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Long-term ethanol consumption impairs reverse cholesterol transport function of high-density lipoproteins by depleting high-density lipoprotein sphingomyelin both in rats and in humans

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Abstract

Moderate alcohol consumption has been linked to lower incidence of coronary artery disease due to increased plasma high-density lipoprotein (HDL), whereas heavy drinking has the opposite effect. Because of the crucial role of HDL in reverse cholesterol transport and positive correlation of HDL sphingomyelin (SM) content with cholesterol efflux, we have compared HDL SM content with its reverse cholesterol transport capacity both in rats fed ethanol on long-term basis and alcoholic individuals. In rats, SM HDL content was decreased in the ethanol group (−15.4%, $P < .01$) with a concomitant efflux decrease (−21.0%, $P < .01$) compared to that in controls. Similarly, HDL from the ethanol group, when compared with HDL from the control group, exhibited 13.8% ($P < .05$) less cholesterol uptake with control-group hepatocytes and 35.0% ($P < .05$) less cholesterol uptake with ethanol-group hepatocytes. Conversely, hepatocytes from the ethanol group, when compared with hepatocytes from the control group, exhibited 31.0% ($P < .01$) less cholesterol uptake with control-group HDL and 48.0% ($P < .01$) less with ethanol-group HDL. In humans, SM content in plasma HDL was also decreased in chronically alcoholic individuals without liver disease (−51.5%, $P < .01$) and in chronically alcoholic individuals with liver disease (−51.3%, $P < .01$), compared with nondrinkers. Concomitantly, in alcoholic individuals without liver disease, both efflux and uptake were decreased by 83.0% and 54.0% ($P < .01$), respectively, and in chronically alcoholic individuals with liver disease by 84.0% and 61.0% ($P < .01$), respectively, compared with nondrinkers. Based on these findings, we conclude that long-term ethanol consumption significantly impairs not only cholesterol efflux function of HDL by decreasing its SM content but also cholesterol uptake by affecting presumably hepatocyte receptors for HDL.

1. Introduction

Ethanol consumption induces a change in lipid homeostasis. It is now accepted that both intracellular lipid carrier proteins and lipoproteins are targets of short- and long-term ethanol consumption [1–3]. Reverse cholesterol transport (RCT) is the process through which cholesterol is transported by high-density lipoprotein (HDL) from the peripheral tissues back

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to the liver (for review, see reference [4]). In this process, HDL maturation begins with the secretion of lipid-poor apolipoprotein (apo) A-I by the liver and the intestine, followed by the acquisition of cholesterol and phospholipids (PLs) via adenosine triphosphate–binding cassette A1 (ABCA1)–mediated efflux from the liver and transfer of cholesterol, PL, and apolipoproteins from chylomicrons and very low-density lipoprotein during lipoprotein lipase–mediated lipolysis to form nascent pre-β HDL particles. These acquire additional cholesterol and PLs from extrahepatic tissues via the ABCA1-mediated efflux leading to cholesterol-enriched HDL_3 particles. The lecithin cholesterol acyltransferase then esterifies the free cholesterol in HDL_3 leading to cholesterol ester–enriched less dense HDL_2 particles. HDL_2 cholesterol can be transferred to apo B–containing particles via cholesteryl ester transfer protein and subsequent uptake by the low-density lipoprotein (LDL) receptor, or can be directly taken up by the HDL receptor scavenger receptor class B, type I (SR-BI) [5]. A very recent study [6], using SR-BI knockout mice, elegantly showed that SR-BI is the sole molecule mediating the selective uptake of cholesterol esters from HDL_2 by the liver. Several studies showed that the RCT function of HDL is affected by its PL content. Particularly, it was shown that cholesterol efflux is positively correlated with sphingomyelin (SM) content in HDL in humans with high serum HDL cholesterol (>67.7 mg/dL) [7]. Thus, factors that regulate the local modification of HDL PL composition may have a large impact on RCT. Also, it was demonstrated that the scavenger receptor class BI (SR-BI)–mediated free cholesterol flux is very sensitive to the phosphatidylcholine (PC) and SM composition of HDL and that HDL PL composition alters the steady-state distribution of cholesterol between cells expressing SR-BI and HDL. Also, SM enrichment of HDL enhances the net efflux of cholesterol from SR-BI–expressing COS-7 cells by decreasing the influx of HDL cholesterol [8].

