Effect of Dietary Omega-3 Fatty Acids and Chronic Ethanol Consumption on Reverse Cholesterol Transport in Rats

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We previously showed that chronic ethanol feeding leads to a decrease of apolipoprotein E (apoE) in high-density lipoprotein (HDL), whereas supplementing this diet with 2.8% of total dietary calories as ω3-fatty acids (ω3FAs) restores HDL-apoE to the control values. Since HDL containing apoE plays a major role in reverse cholesterol transport (RCT), we measured the effects chronic ethanol intake and ω3-FAs on RCT in the present study. Four groups of rats, control normal fat (CN), alcohol-normal fat (AN), control ω3FA fat (CF), and alcohol-ω3FA fat (AF), were fed their respective diets for 8 weeks, after which hepatocytes and HDLs from each group were evaluated for RCT capacity (cholesterol efflux from macrophages and uptake by liver cells). Compared with the control diet (CN), chronic ethanol (AN) feeding inhibited the cholesterol efflux capacity of HDL by 21% ($P < .01$), whereas ω3FA feeding (2.8% of total dietary calories) stimulated this capacity by 79% ($P < .01$) and 25% ($P < .01$) in CF and AF rats, respectively. With respect to cholesterol uptake by the liver, there were no significant 3-way or 4-way interactions between the 4 factors, HDL-alcohol, HDL-fish oil, hepatocyte-alcohol, and hepatocyte-fish oil. The main effects for HDL-alcohol, HDL-fish oil, and hepatocyte-alcohol were all highly significant ($P = .0001$, .0001, and .007, respectively). There was a significant HDL-alcohol and HDL-fish oil interaction ($P = .0001$). Hepatocyte-alcohol was not a factor in any 2-way interactions. Our study indicates no evidence of an interaction between the effects of ω3FAs and the effects of alcohol on hepatocytes in terms of RCT function. Thus, feeding as little as 2.8% of the total dietary calories as ω3FA not only restored the impaired RCT function of HDL caused by chronic ethanol intake, but also enhanced by severalfold the ability of HDL to promote RCT even in normal animals.

THE BENEFICIAL EFFECT of fish oil rich in ω3-fatty acids (ω3FAs) on the 20-year mortality from coronary heart disease is well known.[1] Fish oil, which is rich in ω3FAs, also has been shown to have a pronounced hypolipidemic effect.[2-7] Members of the ω3FA and ω6FA family are considered essential fatty acids, as the body cannot synthesize these de novo.[5] Both types of polyunsaturated fat diets reduce cholesterol in the plasma and in low-density lipoprotein (LDL).[3] Neither diet has any effect on cholesterol in plasma high-density lipoprotein (HDL). On a unit-weight basis, the ω3FAs have a greater hypocholesterolemic effect than the ω6FAs. Some of the possible mechanisms involve inhibition of lipid synthesis via acetyl coenzyme A carboxylase and FA synthetase[8-10] and very–low-density lipoprotein (VLDL) synthesis.[11] Phillipson et al[12] have shown the lipid-lowering effects of fish oil rich in ω3FAs in humans.

However, another study has demonstrated that ω3FAs increased the susceptibility of LDL to oxidation and uptake by macrophages in vitro and has recommended dietary supplementation of α-tocopherol as an antioxidant.[13] Tsukamoto et al[14] have shown that chronic alcohol feeding by the intragastric method caused more liver damage when polyunsaturated fatty acids (PUFAs) from either vegetable oil (ω6 family) or fish oil (ω3 family) replaced saturated fat in the diet.[15] In this study, fish oil constituted 35% of the total calories in the diet, with 13.8% of the total dietary calories as ω3-FA. In contrast, we showed[16] that the presence of only 2.8% of total dietary calories as ω3-FA resulted in lower plasma and liver lipids in both chronic ethanol-fed and control rats. Furthermore, the same low level of dietary ω3FA restored the decreased apoE content of plasma HDL in chronic ethanol-fed rats. It is well established that the apoE component of HDL plays a major role in the reverse cholesterol transport (RCT) function of HDL.[17,18]

Because of the known deleterious effects of chronic ethanol intake on lipoprotein metabolism[19] and the beneficial effect of ω3FAs on the lipid profile and apoE content of HDL,[16] we have examined the effects of ω3FAs (2.8% of total dietary calories) and chronic alcohol intake (36% of total dietary calories) on the RCT function of HDL. It is shown in this study that the presence of 2.8% of total dietary calories as ω3FAs in the alcohol diet not only can completely reverse the impaired RCT function of HDL in chronic ethanol feeding, but also can enhance this HDL function in normal animals.

