritional characteristics, energy expenditure, and essential laboratory parameters are listed in Table 1. There were no significant differences in age and body mass index (BMI) between groups 1 and 2, whereas persons in group 3 differed slightly, but significantly, in age and BMI. The proportion of smokers did not exceed 20% in each group. The comparison of drinking patterns showed a higher proportion of beer drinkers in group 3 and a high proportion of mixed consumption in group 2. According to regional drinking habits, hard liquors and beverages other than beer or wine comprised only a minor portion of the alcohol consumption. The nutritional profile, except for a lower intake of dietary fiber in group 3, and the data for

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 1. Basic anthropometric, nutritional, and laboratory characteristics of the groups

Variable	Group (Alcohol Consumption)		
	Group 1 (0–5 g/day)	Group 2 (5.1–30 g/day)	Group 3 (30.1–75 g/day)
Number	62	172	45
Age (years)	42.4 ± 8.7	43.2 ± 10.5	48.9 ± 10.2[a,b]
BMI (kg/m^2)	25.4 ± 3.5	24.7 ± 3.1	26.6 ± 3.9[b]
Smokers (⩾1 cigarette/day)	16.1%	9.3%[c]	20.0%[d]
Alcohol consumption			
Alcohol (kcal/d)	17 ± 11	113 ± 50[a]	317 ± 82[a,b]
Alcohol (g/d)	2.4 ± 1.5	15.9 ± 7.1[a]	44.6 ± 11.5[a,b]
In beer (g/d)	0.9 ± 1.2	7.3 ± 5.8[a]	24.4 ± 20.4[a,b]
In wine (g/d)	0.9 ± 1.2	7.5 ± 5.9[a]	18.9 ± 17.8[a,b]
In hard liquors/others (g/d)	0.6 ± 0.6	1.1 ± 1.6	1.3 ± 2.9
Consumption pattern (n)			
Abstainers	14	—	—
⩾80% beer	7	25	18
⩾80% wine	7	38	13
Mixed consumption	34	109	14
Nutritional intake			
Energy intake without alcohol (kcal)	2,640 ± 385	2,595 ± 440	2,565 ± 410
Protein (kcal)	410 ± 75	405 ± 75	405 ± 70
Carbohydrates (kcal)	1,215 ± 255	1,170 ± 260	1,140 ± 215
Lipids (kcal)	1,060 ± 215	1,065 ± 235	1,070 ± 245
Cholesterol (mg)	342 ± 104	349 ± 81	374 ± 89
Dietary fiber (g)	26 ± 8	25 ± 6	22 ± 7[c,d]
Energy output			
Resting energy expenditure (kcal)	1,780 ± 190	1,765 ± 185	1,780 ± 200
PAL (kcal)	885 ± 230	905 ± 240	955 ± 205
Energy demand (kcal)	2,820 ± 355	2,830 ± 380	2,895 ± 360
Laboratory profile			
Leukocytes (1,000/μl)	6.1 ± 1.6	5.7 ± 1.6	6.4 ± 1.7
Hematocrit (%)	45 ± 3	45 ± 2	45 ± 2
Mean corpuscular volume (fl)	88 ± 3	89 ± 3[c]	92 ± 4[a,b]
Platelets (1,000/μl)	247 ± 50	253 ± 60	247 ± 57
Glucose (mg/dl)	90 ± 14	89 ± 8	95 ± 16[b]
Creatinine (mg/dl)	0.97 ± 0.12	0.98 ± 0.12	0.95 ± 0.11
Aspartate aminotransferase (U/l)	12 ± 3	13 ± 4	14 ± 3[c]
Alanine aminotransferase (U/l)	17 ± 6	17 ± 8	19 ± 7
γ-Glutamyl transferase (U/l)	13 ± 7	14 ± 9	33 ± 43[a,b]
Alkaline phosphatase (U/l)	89 ± 21	84 ± 21	93 ± 19[d]
Bilirubin (mg/dl)	0.9 ± 0.5	0.9 ± 0.4	0.8 ± 0.3
Lipase (U/l)	108 ± 88	103 ± 61	95 ± 53
Creatinine kinase (U/l)	64 ± 47	64 ± 41	71 ± 52
Fibrinogen (mg/dl)	283 ± 46	282 ± 53	300 ± 68
C-reactive protein (mg/dl)	0.83 ± 0.47	0.89 ± 0.64	0.89 ± 0.90

BMI, body mass index; PAL, physical activity level. Values shown are means ± SD.
[a] $P < 0.005$ versus group 1.
[b] $P < 0.005$ versus group 2.
[c] $P < 0.05$ versus group 1.
[d] $P < 0.05$ versus group 2.

energy expenditure were comparable between all three groups. The evaluation of basic laboratory data showed no differences between groups 1 and 2, whereas in group 3, a significant increase of the markers for alcohol consumption (mean corpuscular volume and γ-glutamyl transferase) became evident.

Overall lipid analysis and lipid fractions

In nonfractionated plasma, plasma cholesterol, PLs, and TGs were similar between groups 1 and 2 (**Table 2**). Higher alcohol consumption (group 3) was associated with slightly increased cholesterol levels compared with group 1. TG concentrations exhibited great variability. In group 3, increased TG levels compared with group 2 were measured.

For HDL-CH, we found a significant increase in persons of groups 2 and 3 compared with group 1 (Table 2). The increase amounted to 16% in group 2 and 25% in group 3. Low density lipoprotein-cholesterol (LDL-CH) levels were nearly identical in all three groups. Because VLDL particles constitute the major vehicle for plasma TG, the differences between the groups resemble those observed for TG levels in nonfractionated plasma.