Sphingomyelin is the second most abundant PL in mammalian plasma and appears in all major lipoproteins where it is part of the monolayer of polar lipids and cholesterol that surrounds a core of neutral lipids. Up to 18% of the total plasma PL occurs as SM [9], and the ratio of PC/SM varies widely among lipoprotein subclasses [10,11]. Sphingomyelin content of the cell membrane is thought to contribute to the maintenance of cellular cholesterol homeostasis because the cholesterol content of the cell membrane is positively correlated with SM content [12]. Importantly, SM is thought to bind cholesterol with high affinity and inhibit its efflux from the plasma membrane by preventing cholesterol desorption [13]. In addition, SM prevents the exchange of cholesterol between the plasma membrane and intracellular pools [14] and was shown to be a risk factor for coronary artery disease [15]. We showed recently, in a well-defined in vitro system, that increasing SM content in HDL has opposite effects on cholesterol uptake and efflux, depending on the saturation levels of the fatty acid in the PC component [16].

In the present work, we have compared the alterations in plasma SM concentration associated with HDL caused by long-term alcohol consumption with the RCT function of the corresponding plasma HDL both in rats and in humans.

2. Methods

2.1. Materials

Materials were obtained from the following sources: mouse macrophages (J774) and human hepatoma (HepG2) from the American Type Cell Collection (Manassas, VA); Dulbecco modified Eagle medium, Eagle minimum essential medium (EMEM), glutamine, and penicillin/streptomycin from Invitrogen (Carlsbad, CA); fetal calf serum (FCS) from Life Technologies (Gaithersburg, MD); insulin from Eli Lilly (Indianapolis, IN); bovine brain SM, collagenase, L-α-phosphatidylcholine dipalmitoyl (lecithin), 4-aminoantopyrine, choline oxidase (from *Arthrobacter globiformis*), horseradish peroxidase, alkaline phosphatase (from *Escherichia coli*), and sphingomyelinase (from *Bacillus cereus*) from Sigma-Aldrich (St Louis,

MO); [^{3}H]cholesteryl oleate from Amersham Biosciences (Piscataway, NJ); tissue culture plasticware from Falcon (Lincoln, NJ); Zwittergent 3–10 from EMD Bioscience (San Diego, CA); and 2-hydroxy-3,5-dichlorobenzenesulfonic acid from Research Organic (Cleveland, OH). All other reagents used were of analytical grade.

2.2. Study subjects

The study subjects were enrolled at the Liver Clinic at the Veterans Health Administration Medical Center in Washington, DC. The study was approved by the institutional review board, and thus informed consents were obtained from all patients to draw their blood for this study. The study subjects were divided into the following 3 groups: ALC, chronically alcoholic individuals without liver disease (n = 8); ALD, chronically alcoholic individuals with liver disease (n = 6); and control subjects were nondrinkers who were age-matched to the study subjects (n = 9). The average period of long-term alcohol consumption in ALC and ALD groups was 21 years. Most of the study subjects were males of African descent. Subjects in the ALC group exhibited only fatty liver, whereas those in the ALD group had clinically defined liver disease. Subjects in the control group consumed less than 8 g of ethanol per day for 6 months or longer, whereas those in the ALC and ALD groups were identified as consuming more than 80 g of ethanol per day. Inclusion criteria were ages between 30 and 59 years. Exclusion criteria were abusers of substances other than alcohol, patients on any lipid-lowering drugs, and patients with severe degenerative complications, or concomitant illness, such as hepatic failure or neoplasia. Fasting blood was collected in EDTA-containing tubes for plasma preparation.