MATERIALS AND METHODS

Reagents

All reagents and chemicals were of analytical grade. Diet components were from ICN Biomedicals (Costa Mesa, CA) or Dyets (Bethlehem, PA).

Animals and Feeding Regimen

Male Wistar-Furth rats (body weight, 125 to 150 g) were procured from Charles River (Wilmington, MA). After 1 week of acclimatization, they were divided into 4 groups and fed for 8 weeks on one of the following diets: control regular (CN), ethanol regular (AN), control ω3FA (CF), and ethanol ω3FA (AF). The AN diet was essentially the same as that used by Lieber and DeCarli[20] and had the following nutrient composition (in g/L): 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 carbohydrate. The corresponding CN diet contained equicaloric amounts of dextrin maltose in place of ethanol. The regular fat content of these diets was

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replaced by an oil mixture of olive oil, cod-liver oil, and menhaden fish oil (gift from Zapata Haynie, Reedville, VA) in a ratio of 67:8:25 in the ω3FA diets. The ω3FA content of the regular fat diets was 0.6% of the total FAs, while that of the ω3FA diets was 7% (3.88% C20:5 and 3.03% C22:6) based on gas chromatography analysis. Thus, the ω3FA content was 0.24% of the total dietary calories in the regular fat diet and 2.8% in the fish oil diet. Rats in the control group were pair-fed with the animals in the corresponding ethanol group. At the end of 8 weeks, all rats (except those saved for liver perfusion) were killed by aortic exsanguination under pentobarbital anesthesia (50 mg/kg body weight). The plasma from these rats was saved and used for isolation of HDL.

Isolation of Rat HDL From Rat Plasma

HDLs from the CN, AN, CF, or AF groups were isolated from the plasma by the precipitation method of Gidez et al.[21] Briefly, 0.1 vol heparin-$MnCl_2$ solution was added to 1.0 vol plasma and incubated for 30 minutes at room temperature. Next, the samples were centrifuged for 1 hour at 3,000 × *g* at 4°C. The supernatant of heparin-Mn^{2+} was taken for precipitation of HDL by addition of 0.1 vol dextran sulfate (final concentration, 0.65% wt/vol). After thorough mixing, the sample was left at room temperature for 30 minutes and then centrifuged for 30 minutes at 4°C as before. The HDL pellet thus obtained was solubilized in 10 mmol/L sodium bicarbonate, pH 8.3, and dialyzed extensively against physiological saline buffered with 10 mmol/L phosphate-buffered saline, pH 7.4 (PBS). Total protein was determined by the Bradford method (Bio-Rad, Hercules, CA) and cholesterol was determined according to the method of Zlatkis and Zak.[22]

Labeling of HDL With [^{3}H]Cholesterol Oleate

A 2- to 5-μCi quantity of [^{3}H]cholesterol oleate in acetone was solubilized in 200 μL dimethylsulfoxide, followed by addition of approximately 1.0 mL saline/EDTA drop-wise with continuous mixing and incubation at 37°C for 10 minutes. Then, 0.5 mL HDL (2 mg/mL) was added again in a drop-wise manner with continuous mixing. The reaction was incubated at 37°C for 3 hours. This was followed by extensive dialysis of the labeled HDL against saline/EDTA. Aliquots of the labeled HDL samples were analyzed for radioactivity and the specific activity was determined.

Isolation of LDL and Its Modification

LDL (density, 1.019 to 1.063 g/mL) was isolated from human plasma by differential ultracentrifugation.[23] LDL was chemically modified by acetylation using the method of Basu et al.[24] Briefly, 1 mL 0.15-mol/L NaCl containing 4 to 8 mg LDL protein was added to an equal volume of saturated solution of sodium acetate with continuous stirring on ice. Next, acetic anhydride was added in multiple small aliquots (2 μL) over a period of 1 hour with continuous stirring. After the addition of a total mass of acetic anhydride equal to 1.5 times the mass of LDL protein used, the mixture was stirred for an additional 30 minutes. The reaction was then dialyzed with several changes against 3 L saline/EDTA solution at 4°C.