Changes in the composition of HDL (HDL_2 vs. HDL_3)

The density of HDL particles is determined mainly by the lipid-to-protein ratio. Within HDL, the dense HDL_3 subfraction containing a low percentage of lipids can be separated from HDL_2, which contains intermediate HDL_{2a} and buoyant HDL_{2b}. Because the antiatherogenic effect of

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 2. Concentrations of cholesterol, PL, and TG in plasma and in HDL, LDL, and VLDL

Variable	Group (Alcohol Consumption) Group 1 (0–5 g/day)	Group 2 (5.1–30 g/day)	Group 3 (30.1–75 g/day)
Plasma			
Cholesterol	202 ± 33	205 ± 43	221 ± 34[a]
PL	213 ± 32	223 ± 37	249 ± 37
TG	140 ± 105	117 ± 76	166 ± 127[b]
Fractions			
HDL-CH	44 ± 10	51 ± 11[c]	55 ± 11[c]
HDL-PL	62 ± 14	75 ± 18[c]	86 ± 17[c,d]
HDL-TG	11 ± 4	13 ± 5	15 ± 6[c,e]
LDL-CH	112 ± 23	110 ± 30	109 ± 26
LDL-PL	74 ± 15	73 ± 20	74 ± 17
LDL-TG	22 ± 11	20 ± 9	23 ± 9[b]
VLDL-CH	28 ± 23	24 ± 22	36 ± 35[e]
VLDL-PL	30 ± 26	25 ± 20	38 ± 33[e]
VLDL-TG	89 ± 90	69 ± 66	107 ± 110[b]

HDL-CH, high density lipoprotein cholesterol; PL, phospholipid; TG, triglyceride. Values shown are means ± SD (mg/dl).
[a] $P < 0.05$ versus group 1.
[b] $P < 0.05$ versus group 2.
[c] $P < 0.0005$ versus group 1.
[d] $P < 0.0005$ versus group 2.
[e] $P < 0.005$ versus group 2.

each of these subfractions may differ, it was of interest to examine the association between alcohol consumption and the cholesterol content of each of these subfractions.

In each of the HDL subfractions, the cholesterol content was increased in alcohol consumers (groups 2 and 3) compared with group 1 (**Table 3**). However, differences between the dose responses of the HDL subfractions were observed. Comparing group 2 and group 1, HDL_{2b}-CH was increased by 24% and HDL_{2a}-CH was increased by 25%, whereas HDL_3-CH showed an increase of only 10%. In group 3, this dissociation was even more pronounced (HDL_{2b}-CH, 28%; HDL_{2a}-CH, 37%; HDL_3-CH, 11% increase vs. group 1).

Translated into ratios of HDL_{2a+2b}-CH to HDL_3-CH, and analogous ratios of PLs and TGs, a substantial shift toward the HDL_2 subfraction was measured (Table 3).

TABLE 3. Concentrations of cholesterol, PLs, and TGs in HDL subfractions 2b, 2a, and 3, and comparison of the balance between HDL_{2a+2b} and HDL_3, expressed as cholesterol, PL, and TG ratios

Variable	Group (Alcohol Consumption) Group 1 (0–5 g/day)	Group 2 (5.1–30 g/day)	Group 3 (30.1–75 g/day)
HDL subfractions			
HDL_{2b}-CH	12.0 ± 4.3	14.9 ± 6.0[a]	15.4 ± 5.4[b]
HDL_{2b}-PL	15.2 ± 5.8	19.9 ± 8.8[a]	22.1 ± 7.8[a]
HDL_{2b}-TG	3.4 ± 1.7	3.8 ± 2.1	4.8 ± 2.9[c]
HDL_{2a}-CH	15.1 ± 4.2	18.9 ± 4.4[a]	20.7 ± 4.5[a,d]
HDL_{2a}-PL	22.4 ± 7.0	28.7 ± 8.0[a]	33.6 ± 8.2[a,e]
HDL_{2a}-TG	3.8 ± 1.2	4.5 ± 1.8[c]	5.7 ± 2.2[a,f]
HDL_3-CH	14.4 ± 2.9	15.9 ± 2.9[b]	16.0 ± 3.5[c]
HDL_3-PL	21.3 ± 4.9	24.0 ± 4.6[a]	25.6 ± 5.3[a]
HDL_3-TG	3.4 ± 1.2	3.5 ± 1.2	3.9 ± 1.3
HDL_2 versus HDL_3 (ratios)			
HDL_2-CH/HDL_3-CH	1.92 ± 0.55	2.14 ± 0.64[b]	2.34 ± 0.67[c]
HDL_2-PL/HDL_3-PL	1.83 ± 0.58	2.07 ± 0.66[b]	2.24 ± 0.66[b]
HDL_2-TG/HDL_3-TG	2.20 ± 0.79	2.46 ± 0.85	2.75 ± 1.02[b]

Values shown are means ± SD (mg/dl).
[a] $P < 0.0005$ versus group 1.
[b] $P < 0.005$ versus group 1.
[c] $P < 0.05$ versus group 1.
[d] $P < 0.05$ versus group 2.
[e] $P < 0.005$ versus group 2.
[f] $P < 0.0005$ versus group 2.