2.3. Animals and dietary feeding regimen

The animal component of the research protocol used for this study was approved by the Institutional Animal Care and Use Committees (IACUCs) of both the Veterans Affairs Medical Center and the George Washington University (both in Washington, DC). Male Wistar-Furth rats (body weight, 125–150 g) were obtained from Charles River (Wilmington, MA). After a week of acclimatization, the animals were divided into 2 groups (8 animals per group) and they were fed for 8 weeks with one of the following diets: control or ethanol. The ethanol diet was essentially identical as that of used by Lieber and DeCarli [17] and had the following nutrient composition expressed in grams per liter: oil mixture (olive oil, cod liver oil, and corn oil in a ratio of 67:8:25), 126.75; ethanol, 50; casein, 47; and dextrin maltose, 11.36. Thus, 40% of the total energy of this diet was from fat, 36% from ethanol, 20% from protein, and the rest from carbohydrates. The corresponding control diet contained equivalent energy amounts of dextrin maltose in place of ethanol. The rats in control group were pair-fed with the animals in the corresponding ethanol group. On an average, each animal consumed 70-mL diet per day in both groups. This amounted to a consumption of 14 g of ethanol per kilogram of body weight per day by each animal in the ethanol group. At the end of 8 weeks, all the rats (except those saved for liver perfusion) were killed by aortic exsanguination under pentobarbital anesthesia (50 mg/kg of body weight). Blood ethanol concentration at the time of sacrifice varied widely (~5–25 mmol/L) in the ethanol-fed group depending upon when the animal had his last binge before sacrifice. This aspect was not critical for the present study because plasma HDL level is dependent upon the long-term effect of alcohol and not on its immediate effect.

2.4. Isolation of hepatocytes from rats by liver perfusion

Hepatocytes were isolated from rats fed with control or ethanol diet for 8 weeks and were used in the cholesterol uptake experiments with rat HDLs. Liver perfusion was performed essentially according to the method as described by us previously [18], which was based on the method of Hems et al [19]. The only significant modification in this procedure was that, as soon as the cannula was introduced into the portal vein, the liver perfusion was started and the perfusion fluid flushed the liver out uniformly and completely drained through the inferior vena cava

below the liver that was cut open. By this technique there was virtually no anoxic period for the liver, and at the same time the use of heparin was avoided. Once the chest wall was opened and the inferior vena cava above the liver was cannulated, the one below the liver was ligated so that a recirculation system was set up for perfusion of the liver. Hepatocytes were isolated during the midday using the method of Berry and Friend [20] with modifications as described previously [21], except that glucose was added to the final perfusion medium (20 mmol/L) in order to minimize hepatic glycogenolysis [22]. These hepatocytes were 90% to 95% viable as tested by trypan dye exclusion and satisfied the criteria for intact, viable, and metabolically active hepatocytes defined by Krebs et al [21].

2.5. Isolation of HDL from plasma

High-density lipoprotein was isolated from human or rat plasma by precipitation [23]. Briefly, 0.1 volume of a solution containing 2.264 USP U per milliliter of heparin and 1.0 mol/L $MnCl_2$ was added to 1.0 volume of plasma. The mixture was vortexed briefly, incubated for 30 minutes on ice, and then centrifuged for 20 minutes at 14000*g* (4°C). One volume of very low-density lipoprotein/LDL-free supernatant was mixed with 0.1 volume of 7.8% (wt/vol) dextran sulfate (15 kd) and 0.1 volume of 2.4 mol/L $MnCl_2$, vortexed briefly, incubated for 30 minutes on ice, and then centrifuged as before. The HDL pellet thus obtained was solubilized in the initial sample's volume with saline/EDTA (0.154 mol/L NaCl, 0.3 mmol/L EDTA) and dialyzed extensively against the same solution. Protein concentration was determined colorimetrically, using bovine serum albumin (BSA) as a standard [24]. Cholesterol [25], PL [26], and triglyceride [27] concentrations were also determined according to established procedures.