Labeling of Modified LDL Preparations

Acetylated LDL was labeled with [^{3}H]cholesterol oleate as previously described,[24] similar to the procedure for labeling HDL described earlier.

Cell Culture

Mouse J774.A macrophages were obtained from the American Type Culture Collection (Rockville, MD). Dulbecco's modified Eagle's medium (DMEM) (high-glucose) was obtained from Life Technologies (Gaithersburg, MD). Heat-inactivated fetal bovine serum, glutamine, and antibiotics were obtained from Bio-Whittaker (Walkersville, MD). All media were supplemented with 2 mmol/L glutamine.

Cholesterol Efflux Experiments

Macrophages were plated 24 to 48 hours prior to efflux experiments in 6-well plates at a density of 1×10^6 cells per well with 4 mL DMEM supplemented with 10% heat-inactivated fetal bovine serum. The cells were incubated at 37°C in a humidified incubator in an atmosphere of 5% CO_2. Pretreatment was started when the cells were about 90% confluent. Cells were labeled by removing the spent medium and adding fresh medium containing the indicated amounts of labeled acetylated LDL. Incubations were continued for another 24 hours, during which time approximately 50 to 60% of the added label was loaded on the macrophages. Next, the cell layers were washed 3 times with PBS to completely remove the labeled acetyl LDL that was not taken up by the cells. These labeled acetyl LDL–loaded cells were next incubated for another 24 hours in the presence of serum-free DMEM containing the indicated amounts of HDL isolated from CN, AN, CF, or AF rat plasma or an equal amount of rat albumin as a negative control. Efflux was calculated by counting the [^{3}H]cholesterol label effluxed into the culture medium in an aliquot of the cell-free culture supernatant.

Time Course and HDL Concentration Effects on Cholesterol Efflux

We already established in our preliminary experiments that the time course of cholesterol efflux in the macrophage system was linear over a period of 48 hours and up to 200 μg HDL (regardless of the source of HDL) per incubation mixture under the above-mentioned conditions (data not shown). Therefore, all subsequent efflux experiments were performed for 24 hours with 100 μg HDL from the various groups to determine the efflux in the linear range of the efflux process.

Isolation of Hepatocytes by Liver Perfusion

The liver perfusion was performed essentially according to the method described by our group previously,[25] which is based on the method of Hems et al.[26] 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 uniformly and completely drained the blood through the inferior vena cava below the liver which 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 by the method of Berry and Friend[27] with modifications as described previously,[28] except that glucose was added to the final perfusion medium (20 mol/L) to minimize hepatic glycogenolysis.[29] These hepatocytes were 90% to 95% viable as tested by trypan dye exclusion and satisfied the criteria for intact, viable, and metabolically active hepatocytes as defined by Krebs et al.[28]

Time Course and HDL Concentration Effects on Cholesterol Uptake

We already established in our preliminary experiments that the time course of cholesterol uptake in the hepatocyte system was linear over a period of 2 hours and up to 200 μg HDL (regardless of the source of HDL) per incubation mixture under the above-reported conditions (data not shown). Therefore, all subsequent uptake experiments were performed for 90 minutes with 60 μg HDL from various groups to determine the uptake in the linear range of the uptake process.

Cholesterol Uptake Experiments

The incubation procedure for measurement of the rate of [^{3}H]cholesterol oleate uptake was as follows. Hepatocytes (CN, AN, CF, or AF)

equivalent to about 100 mg wet weight 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]cholesterol oleate–labeled HDL fractions (from CN, AN, CF, or AF rat plasma), when added, were in NaCl/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. The uptake of [^{3}H]cholesterol oleate label was monitored by centrifuging 200 μL cell suspension and washing the cell pellet 3 times with saline/EDTA. The cell pellet was solubilized in Scintisol (Packard, Downers Grove, IL) and incorporation of the tritium label was measured in a Beckman LS 6500 scintillation counter. The difference in counts at time 0 and after 2 hours was used to calculate the uptake of [^{3}H]cholesterol oleate.