Increase of the PL component

Because PLs, along with apoA-I, apoA-II, and FC, constitute the outer shell of HDL, presumably interacting with other receptors or soluble enzymes, it was of particular interest to study the ratios between these components in total HDL and its subfractions (**Table 4**). We found a stepwise increase of PL versus apoA-I in the alcohol-consuming groups, whereas the ratios between other surface components (i.e., FC, apoA-I, and apoA-II) remained constant. Consequently, the PL/FC ratio was increased. Analysis of the subfractions indicates that in dense HDL_3 and intermediate HDL_{2a}, the alcohol-associated increase of the PL portion was attributable to an increase of PL (compared with apoA-I), without major changes of the ratio of FC to apoA-I, as opposed to HDL_{2b}, in which the relative enrichment of PL obviously results from both a depletion of FC and an increase of PL.

To assess the atherogenic potency of HDL-CH, it was also of interest to examine the ratio between the core component cholesteryl ester (CE) and FC in HDL and its subfractions. However, these ratios were constant in all three groups. The relations of TG, the other core component, to CE, FC, and apoA-I remained unchanged (data not shown).

Multivariate analysis: influence of various factors on HDL components

We defined a multivariate model describing HDL components as a linear function of various nutritional, anthropometric, and lifestyle variables (**Table 5**). Parameters that significantly increased cholesterol, PL, and apoA-I in HDL were alcohol consumption and PAL, whereas BMI was negatively related to HDL-CH. Alcohol consumption was the only parameter increasing TG and apoA-II concentrations in the HDL fraction. No significant effects of the type of beverage (beer vs. wine), age, or smoking were observed. Calorie intake significantly decreased PL and apoA-I content, whereas PAL was associated with an increase of these variables. In an extended analysis, the influence of carbohydrate, fat, protein, and fiber uptake was examined, but none of these parameters had any statistical effect on each of the HDL components (data not shown).

Multivariate analysis: qualitative changes

Using analogous multivariate models, the influence of alcohol consumption on qualitative changes of the HDL fraction was examined (**Table 6**). The PL/cholesterol ratio showed a highly significant dependence on alcohol consumption. Interestingly, BMI and PAL were also positively

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 4. Analysis of qualitative changes of surface and core components in HDL and HDL subfractions 2b, 2a, and 3

Variable		Group (Alcohol Consumption)		
		Group 1 (0–5 g/day)	Group 2 (5.1–30 g/day)	Group 3 (30.1–75 g/day)
Surface				
FC/apoA-I	HDL	0.059 ± 0.009	0.061 ± 0.009	0.059 ± 0.016
	HDL_{2b}	0.136 ± 0.038	0.133 ± 0.041	0.122 ± 0.041
	HDL_{2a}	0.067 ± 0.010	0.067 ± 0.009	0.065 ± 0.008
	HDL_3	0.035 ± 0.004	0.036 ± 0.008	0.034 ± 0.008[a]
PL/apoA-I	HDL	0.62 ± 0.06	0.64 ± 0.06[f]	0.69 ± 0.06[b,c]
	HDL_{2b}	1.01 ± 0.16	1.02 ± 0.18	1.04 ± 0.12
	HDL_{2a}	0.76 ± 0.08	0.78 ± 0.08	0.82 ± 0.07[b,d]
	HDL_3	0.45 ± 0.05	0.47 ± 0.06[f]	0.49 ± 0.05[a,b]
PL/FC	HDL	10.6 ± 1.6	10.7 ± 1.3	11.7 ± 2.3[d,e]
	HDL_{2b}	7.7 ± 1.3	8.1 ± 1.4	9.2 ± 2.2[b,d]
	HDL_{2a}	11.5 ± 1.7	11.7 ± 1.3	12.7 ± 1.8[b,c]
	HDL_3	13.0 ± 1.7	13.2 ± 1.8	14.7 ± 2.4[b,c]
ApoA-I/apoA-II	HDL	2.50 ± 0.34	2.59 ± 2.52	2.52 ± 0.38
	HDL_{2b}	4.35 ± 0.83	4.55 ± 1.33	4.18 ± 0.82
	HDL_{2a}	2.26 ± 0.36	2.35 ± 0.48	2.28 ± 0.38
	HDL_3	2.10 ± 0.27	2.10 ± 0.32	2.04 ± 0.35
Core versus surface				
CE/FC	HDL	10.8 ± 1.2	10.7 ± 1.3	10.7 ± 1.6
	HDL_{2b}	8.6 ± 1.2	8.6 ± 1.2	9.0 ± 1.4
	HDL_{2a}	11.5 ± 1.6	11.4 ± 1.4	11.6 ± 1.5
	HDL_3	13.3 ± 1.5	13.1 ± 1.9	13.7 ± 1.8

ApoA-I, apolipoprotein A-I; CE, cholesteryl ester; FC, free cholesterol. Indicated are ratios of surface components FC/apoA-I, PL/apoA-I, PL/FC, and apoA-I/apoA-II, and the ratio of core CE versus surface FC. Values shown are means ± SD.

[a] $P < 0.05$ versus group 2.
[b] $P < 0.0005$ versus group 1.
[c] $P < 0.0005$ versus group 2.
[d] $P < 0.005$ versus group 2.
[e] $P < 0.005$ versus group 1.
[f] $P < 0.05$ versus group 1.

associated with PL enrichment in HDL, whereas energy intake had a negative influence.

The relationship between apoA-I and apoA-II in the HDL fraction constitutes an example of alcohol-independent qualitative changes. Age and PAL shifted the ratio toward apoA-I, whereas a high BMI was associated with increased apoA-II. Finally, the HDL_2-CH/HDL_3-CH ratio was assessed. In this mathematical model, BMI was the only factor with a significant (inverse) effect on this ratio. The influence of alcohol on this ratio did not reach statistical significance ($P = 0.06$).