2.6. Labeling of HDL and acetylated LDL with [^{3}H]cholesteryl oleate

This procedure was performed essentially as described previously [28,29]. High-density lipoprotein was obtained as described above. Low-density lipoprotein was obtained from human pooled plasma by sequential flotations [30] and oxidized by acetylation [28], as described in details previously [2]. Acetylation of LDL (ie, LDL oxidation) was necessary to promote cholesterol uptake by the macrophage during the loading step of the cholesterol efflux experiments. Seventy-four kilo becquerels (74 kBq) of [^{3}H]cholesteryl oleate (per milligram of lipoprotein protein), originally in acetone, was evaporated to dryness using nitrogen or helium. Dried cholesterol was solubilized in 1 volume (corresponding to one fifteenth of the lipoprotein volume) of dimethyl sulfoxide. This was followed by the addition of 4 volumes of saline/EDTA (0.154 mol/L NaCl, 3.0 mol/L EDTA) in a dropwise manner with continuous mixing. This clear solution was incubated at 37°C for 10 minutes and was then mixed with 15 volumes of HDL or LDL, again in a dropwise manner with continuous mixing. The lipoprotein plus radioactive cholesteryl oleate solution was then incubated at 37°C for 3 hours, followed by extensive dialysis against at least 500 volumes of saline/EDTA. Aliquots of the dialyzed, labeled lipoproteins were counted for radioactivity and their specific activity determined.

2.7. Cholesterol efflux experiments

Cholesterol efflux assays with HDLs from various experimental groups of humans and rats were carried out as described previously [2]. Briefly, mouse macrophages (J774) were maintained in Dulbecco modified Eagle medium supplemented with 2 mmol/L L-glutamine, 1% penicillin/streptomycin, 10% (vol/vol) FCS, and 0.1 mU% insulin in 37°C in 5% CO_2 air humidified atmosphere. When needed, cells were split and grown as monolayer in 12-well culture dishes (1×10^6 cells per well in 4 mL culture medium). Confluence of the cell layers was observed after 24 to 36 hours. All experiments were performed in the postconfluent proliferating phase of cells. Macrophages were first incubated with [^{3}H]cholesteryl oleate–labeled acetylated LDL (eg, 2.22 kBq and 115.5 μg of LDL protein per well) in 2 mL of culture

medium for 24 hours. After washing 3 times with phosphate-buffered saline (PBS, pH 7.4), the macrophages were then incubated with 75 μg protein of indicated HDL per well in culture medium without FCS for 24 hours. Efflux was measured by counting the radioactivity in 0.2 mL of cell-free medium mixed with 4.0 mL of liquid scintillation solution (UltimaGold, Perkin Elmer, Boston, MA) in a Beckman LS6500 liquid scintillation counter (Beckman Coulter, Fullerton, CA) and was expressed as percentage of efflux.

2.8. Cholesterol uptake experiments

2.8.1. Uptake with rat HDLs and hepatocytes—The incubation procedure for the measurement of the rates of [^{3}H]cholesteryl oleate uptake was carried out as described previously [31]. Hepatocytes (either control or ethanol rats) equivalent to about 100 mg wet weight of cells (~10×10^6 cells) were incubated in 25-mL Erlenmeyer flasks in Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 1.5% (wt/vol) dialyzed albumin in a final volume of 2 mL. [^{3}H]cholesteryl oleate–labeled HDL fractions (from either control or ethanol rat plasmas), when added, were in saline/EDTA. After gassing with an O_2/CO_2 (95:5) mixture, the flasks were tightly capped and incubated at 37°C in a shaking water bath (100 oscillations per minute) for 90 minutes. To measure cholesterol uptake, 0.2 mL of cell suspension was sampled and centrifuged, and the pellet was washed 3 times with saline/EDTA. The final washed cell pellet was solubilized in 4.0 mL of liquid scintillation solution and counted as described above. The difference in counts at "0" time and after 90 minutes was used to calculate the uptake of [^{3}H] cholesteryl oleate.