Statistical Analyses

All statistical analyses were performed using SAS software (SAS Institute, Cary, NC). A 4-way ANOVA was performed on the logarithmically transformed values. The residual from the ANOVA was tested for normality with the Wilk-Shapiro test and the variance appeared uniform on residual plots. The 4 factors were HDL as a function of alcohol (HDL-alcohol), HDL as a function of fish oil (HDL-fish oil), hepatocytes as a function of alcohol (hepatocyte-alcohol), and hepatocytes as a function of fish oil (hepatocyte-fish oil).

RESULTS AND DISCUSSION

Effect of ω3FAs and Ethanol on Efflux of Cholesterol From Macrophages

Figure 1 depicts the ability of various HDL preparations from different dietary groups for ^{3}H-cholesterol efflux from mouse macrophages. The results are expressed as ^{3}H-cholesterol efflux into the culture medium as a percent of the initial ^{3}H-cholesterol

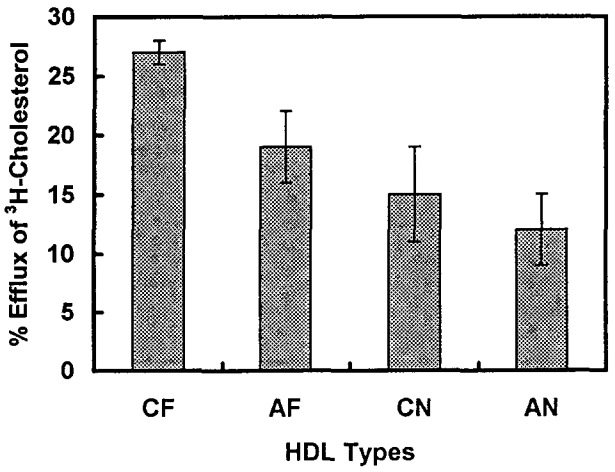

Fig 1. Effect of ω3FA and ω3FA-alcohol diet on the function of HDL in cholesterol efflux during RCT. Mouse J774.A macrophages (1 million cells per well) were first loaded with ^{3}H-cholesterol oleate–labeled acetylated LDL for 24 hours. Next, the cell layers were washed with PBS 3 times to completely remove the labeled acetylated LDL that was not taken up by the cells. The cell layers were fed with 4 mL serum-free fresh medium supplemented with 100 μg of the indicated HDL samples per well in duplicate and incubated for another 24 hours at 37°C. A small aliquot of culture medium was removed at time 0 and later after 24 hours and effluxed labeled LDL was counted, and based on these counts, the % efflux was calculated and compared between CF, AF, CN, and AN rats. Each value is the mean ± SD of 3 independent HDL samples from each group.