DISCUSSION

This is, to the best of our knowledge, the first cross-sectional study in a large and well-characterized group of healthy working males comparing the influence of non-

TABLE 5. Coefficients of the multivariate analyses (linear model) for HDL-CH, PLs, TGs, apoA-I, and apoA-II

Dependent Variable	Cholesterol	PL	TG	ApoA-I	ApoA-II
			mg/dl		
Alcohol (g/day)	**0.24 ± 0.04**[a]	**0.48 ± 0.07**[a]	**0.06 ± 0.02**[b]	**0.51 ± 0.08**[a]	**0.22 ± 0.03**[a]
$RC_{beer\ vs.\ wine}$ (%)	−0.038 ± 0.019	−0.032 ± 0.032	−0.008 ± 0.009	−0.033 ± 0.037	0.007 ± 0.013
Age (years)	−0.001 ± 0.063	0.08 ± 0.10	0.05 ± 0.03	0.11 ± 0.12	−0.07 ± 0.04
BMI (kg/m^2)	**−1.1 ± 0.2**[a]	**−1.2 ± 0.3**[a]	0.10 ± 0.09	**−2.0 ± 0.4**[a]	−0.19 ± 0.13
Cigarettes (n)	−0.21 ± 0.11	−0.24 ± 0.17	0.01 ± 0.05	−0.39 ± 0.21	−0.11 ± 0.07
Energy intake without alcohol (kcal)	−3.2 ± 2.0 ($\times 10^{-3}$)	**−8.0 ± 3.3**[c] **($\times 10^{-3}$)**	−1.7 ± 0.9 ($\times 10^{-3}$)	**−8.3 ± 3.8**[c] **($\times 10^{-3}$)**	−2.7 ± 1.4 ($\times 10^{-3}$)
PAL (kcal)	**9.3 ± 3.6**[c] **($\times 10^{-3}$)**	**19.4 ± 6.0**[b] **($\times 10^{-3}$)**	1.8 ± 1.7 ($\times 10^{-3}$)	**21.4 ± 7.0**[b] **($\times 10^{-3}$)**	4.0 ± 2.4 ($\times 10^{-3}$)
Intercept	**75.1 ± 6.2**[a]	**97.8 ± 10.1**[a]	**10.2 ± 2.9**[a]	**154 ± 12**[a]	**53 ± 4**[a]

For each of these variables, the coefficients (±SEM) describing the dependence on various anthropometric, nutritional, and lifestyle parameters and the intercept are listed. $RC_{beer\ vs.\ wine}$ denotes relative consumption of beer versus wine (see Subjects and Methods). All coefficients with $P < 0.05$ are shown in boldface.

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 6. Estimation of the qualitative changes of HDL composition in a multivariate model: PL/cholesterol ratio, apoA-I/apoA-II ratio, and HDL_2-CH/HDL_3-CH ratio

Variable	PL/Cholesterol	ApoA-I/ApoA-II	HDL_2-CH/HDL_3-CH
Alcohol (g/day)	**2.62 ± 0.69 ($\times 10^{-3}$)**[a]	−0.45 ± 1.48 ($\times 10^{-3}$)	11.4 ± 6.0 ($\times 10^{-3}$)
$RC_{beer\ vs.\ wine}$ (%)	0.48 ± 0.31 ($\times 10^{-3}$)	−1.1 ± 0.1 ($\times 10^{-3}$)	−3.5 ± 2.7 ($\times 10^{-3}$)
Age (years)	1.7 ± 1.0 ($\times 10^{-3}$)	**6.8 ± 2.2 ($\times 10^{-3}$)**[b]	7.4 ± 8.8 ($\times 10^{-3}$)
BMI (kg/m^2)	**7.5 ± 3.1 ($\times 10^{-3}$)**[c]	**−33 ± 7 ($\times 10^{-3}$)**[a]	**−62.7 ± 26.7 ($\times 10^{-3}$)**[c]
Cigarettes (n)	1.2 ± 1.8 ($\times 10^{-3}$)	−3.3 ± 3.8 ($\times 10^{-3}$)	26.1 ± 15.2 ($\times 10^{-3}$)
Energy intake without alcohol (kcal)	**−0.065 ± 0.032 ($\times 10^{-3}$)**[c]	−0.034 ± 0.070 ($\times 10^{-3}$)	0.036 ± 0.282 ($\times 10^{-3}$)
PAL (kcal)	**0.12 ± 0.06 ($\times 10^{-3}$)**[c]	**0.27 ± 0.13 ($\times 10^{-3}$)**[c]	0.46 ± 0.51 ($\times 10^{-3}$)
Intercept	**1.20 ± 0.10**[a]	**3.00 ± 0.21**[a]	**3.09 ± 0.86**[a]

For the calculation of each linear model, the same variables were used as in Table 5 (coefficients ± SEM). All coefficients with $P < 0.05$ are shown in boldface.

[a] $P < 0.001$.

[b] $P < 0.01$.

[c] $P < 0.05$.

addictive alcohol consumption on the composition of HDL and its subfractions HDL_{2b}, HDL_{2a}, and HDL_3. Apart from a highly significant alcohol-associated increase of lipids and apolipoproteins in HDL and its subfractions, we observed two qualitative changes that are likely to influence atherogenicity: a lipid enrichment of HDL, as shown by an increase of the HDL_2-CH/HDL_3-CH ratio, and an increase of the PL/cholesterol ratio in all HDL subclasses. The latter change can be traced back to PL enrichment in the intermediate density HDL_{2a} and in the dense HDL_3 subfractions and additional depletion of FC in the buoyant HDL_{2b} subfraction.