2.8.2. Uptake with human HDLs and HepG2 cells—The procedure was as described previously with slight modifications [32]. Cells were maintained, at 37°C in 5% CO_2 air humidified atmosphere, in EMEM supplemented with 1% nonessential amino acids, 2 mmol/L L-glutamine, 1% penicillin/streptomycin, and 10% (vol/vol) FCS, according to the supplier's instructions. When needed, cells were subcultured and grown as monolayers in 6-well culture dishes, at 1×10^6 cells per well (4 mL EMEM), at 37°C in 5% CO_2 air humidified atmosphere. Confluence of the cell layers was observed after 36 to 48 hours. All experiments were performed with confluent cells. An adequate volume of dialyzed, labeled HDL was mixed with EMEM (4 mL final volume; 100 μg/mL HDL protein) and added as a sterile solution (by filtration through 0.22-μmol/L sterile filters) to each culture dish containing confluent HepG2 cells. An aliquot (0.1 mL) of the incubation medium was analyzed for the total esterified cholesterol radioactivity added to each dish at the beginning of the incubation. The cells were incubated for 24 hours at 37°C. The uptake of labeled esterified cholesterol after 24 hours was determined by counting the cell-associated radioactivity as follows: the cell medium was completely removed and, after thorough washing of the cells 3 times with PBS to remove the radioactivity in the medium, the cells were solubilized in 1 mL PBS containing 0.1% SDS and 1% Triton X-100. An aliquot of the dissolved cells was mixed with 4 mL of liquid scintillation solution and counted as described above. Cholesterol uptake, expressed in micrograms of cholesterol oleate per gram of hepatocytes per hour, was calculated by taking into account the specific activity values of the [^{3}H]cholesterol oleate–labeled HDL.

2.9. Sphingomyelin assay on HDL

An assay was performed according to a colorimetric method previously reported [33], as follows: to extract lipids, 100 μL of each HDL solution was mixed with 300 μL of ice-cold chloroform-methanol (2:1, vol/vol), vortexed for 2 minutes, and centrifuged at 1000*g* for 5 minutes (4°C). The entire chloroform layer (bottom layer) was transferred to a new tube, and the solvent was evaporated at 65°C in a water bath. The sample was allowed to cool down, then 500 μL of chloroform-methanol (2:1, vol/vol) was added, mixed briefly to dissolve lipid residue, and the sample was stored at −20°C until use. For the assay, 100 μL of the sample lipid extract was mixed with 200 μL of 0.5 mmol/L lecithin in chloroform, evaporated at 65°

C under nitrogen, cooled down at room temperature, mixed with 200 μL of 48 mmol/L Zwittergent 3–10, vortexed briefly, and then returned at 65°C water bath for 5 minutes. Samples were then promptly vortexed for about 1 minute, followed by a centrifugation at 6500*g* for 10 minutes. Insoluble material formed a thin layer on top of the solution, and the clear subnatant was used for the assay. Sphingomyelin standards were prepared similarly in 100 μL of 48 mmol/L Zwittergent 3–10 to have the following concentrations: 5, 10, 20, and 30 μmol/L. All standards also contained 250 μmol/L of lecithin. Sphingomyelin reagent composition was as follows: 50 mmol/L Tris-HCl (pH 7.8), 0.75 mmol/L 4-aminoantopyrine, 3 mmol/L 2-hydroxy-3,5-dichlorobenzenesulfonic acid, 3 U/mL choline oxidase, 1 U/mL sphingomyelinase, 5 U/mL peroxidase, and 1 U/mL alkaline phosphatase. Fifty microliters of each standard and samples were distributed in separate wells in a 96-well microplate kept ice-cold and 100 μL of SPM reagent was added promptly to all used wells, and kinetics was measured immediately in a Spectramax microplate reader (Molecular Devices, Sunnyvale, CA). Enzymatic kinetics was monitored at 510 nm for 30 minutes at 37°C. Kinetics data were exported into a Excel spreadsheet software (Microsoft, Redmond, WA) for samples' SM content determination expressed in micromoles per liter of HDL SM in plasma.