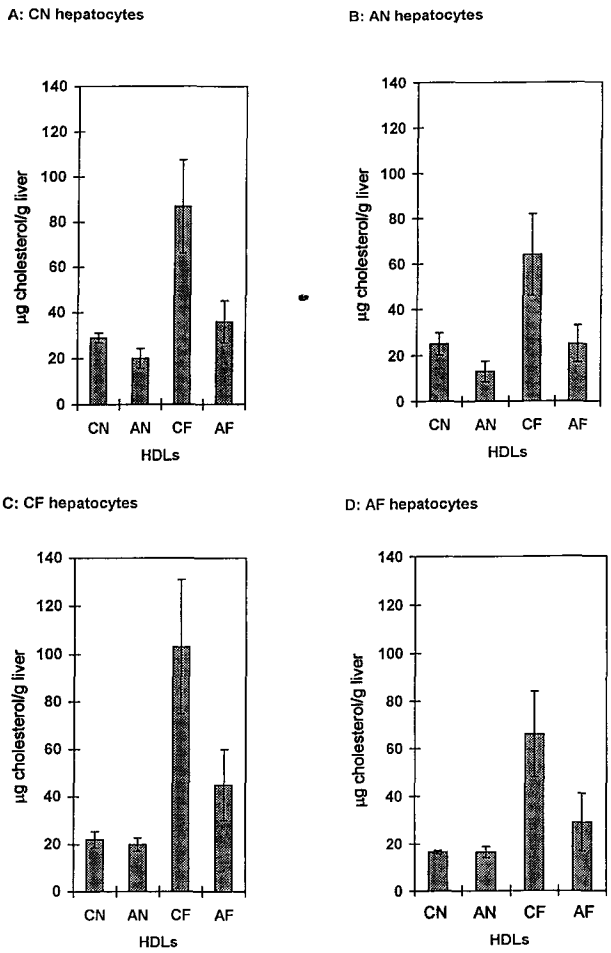

Fig 2. Effect of ω3FA and ω3FA-alcohol diet on the function of HDL in cholesterol uptake by hepatocytes during RCT. Animals were fed their respective diets for 8 weeks. At the end of 8 weeks, hepatocytes and HDLs were prepared from each group of rats. Hepatocytes from CN (A), AN (B), CF (C), or AF (D) perfused livers were prepared. Hepatocyte cell suspension (2 mL) was incubated with 60 μg of the indicated ^{3}H-cholesterol oleate–labeled HDL samples (CN, AN, CF, or AF HDL) in duplicate at 37°C for 90 minutes with constant shaking in a water bath. Later, an aliquot of cell suspension was removed and washed 3 times with PBS, and the final washed cell pellet was solubilized in 1% sodium dodecyl sulfate, 10 mmol/L Tris hydrochloride, 1 mmol/L EDTA (pH 7.4) buffer and analyzed for radioactivity. Using the specific activity of the labeled HDL, the uptake of HDL was calculated in terms of micrograms of cholesterol. Each value is the mean ± SD of 3 independent HDL samples from each group. A 4-way ANOVA was performed to determine the interaction between alcohol, ω3FAS, hepatocytes, and the different HDL samples.

inside the cells. Compared with the control diet (CN), chronic ethanol feeding (AN) inhibited the cholesterol efflux capacity of HDL by 21% ($P < .01$), whereas ω3FA feeding (2.8% of total dietary calories) stimulated this capacity by 79% ($P < .01$) and 25% ($P < .01$) in control (CF) and alcohol-fed (AF) rats, respectively.

Effect of ω3FAs and Ethanol on Uptake of Cholesterol by Hepatocytes

As described earlier, experiments were performed with all different combinations of hepatocytes (isolated from CF, AF,

CN, or AN rats) and the effects of ω3FAs and alcohol on hepatic cholesterol uptake were determined. The results are shown in Fig 2A to D. A 4-way ANOVA of the data showed that overall ω3FAs affected HDL properties more than that of the hepatocytes during RCT.

Previously, we have shown[16] that the effects of ω3FAs and chronic ethanol intake on plasma apoA and apoE were significant. We showed that plasma apoA was increased by ethanol ($P < .001$) and decreased by ω3FAs ($P < .002$) without any interaction between the two treatments.[16] In this study, we have examined the effect of very low dietary ω3FA (2.8% of total calories) on the capability of HDL to promote RCT in AF rats compared with the capability of HDLs from CN, AN, and CF groups. There were no significant 3-way or 4-way interactions. The main effects for HDL-alcohol, HDL-fish oil, and hepatocyte-alcohol were all highly significant ($P = .0001$, .0001, and .007, respectively). There was a significant interaction between HDL-alcohol and HDL-fish oil ($P = .0001$). Hepatocyte-alcohol was not a factor in any 2-way interactions.

Our study indicates no evidence of an interaction between the effects of ω3FAs and the effects of alcohol on the properties of hepatocytes in terms of RCT function. However, it appears that ω3FAs significantly altered the properties of HDL in such a way that HDL isolated from the CF group was severalfold more efficient in the RCT function compared with HDL isolated from any of the other groups. Thus, feeding as little as 2.8% of the total dietary calories as ω3FAs not only restored the impaired RCT function of HDL caused by chronic ethanol intake, but also enhanced by severalfold the ability of HDL to promote RCT even in normal animals.