Several case-control and epidemiological studies indicate that moderate alcohol consumption reduces cardiovascular mortality and that this effect is associated with the increase of HDL-CH (5, 11). In their meta-analysis, Rimm et al. (5) calculated an increase of HDL-CH of 0.134 mg/dl per gram of alcohol of daily consumption for men (for women, it was 0.095 mg/dl per gram of alcohol). In our study, the coefficient reached 0.24 mg/dl per gram of alcohol of daily consumption for healthy male persons. Several factors may account for this difference. Rimm et al. (5) did not adjust their model for BMI and smoking, both of which have decreasing effects on HDL-CH. Also, we could not find evidence for their statement that the effect of alcohol on HDL-CH was more pronounced in subjects with a sedentary lifestyle. In our multivariate analysis, physical activity had an independent and significantly positive influence on HDL-CH levels.

The quantitative change of HDL-CH is thought to be the result of increased hepatic production or an increased transport rate of apoA-I and apoA-II (12–14). Other data indicate that alcohol might interfere with the activity of the cholesteryl ester transfer protein (CETP), which mediates cholesterol transfer from HDL to LDL (15). Additionally, it has been shown by ex vivo experiments that moderate alcohol consumption increases the efflux of FC from macrophages to HDL, mainly via the ATP binding cassette transporter 1 (16). Large amounts of alcohol, on the other hand, may result in impaired cholesterol efflux (17).

One of the major findings of our study is the divergence of the dose relationships between the HDL subclasses. Compared with controls, the alcohol-related increase of cholesterol, PL, and TG in HDL_{2a} was more than twice that of the increase in the HDL_3 subclass. The changes in HDL_{2b} were similar to those in HDL_{2a}, but somewhat less pronounced. The shift toward HDL_2 indicates a higher HDL lipid load in alcohol consumers (Table 3). This phenomenon is confirmed by other examinations (18–21), whereas some groups found a predominant increase of HDL_3 (22, 23) or no relevant change in the relation of HDL_2 to HDL_3 components (11, 24). These discrepancies might be explained by variant study design and the disregard of powerful confounders, such as obesity and smoking.

The increased lipidation of HDL, which may be attributable to an increased cholesterol efflux from peripheral cells (16), could indicate an augmentation of reverse cholesterol transport. This can be inferred from studies showing that risk factors such as obesity or hypertriglyceridemia are associated with the opposite change (i.e., a decrease of large HDL_2 vs. an increase of small preβ HDL or HDL_3 particles) (25, 26), which has been confirmed for obesity by the multivariate analysis of our study. Interestingly, the influence of alcohol on the HDL_2-CH/HDL_3-CH balance seems to "override" the opposing influence of high BMI or increased TGs, as subjects with an alcohol consumption of >30 g/day (group 3) exhibited the highest HDL_2-CH/HDL_3-CH ratios despite the increased BMI and TG values (Tables 1–3).

It could be argued that an increase of HDL_2 cholesterol might be ambiguous, because this fraction is the substrate of cholesterol transfer protein, which shifts CEs to proatherogenic LDL particles. Thus, we examined the balance between CEs and FC in HDL but found no significant change.

From a clinical point of view, the cardioprotective relevance of HDL_2 is supported by several population studies, such as the Québec and Kuopio trials (27, 28). In other population studies, such as the Physicians' Health Study (29) and the Caerphilly and Speedwell trials (30), HDL_3 was the strongest predictor, but risk reduction was shown for HDL_2 as well.

The other qualitative change of HDL apart from the increase in lipid-rich particles is the alcohol-induced relative

Downloaded from www.jlr.org by guest, on June 14, 2012

increase of the PL components compared with the other HDL components (cholesterol, TG, apoA-I, and apoA-II). This change was observed in all HDL subfractions and was most pronounced in persons with increased alcohol consumption. In the current multivariate analysis, a highly significant association between alcohol consumption and an increase of the PL/cholesterol ratio was detected, although other factors (BMI, energy intake, and physical activity) seemed to be involved in the regulation of this balance as well.

The relative increase of the PL component may have important implications concerning the antiatherosclerotic effects of HDL. Because this change occurs in the surface layer of HDL that interacts with cellular receptors or serum components, it may be of particular importance in two respects: the increase of PL may reduce the inflammatory process in the vessel wall, because HDL particles reconstituted with PLs inhibited the cytokine-induced activation of endothelial cells in vitro (31); and PLs are obviously required for an effective cholesterol efflux from peripheral cells to ApoA-I-containing lipoprotein particles (32).

In this context, it could be argued that the PL portion might contain compounds associated with an increased atherogenic risk, such as sphingomyelin (SM). However, it is very unlikely that SM plays such a role, for the following reasons. *i*) HDL-like LpA1 containing SM may be even more effective at effluxing cholesterol from fibroblasts than LpA1 without SM (33). *ii*) There is evidence from animal experiments that excess SM induces an increase of VLDL-CH and LDL-CH but a decrease of HDL-CH (33, 34). This situation is contrary to that found in the alcohol consumers of our study, so a general increase of atherogenic SM is unlikely. *iii*) In alcohol-consuming humans, no change of the SM content in platelets occurred, and even a decrease of SM in erythrocyte membrane occurred (35, 36). Despite this circumstantial evidence, further studies are needed to delineate the influence of alcohol on PL composition in HDL.