2.10. Statistical analyses

The significance of the various effects was evaluated by a 4-way analysis of variance (ANOVA) and 1-way ANOVA with post hoc Tukey test using SAS software (SAS Institute, Cary, NC).

3. Results and discussion

Sphingomyelin content of HDL seems to play a critical role in RCT function [7]. We recently showed that HDL PL composition, that is, saturation of the fatty acyl chain of the PC and SM content was critical in regulating HDL RCT function [16]. This previous study with reconstituted HDLs with known concentrations of SM clearly showed that cholesterol efflux was markedly affected by the depletion of SM content of HDL [16]. In an attempt to rationalize the inhibitory effect of long-term ethanol consumption both in rats and in humans on the RCT function of HDL, our previous work is extended herein in vivo to compare the influence of long-term alcohol consumption on the RCT capacity of HDL with the alteration in HDL SM content both in rats fed ethanol on long-term basis and in alcoholic individuals.

3.1. Effect of ethanol on SM content in rat HDL

Thus, in the present study, 2 groups of male Wistar rats were pair-fed either the isoenergetic control liquid diet or the long-term ethanol (36% of ethanol dietary calories [EDC]) liquid diet for a period of 8 weeks. High-density lipoprotein was isolated and characterized from the animals of both groups. Results of the analysis of plasma HDL components, that is, SM, PL, cholesterol, and triglycerides, are listed in Table 1. As expected, there was a significant increase in plasma HDL cholesterol in the ethanol group compared with that in the control group (+41.0%, $P < .02$). Also, HDL PL content was significantly increased by 55.3% ($P < .05$) in the ethanol group compared to that in the control group. Significantly, plasma HDL SM content was decreased in the ethanol group (15.4%, $P < .01$), with a relative decrease of 51.3% (SM/PLs) ($P < .01$). We have previously shown that long-term ethanol consumption increased plasma cholesterol, apo A-I, and triglycerides [34,35], indicating the specific effect of ethanol on HDL composition. Now we have extended our findings demonstrating that long-term ethanol consumption induces the preferential depletion of SM relative to that of total PLs in rat plasma HDL.

3.2. Cholesterol efflux

To compare HDL SM content with RCT function, we first measured the ability of HDL from various groups to promote efflux, which is the release of cholesterol from macrophages in an

in vitro system. Results, listed in Table 2, are expressed as [^{3}H]cholesterol efflux into the culture medium as a percentage of the initial [^{3}H]cholesterol loaded inside the macrophages. Results showed that there was a 20% decrease ($P < .05$) of cholesterol efflux with HDL obtained from ethanol-treated animals than with HDL obtained from control animals. Thus, HDL with ethanol-induced lower SM content has a significant diminished ability to carry out cholesterol efflux.

3.3. Cholesterol uptake

To continue our analysis of the relationship between HDL SM content and RCT function, we then measured the ability of HDL from various rat groups to promote uptake, which is the transfer of cholesterol from HDL into hepatocytes. Cholesterol uptake capacity was measured with all different combinations of radiolabeled HDLs and isolated hepatocytes from both rat groups. The results are shown in Table 3. Compared with that in the control group, HDLs from the ethanol group promoted 13.8% ($P < .05$) and 35.0% ($P < .05$) less uptake, with control and ethanol hepatocytes, respectively. Conversely, compared with the control group, hepatocytes from the ethanol group promoted 31.0% ($P < .01$) and 48.0% ($P < .01$) less uptake, with control and ethanol HDLs, respectively. Importantly, further analysis of the data by a 4-way ANOVA showed that overall long-term ethanol consumption affected hepatocyte properties more than that of HDL during cholesterol uptake.