Thus, ω3FA has a beneficial effect at a dietary level of 2.8% of total calories, whereas a 5-fold increase in ω3FA (compared with our study) seems to have a detrimental effect on the liver.[15] In view of these findings, it seems that the ω3FA concentration of the diet should be moderate to observe its beneficial effects. Perhaps dietary supplementation of α-tocopherol as an antioxidant is another prerequisite (our diet had 120 IU α-tocopherol/L) for ω3FA intervention studies in order to observe its beneficial effects. It is recognized that the daily ethanol consumption amounts to 47% of the total calories in the intragastric model,[14] whereas it is usually 36% of the total calories in the Lieber-DeCarli model of ethanol feeding. This added ethanol consumption also could have caused the deleterious effects when a PUFA-rich diet was fed. Interestingly, long-term PUFA containing lecithin supplementation, but not saturated FAs containing lecithin, has been demonstrated to prevent alcohol-induced hepatic fibrosis in baboons.[30,31] Obviously, the PUFA-rich lecithin must confer some specificity for its beneficial effect in preventing ethanol-mediated fibrosis, although the underlying mechanism remains to be determined. The beneficial effects of ω3FAs (another class of PUFAs) on the RCT function of HDL found in the present study are consistent with these findings, although it seems that ω3FAs should be included in the diet in moderation.

Possible Actions of Ethanol and ω3FAs

Based on the present study and the current status in this field, our hypotheses are as follows: ethanol exposure leads to increased VLDL production and sialic acid–deficient apoE that has a higher affinity for VLDL than for HDL.[32] This results in a depletion of the apoE component of HDL[16] and a consequently impaired RCT after chronic alcohol exposure (present study). In contrast, ω3FAs seem to have the opposite effect, as evidenced by the restoration of the apoE component of HDL[16] and RCT by ω3FAs in ethanol-fed animals. Furthermore, the fact that ω3FAs significantly stimulated the RCT function of HDL severalfold even in normal animals (Fig 2) implies that restoration of the apoE component of HDL is not the only mechanism by which ω3FAs have a beneficial effect on RCT. This raises other possible mechanisms of action of ω3FAs in regulating the RCT function of HDL. In this regard, it is well known that HDL phospholipid acyl-chain composition is known to influence cholesterol efflux.[33] It is possible that ω3-FAs alter the FA composition of HDL phospholipids that may influence its RCT function. A study is currently in progress to test this hypothesis.

REFERENCES

1. Kromhout D, Bosschieter EB, de Lezenne Coulander C: The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 312:1205-1209, 1985

2. Harris WS, Connor WE: The effects of salmon oil upon plasma lipids, lipoproteins, and triglyceride clearance. Trans Assoc Am Phys 93:148-155, 1980

3. Harris WS, Connor WE, McMurry MP: The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: Salmon oil versus vegetable oils. Metabolism 32:179-184, 1983

4. Harris WS, Connor WE, Inkeles SB, et al: Dietary omega-3 fatty acids prevent carbohydrate-induced hypertriglyceridemia. Metabolism 33:1016-1019, 1984

5. Ahrens EH Jr: The management of hyperlipidemia: Whether, rather than how. Ann Intern Med 85:87-93, 1976

6. Endres S, von Schacky C: n-3 Polyunsaturated fatty acids and human cytokine synthesis. Curr Opin Lipidol 7:48-52, 1996

7. Harris WS: n-3 Fatty acids and serum lipoproteins: Human studies. Am J Clin Nutr 65:1645S-1654S, 1997 (suppl)

8. Yang YT, Williams MA: Comparison of C18-, C20- and C22-unsaturated fatty acids in reducing fatty acid synthesis in isolated rat hepatocytes. Biochem Biophys Res Commun 531:133-140, 1978

9. Iritani N, Inoguchi K, Endo M, et al: Identification of shellfish fatty acids and their effects on lipogenic enzymes. Biochim Biophys Acta 618:378-382, 1980

10. Iritani N, Fukuda E, Inoguchi K, et al: Reduction of lipogenic enzymes by shellfish triglycerides in rat liver. J Nutr 110:1664-1670, 1980

11. Nestel PJ, Connor WE, Reardon MF, et al: Suppression by diets rich in fish oil of very low density lipoprotein production in man. J Clin Invest 74:82-89, 1984