The exact mechanisms leading to the increase of the PL/cholesterol ratio remain unclarified. Several transfer enzymes promote the exchange of lipid components between HDL, LDL, and VLDL. High-level alcohol consumption results in depressed CETP activity, which is associated with an increase of HDL_{3b} (medium HDL_3) at the expense of HDL_{2a}. In addition, alcohol-induced phospholipid transfer protein (PLTP) activity, which shifts PL from TG-rich lipoproteins to HDL, appears to increase HDL_{2b} at the expense of HDL_{3a} (large HDL_3) (37). The reduction of CETP and the increase of PLTP were confirmed by another study, which also observed an increase in the net PL transfer from apoB-containing lipoproteins to HDL in alcoholics (38). However, none of these enzymes seems to be changed in moderate alcohol consumers (39–41). In our study, the analysis of HDL_{2a} and HDL_3 composition shows an isolated dose-dependent increase of the PLs compared with apoA-I, whereas the ratio of FC to apoA-I remained unchanged, which argues for a role of PLTP in PL enrichment in these HDL subclasses. In lipid-rich HDL_{2b}, the situation is more complex, because both PL enrichment and FC depletion occur.

Another possible mechanism for the PL enrichment is highlighted by an in vitro study showing that ethanol at concentrations found in heavy drinkers inhibits the incorporation of cholesterol into preβ-like particles containing apoA-I and phosphatidylcholine, whereas this phenomenon was not observed in particles without this PL (42). Thus, alcohol, at least at high concentrations, might influence the balance between PL and cholesterol at this very early step of HDL assembly.

In terms of antiatherogenicity, it is also of interest to study the effect of alcohol consumption on a third feature of HDL, the relation of HDL particles containing only apoA-I, termed LpA1, to particles containing apoA-I and apoA-II (LpA1/A2), because LpA1 has a higher potency than LpA1/A2 in effluxing cellular cholesterol (43, 44). In our study, the concentrations of LpA1 and LpA1/A2 were not measured directly. However, comparison between the groups and multivariate analysis showed no influence of alcohol consumption on the ratio of apoA-I to apoA-II (Tables 4, 6), which allows the conclusion that LpA1 and LpA1/A2 are increased in a similar manner. This conclusion is in accordance with two studies directly showing an alcohol-related increase of both lipoprotein species (24, 45). Yet, other data suggest that alcohol consumption mainly increases LpA1/A2 (18, 46).

It is somewhat surprising that the shift from HDL_3 to HDL_2 does not result in an increase of apoA-I, given the fact that HDL_2 consists mainly of LpA1, with most of LpA1/A2 being associated with HDL_3. Two phenomena may account for the constancy of the apoA-I/apoA-II ratio despite the relative increase of HDL_2 in alcohol consumers (Table 4): *i*) the ratios of the two apolipoproteins in HDL_3 and in HDL_{2a}, the major portion of HDL_2, are almost alike; *ii*) HDL_{2b}, the minor portion of HDL_2, showed a slight, although insignificant, decrease in this ratio in group 3, indicating some loss of LpA1 in this subfraction.

Apart from the mere alcohol effect, we examined whether beer or wine consumption had a differential effect on the composition of the HDL fraction. The multivariate analysis showed no significant differences between relative beer and wine consumption on all HDL components and on qualitative changes. Thus, our data are in accordance with the general assumption (5) that the increase of HDL-CH is an effect of the alcohol itself, particularly in the range of moderate alcohol consumption.

The effect of alcohol must be interpreted in the context of other lifestyle and anthropometric parameters. In our multivariate model, obesity and factors predisposing to it, such as high caloric intake and scarce physical activity, are associated with a reduction in essential HDL components; thus, they counteract the influence of alcohol on HDL (Table 4). Beyond this, obesity tilts the balance between apoA-I and apoA-II toward the latter, resulting in a relative increase of LpA1/A2 at the cost of LpA1, which is an additional indication of impaired reverse cholesterol transport (Table 5). Cigarette consumption had no statistically significant effect, probably because of the low percentage of

Downloaded from www.jlr.org by guest, on June 14, 2012

smokers in this study, whereas age, interestingly, is associated with a "favorable" increase of the apoA-I/apoA-II ratio.

In summary, this study supports previous data showing a powerful influence of moderate (5–30 g/day) and increased (30–75 g/day) alcohol consumption on the concentration of HDL-CH. An additional effect, which deserves further research, is the compositional and qualitative change of HDL particles in alcohol-consuming persons, such as PL enrichment, which may inhibit the inflammatory process associated with the formation of atheromatous plaques, and the relative increase of lipid-rich HDL_2 versus HDL_3, which indicates a more effective reverse cholesterol transport.

This study was supported by Grant B-50 from the Wissenschaftsförderung der Deutschen Brauwirtschaft. The authors thank Ms. I. Friedrich for excellent technical assistance and J. Dippon, Ph.D., University of Stuttgart, for statistical support. The authors thank the companies that supported this work by recruiting study subjects.