3.4. Effect of ethanol on SM content in human HDL

To extend the above study in humans, we then studied HDL SM content and RCT in 3 subjects groups, namely, nondrinkers, ALC, and ALD. Our previous studies in these subject groups showed that total cholesterol and LDL cholesterol did not differ significantly among the various groups. However, HDL cholesterol tended to increase in alcoholic groups (ie, ALC and ALD) compared with that in nondrinking controls [2], which is comparable to the results obtained in our animal system (see Table 1). Measurement of SM content was carried out in the HDL fractions obtained from the plasma of the study subjects, and results are listed in Table 4. It can be seen that there was a dramatic decrease of SM content in HDL by 51.5% ($P < .01$) in subjects classified as ALC and by 51.3% ($P < .01$) in subjects classified as ALD compared with control (nondrinkers) subjects.

3.5. Cholesterol efflux and uptake with HDLs from humans

Analysis of the RCT function of these subjects was reported previously [2] and the results are summarized in Table 5. It can be seen that cholesterol efflux capacities of HDLs from ALC and ALD groups were inhibited by 83% ($P < .01$) and 84% ($P < .01$), respectively, compared with HDLs from the control group. Also, with regard to cholesterol uptake, HDLs from ALC and ALD groups were less efficient by 54% and 61% ($P < .01$), respectively, compared with HDLs from the control group.

In confirmation of our previous in vitro findings, our present study shows that long-term ethanol feeding leads to the depletion of SM from plasma HDL (Table 1), which is accompanied by a decreased ability of SM-depleted HDLs to carry out their RCT function, both cholesterol efflux and cholesterol uptake (Tables 2 and 3). This effect of ethanol was even more apparent in alcoholic individuals with or without liver disease who showed a more dramatic decrease in plasma HDL SM content with a concomitant impaired RCT capacity of their HDL (Tables 4 and 5). Thus, individuals with heavy alcohol consumption might be at greater coronary artery disease risk due to impaired RCT function.

On the other hand, our finding that hepatocytes from animals fed ethanol on long-term basis had a greater impairment in cholesterol uptake than HDL from the same animals group (compare −31.0% and −48.0% with −13.8% and −35.0%, see Table 3) implies that ethanol

affects the hepatocyte component (eg, cholesterol/HDL receptor uptake mechanism) more than it affects the HDL SM component with regard to the cholesterol uptake function. In fact, we have previously shown that depleting SM in reconstituted HDL (rHDL) had a clear positive regulatory effect on cholesterol uptake [16]. Others have reported the same effect in another experimental system in which HDL enriched in SM decreased the net influx (ie, uptake) of cholesterol in SR-BI–expressing COS-7 cells [8] and CHO cells [36]. Thus, it appears that the effect of long-term ethanol consumption on the hepatocyte uptake mechanism is critical to induce a decrease of cholesterol uptake with SM-depleted HDLs. High-density lipoprotein receptors, such as SR-BI or other intracellular elements involved in promoting the binding of HDL on the hepatic plasma membrane, might be affected by ethanol exposure. Based on these findings, we conclude that long-term ethanol significantly impairs not only cholesterol efflux function of HDL by decreasing its SM content but also cholesterol uptake by affecting presumably hepatocyte receptors for HDL.