12. Phillipson BE, Rothrock DW, Connor WE, et al: Reduction of plasma lipids, lipoproteins, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N Engl J Med 312:1210-1216, 1985

13. Suzukawa M, Abbey M, Howe PR, et al: Effects of fish oil fatty acids on low density lipoprotein size, oxidizability, and uptake by macrophages. J Lipid Res 36:473-484, 1995

14. Tsukamoto H, Towner SJ, Ciofalo LM, et al: Ethanol-induced liver fibrosis in rats fed high fat diet. Hepatology 6:814-822, 1986

15. French SW: Nutrition in the pathogenesis of alcoholic liver disease. Alcohol Alcohol 28:97-109, 1993

16. Lakshman MR, Chirtel SJ, Chambers LL: Roles of omega 3 fatty acids and chronic ethanol in the regulation of plasma and liver lipids and plasma apoproteins A1 and E in rats. J Nutr 118:1299-1303, 1988

17. Mahley RW: Cellular and molecular biology of lipoprotein metabolism in atherosclerosis. Diabetes 30:60-65, 1981

18. Basu SK, Ho YK, Brown MS, et al: Biochemical and genetic studies of the apoprotein E secreted by mouse macrophages and human monocytes. J Biol Chem 257:9788-9795, 1982

19. Lakshman: Effect of chronic ethanol feeding upon the catabolism of protein and lipid moieties of chylomicrons and VLDL in vivo and in the perfused heart system. Alcohol Clin Exp Res 9:327-330, 1985

20. Lieber CS, DeCarli LM: The feeding of alcohol liquid diets: Two decades of applications and 1982 update. Alcohol Clin Exp Res 6:523-531, 1982

21. Gidez LI, Miller GJ, Burstein M, et al: Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. J Lipid Res 23:1206-1220, 1982

22. Zlatkis A, Zak B: Study of a new cholesterol reagent. Anal Biochem 29:143-148, 1969

23. Schumaker VN, Puppione DL: Sequential floatation ultracentrifugation. Methods Enzymol 128:155-170, 1986

24. Basu SP, Goldstein JL, Anderson RGW, et al: Degradation of cationized low density lipoprotein and regulation of cholesterol metabolism in homozygous familial hypercholesterolemia fibroblasts. Proc Natl Acad Sci USA 73:8178-8182, 1976

25. Lakshman MR, Muesing RA, LaRosa JC: Regulation of cholesterol biosynthesis and 3-hydroxy-3-methylglutaryl coenzyme A reductase activity by chylomicron remnants in isolated hepatocytes and perfused liver. J Biol Chem 256:3037-3043, 1981

26. Hems R, Ross BD, Berry MN, et al: Gluconeogenesis in the perfused rat liver. Biochem J 101:284-292, 1966

27. Berry MN, Friend DS: High-yield preparation of isolated rat liver parenchymal cells: A biochemical and fine structural study. J Cell Biol 43:506-520, 1969

28. Krebs HA, Cornell NW, Lund P, et al: Isolated liver cells as experimental material. Presented at the Alfred Benzon Symposium, Copenhagen, Denmark, 1974

29. Harris RA: Studies on the inhibition of hepatic lipogenesis by *N*-6,*O*-2′-dibutyryl adenosine 3′,5′-monophosphate. Arch Biochem Biophys 169:168-180, 1975

30. Lieber CS, DeCarli LM, Mak KM, et al: Attenuation of alcohol-induced hepatic fibrosis by polyunsaturated lecithin. Hepatology 12:1390-1398, 1990

31. Lieber CS, Robins SJ, Li J, et al: Phosphatidylcholine protects against fibrosis and cirrhosis in the baboon. Gastroenterology 106:152-159, 1994

32. Marmillot P, Rao MN, Liu QH, et al: Desialylation of human apolipoprotein E decreases its binding to human high-density lipoprotein and its ability to deliver esterified cholesterol to the liver. Metabolism 48:1184-1192, 1999

33. Davidson WS, Gillotte KL, Lund-Katz S, et al: The effect of high density lipoprotein phospholipid acyl chain composition on the efflux of cellular free cholesterol. J Biol Chem 270:5882-5890, 1995