REFERENCES

1. Moore, R. D., and T. A. Pearson. 1986. Moderate alcohol consumption and coronary artery disease. A review. *Medicine (Baltimore).* **65:** 242–267.
2. Maclure, M. 1993. Demonstration of deductive meta-analysis: ethanol intake and risk of myocardial infarction. *Epidemiol. Rev.* **15:** 328–351.
3. Gaziano, J. M., and J. E. Buring. 1998. Alcohol intake, lipids and risks of myocardial infarction. *Novartis Found. Symp.* **216:** 86–95; discussion 95–110.
4. Marmot, M. G. 2001. Alcohol and coronary heart disease. *Int. J. Epidemiol.* **30:** 724–729.
5. Rimm, E. B., P. Williams, K. Fosher, M. Criqui, and M. J. Stampfer. 1999. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. *BMJ.* **319:** 1523–1528.
6. Rimm, E. B., A. Klatsky, D. Grobbee, and M. J. Stampfer. 1996. Review of moderate alcohol consumption and reduced risk of coronary heart disease: is the effect due to beer, wine, or spirits? *BMJ.* **312:** 731–736.
7. Di Castelnuovo, A., S. Rotondo, L. Iacoviello, M. B. Donati, and G. De Gaetano. 2002. Meta-analysis of wine and beer consumption in relation to vascular risk. *Circulation.* **105:** 2836–2844.
8. Landig, J., J. G. Erhardt, J. C. Bode, and C. Bode. 1998. Validation and comparison of two computerized methods of obtaining a diet history. *Clin. Nutr.* **17:** 113–117.
9. Kleiber, M. 1947. Body size and metabolic rate. *Physiol. Rev.* **27:** 511–515.
10. Winkler, K., T. Konrad, S. Füllert, I. Friedrich, R. Destani, M. W. Baumstark, K. Krebs, H. Wieland, and W. März. 2003. Pioglitazone reduces atherogenic dense LDL particles in nondiabetic patients with arterial hypertension. *Diabetes Care.* **26:** 2588–2594.
11. 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.
12. Moore, R. D., C. R. Smith, P. O. Kwiterovich, and T. A. Pearson. 1988. Effect of lowdose alcohol use versus abstention on apolipoproteins A1 and B. *Am. J. Med.* **84:** 884–890.
13. Savolainen, M. J., and Y. A. Kesaniemi. 1995. Effects of alcohol on lipoproteins in relation to coronary heart disease. *Curr. Opin. Lipidol.* **6:** 243–250.
14. 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.
15. Hannuksela, M., Y. L. Marcel, Y. A. Kesaniemi, and M. J. Savolainen. 1992. Reduction in the concentration and activity of plasma cholesteryl ester transfer protein by alcohol. *J. Lipid Res.* **33:** 737–744.
16. Beulens, J. W. J., A. Sierksma, A. van Tol, N. Fournier, T. van Gent, J-L. Paul, and H. F. J. Hendriks. 2004. Moderate alcohol consumption increases cholesterol efflux mediated by ABCA1. *J. Lipid Res.* **45:** 1716–1723.
17. Rao, M. N., Q. H. Liu, P. Marmillot, L. B. Seef, D. B. Strader, and M. R. Lakshman. 2000. High-density lipoproteins from human alcoholics exhibit impaired reverse cholesterol transport function. *Metabolism.* **49:** 1406–1410.
18. Perret, B., J. B. Ruidavets, C. Vieu, B. Jaspard, J. P. Cambou, F. Terce, and X. Collet. 2002. Alcohol associated with enrichment of high-density lipoprotein particles in polyunsaturated lipids and increased cholesterol esterification rate. *Alcohol. Clin. Exp. Res.* **26:** 1134–1140.
19. Taskinen, M. R., M. Valimaki, E. A. Nikkila, T. Kuusi, C. Ehnholm, and R. Ylikahri. 1982. High density lipoprotein subfractions and postheparin plasma lipases in alcoholic men before and after ethanol withdrawal. *Metabolism.* **31:** 1168–1174.
20. Dai, W. S., R. E. LaPorte, D. L. Hom, L. H. Kuller, J. A. D'Antonio, J. P. Gutai, M. Wozniczak, and B. Wohlfahrt. 1985. Alcohol consumption and high density lipoprotein cholesterol concentration among alcoholics. *Am. J. Epidemiol.* **122:** 620–627.
21. Contaldo, F., E. D'Arrigo, V. Carandente, C. Cortese, A. Coltorti, M. Mancini, M. R. Taskinen, and E. A. Nikkila. 1989. Short term effects of moderate alcohol consumption on lipid metabolism and energy balance in normal men. *Metabolism.* **38:** 166–171.
22. Haskell, W. L., C. Camargo, P. T. Williams, K. M. Vranizan, R. M. Krauss, F. T. Lindgren, and P. T. Wood. 1984. The effect of cessation and resumption of moderate alcohol intake on serum high-density-lipoprotein subfractions. A controlled study. *N. Engl. J. Med.* **310:** 805–810.
23. Sillanaukee, P., T. Koivula, H. Jokela, H. Myllyharju, and K. Seppa. 1993. Relationship of alcohol consumption to changes in HDL-subfractions. *Eur. J. Clin. Invest.* **23:** 486–491.
24. Välimäki, M., K. Laitinen, R. Ylikahri, C. Ehnholm, M. Jauhiainen, J. M. Bard, J. C. Fruchart, and M. R. Taskinen. 1991. The effect of moderate alcohol intake on serum apolipoprotein A-I-containing lipoproteins and lipoprotein (a). *Metabolism.* **40:** 1168–1172.
25. Gou, L., M. Fu, Y. Xu, Y. Tian, B. Yan, and L. Yang. 2005. Alterations of high-density lipoprotein subclasses in endogenous hypertriglyceridemia. *Am. Heart J.* **150:** 1039–1045.
26. Tian, L., L. Jia, F. Mingde, Y. Tian, Y. Xu, H. Tian, and Y. Yang. 2006. Alterations of high density lipoprotein subclasses in obese subjects. *Lipids.* **41:** 789–796.
27. Lamarche, B., S. Moorjani, B. Cantin, G. R. Dagenais, P. J. Lupien, and J. P. Despres. 1997. Associations of HDL2 and HDL3 subfractions with ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1098–1105.
28. Salonen, J. T., R. Salonen, K. Seppanen, R. Rauramaa, and J. Tuomilehto. 1991. HDL, HDL2, and HDL3 subfractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. *Circulation.* **84:** 129–139.
29. Stampfer, M. J., F. M. Sacks, S. Salvini, W. C. Willett, and C. H. Hennekens. 1991. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N. Engl. J. Med.* **325:** 373–381.
30. Sweetnam, P. M., C. H. Bolton, J. W. G. Yarnell, D. Bainton, I. A. Baker, P. C. Elwood, and N. E. Miller. 1994. Associations of the HDL2 and HDL3 cholesterol subfractions with the development of ischemic heart disease in British men. *Circulation.* **90:** 769–774.
31. Baker, P. W., K. A. Rye, J. R. Gamble, M. A. Vadas, and P. J. Barter. 2000. Phospholipid composition of reconstituted high density lipoproteins influences their ability to inhibit endothelial cell adhesion molecule expression. *J. Lipid Res.* **41:** 1261–1267.
32. Zhao, Y., D. L. Sparks, and Y. L. Marcel. 1996. Specific phospholipid association with apolipoprotein A-I stimulates cholesterol efflux from human fibroblasts. *J. Biol. Chem.* **271:** 25145–25151.
33. Park, T-S., R. L. Panek, S. B. Mueller, J. C. Hanselman, W. S. Rosebury, A. W. Robertson, E. K. Kindt, R. Homan, S. K. Karathanasis, and M. D. Rekhter. 2004. Inhibition of sphingomyelin synthesis reduces atherogenesis in apolipoprotein E-knockout mice. *Circulation.* **110:** 3465–3471.
34. Dong, J., J. Liu, B. Lou, Z. Li, X. Ye, M. Wu, and X. C. Jiang. 2006.