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Table 1

Effect of long-term alcohol consumption on the plasma levels of various HDL components in rats

HDL component	Rat groups		Statistics	
	Control	Ethanol	*P* (Tukey test)	% Change in alcohol group
SM (μmol/L)	81.6 ± 5.6	68.6 ± 3.9	<.05	−15.4
Phospholipid (μmol/L)	613 ± 161	953 ± 141	<.05	+55.3
SM/PL	0.043 ± 0.004	0.026 ± 0.002	<.01	−51.5
Cholesterol (μmol/L)	1896 ± 161	2675 ± 255	<.05	+41.0
Triglycerides (μmol/L)	778 ± 27	756 ± 11	NS	
Apolipoprotein AI (mg/dL)	102 ± 17	122 ± 19	NS	

Results are expressed as mean ± SD (n = 8). High-density lipoproteins were obtained by precipitation from plasma of rats fed a control or an ethanol (36% ethanol dietary calories [EDC]) liquid diet for 8 weeks. Various assays are described in the Methods section. NS indicates not significant.

Table 2

Effect of long-term ethanol consumption on cholesterol efflux function in rats

HDL source	Cholesterol efflux (n = 8)	Statistics	
	(% ± SD)	*P* (Tukey test)	% Change in alcohol group
Control	15 ± 2		
Ethanol	12 ± 3	< .05	−20.0

High-density lipoproteins were obtained by precipitation from plasma of rats fed a control or an ethanol (36% EDC) liquid diet for 8 weeks and tested for cholesterol efflux with macrophages, as described in the Methods section. Percentage of efflux was calculated as the proportion of [^{3}H]cholesteryl oleate loaded in the macrophages that was released in the medium after a 24-hour incubation with FCS-free medium containing HDL.

Table 3

Effect of long-term ethanol consumption on cholesterol uptake function in rats

Hepatocyte source	HDL source		Statistics	
	Control	Ethanol	*P* (Tukey test)	% Change in alcohol group
Control	29.0 ± 2.2	25.0 ± 4.8	< .05	−13.8
Ethanol	20.0 ± 4.2	13.0 ± 4.5	< .01	−35.0
P (Tukey test)	< .01	< .01		
% Change in alcohol group	−31.0	−48.0		

Results are expressed as mean ± SD (n = 8). High-density lipoproteins were obtained by precipitation from plasma of rats fed a control or an ethanol (36% EDC) liquid diet for 8 weeks, labeled with [^{3}H]cholesteryl oleate, and then tested for cholesterol uptake with hepatocytes isolated from the same experimental rats, as described in the Methods section. Using the specific activity of the labeled HDL, we calculated the uptake as the amount of cholesterol that was incorporated per gram of liver (micrograms of cholesterol per gram of liver).

Table 4

Sphingomyelin content in plasma HDL of various human groups

Group	n	[HDL SM]	Statistics	
		*μ*mol/L	% Decrease from control	*P* (Tukey test)
ND	9	67.7 ± 12.7	—	—
ALC	8	32.8 ± 7.5	51.5	<.01
ALD	6	33.0 ± 8.2	51.3	<.01

High-density lipoproteins were isolated by precipitation from plasma collected from study subjects belonging to 1 of the 3 study groups: nondrinkers (ND), alcoholic individuals without liver disease (ALC), or alcoholic individuals with liver disease (ALD). Sphingomyelin assay was performed as described in the Methods section. [HDL SM] indicates plasma concentration of SM bound to HDL.

Table 5

Effect of long-term ethanol consumption on cholesterol RCT function (efflux and uptake) of human HDLs

Group	n	Efflux	Statistics		Uptake (μg cholesterol per hour per gram of hepatocytes)	Statistics	
		%	% Decrease from control	*P* (Tukey test)		% Decrease from control	*P* (Tukey test)
ND	9	24.9 ± 2.6	—	—	47.3 ± 6.3	—	—
ALC	8	4.3 ± 0.5	83.0	<.01	21.6 ± 1.7	54.0	<.01
ALD	6	4.0 ± 0.3	84.0	<.01	18.3 ± 4.7	61.0	<.01

High-density lipoproteins were isolated by precipitation from plasma collected from study subjects belonging to 1 of the 3 study groups: ND, alcoholic individuals without liver disease (ALC), or alcoholic individuals with liver disease (ALD). Data are the results of analysis reported previously [2].

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