Downloaded from www.jlr.org by guest, on June 14, 2012

Adenovirus-mediated overexpression of sphingomyelin synthases 1 and 2 increases the atherogenic potential in mice. *J. Lipid Res.* **47:** 1307–1314.
35. Simonetti, P., A. Brusamolino, N. Pellegrini, P. Viani, G. Clemente, C. Roggi, and B. Cestaro. 1995. Evaluation of the effect of alcohol consumption on erythrocyte lipids and vitamins in a healthy population. *Alcohol. Clin. Exp. Res.* **19:** 517–522.
36. Pellegrini, N., P. Simonetti, A. Brusamolino, B. Bottasso, and F. I. Pareti. 1996. Composition of platelet phospholipids after moderate consumption of red wine in healthy volunteers. *Eur. J. Clin. Nutr.* **50:** 535–544.
37. Lagrost, L., A. Athias, B. Herbeth, V. Guyard-Dangremont, Y. Artur, F. Paille, P. Gambert, and C. Lallemant. 1996. Opposite effects of cholesteryl ester transfer protein and phospholipid transfer protein on the size distribution of plasma high density lipoproteins. *J. Biol. Chem.* **271:** 19058–19065.
38. Liinamaa, M. J., M. L. Hannuksela, Y. A. Kesaniemi, and M. J. Savolainen. 1997. Altered transfer of cholesteryl esters and phospholipids in plasma from alcohol abusers. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2940–2947.
39. Nishiwaki, M., T. Ishikawa, T. Ito, H. Shige, K. Tomiyasu, K. Nakajima, K. Kondo, H. Hashimoto, K. Saitoh, M. Manabe, et al. 1994. Effects of alcohol on lipoprotein lipase, hepatic lipase, cholesteryl transfer protein, and lecithin:cholesterol acyltransferase in high-density lipoprotein cholesterol elevation. *Atherosclerosis.* **111:** 99–109.
40. Ito, T., M. Nishiwaki, T. Ishikawa, and H. Nakamura. 1995. CETP and LCAT activities are unrelated to smoking and moderate cholesteryl ester transfer protein by alcohol. *J. Lipid Res.* **59:** 541–546.
41. Riemens, S. C., A. van Tol, K. Hoogenberg, T. van Gent, L. M. Scheek, W. J. Sluiter, and R. P. 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.
42. Avdulov, N. A., S. V. Chochina, U. Igbavboa, and W. G. Wood. 2000. Cholesterol efflux to high-density lipoproteins and apolipoprotein A-I phosphatidylcholine complexes is inhibited by ethanol: role of apolipoprotein structure and cooperative interaction of phosphatidylcholine and cholesterol. *Biochemistry.* **39:** 10599–10606.
43. Puchois, P., A. Kandoussi, P. Fievet, J. L. Fourrier, M. Bertrand, E. Koren, and J. C. Fruchart. 1987. Apolipoprotein A-I-containing lipoproteins in coronary artery disease. *Atherosclerosis.* **68:** 35–40.
44. Rinninger, F., T. Kaiser, E. Windler, H. Greten, J. C. Fruchart, and G. Castro. 1998. Selective uptake of cholesteryl esters from high-density lipoprotein-derived LPA-I and LPA-I:A-II particles by hepatic cells in culture. *Biochim. Biophys. Acta.* **1393:** 277–291.
45. Branchi, A., A. Rovellini, C. Tomella, L. Sciariada, A. Torri, M. Molgora, and D. Sommariva. 1997. Association of alcohol consumption with HDL subpopulations defined by apolipoprotein A-I and apolipoprotein A-II content. *Eur. J. Clin. Nutr.* **51:** 362–365.
46. Luc, G., J. M. Bard, A. Evans, D. Arveiler, J. B. Ruidavets, P. Amouyel, and P. Ducimetiere. 2000. The relationship between apolipoprotein AI-containing lipoprotein fractions and environmental factors: the Prospective Epidemiological Study of Myocardial Infarction (PRIME study). *Atherosclerosis.* **152:** 399–405.

Downloaded from www.jlr.org by guest, on June 14, 2012