Fish Oil, Alcohol, and Liver Pathology: Role of Cytochrome P450 2E1 (43807)

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Abstract. **Rats were fed ethanol in combination with fish oil or a corn diet in order to evaluate the effect of fish oil feeding on liver injury, microsomal ethanol oxidation, and NADPH-dependent lipid peroxidation. The rats were maintained on the dietary regimen for 72 days, and for comparison, pair-fed controls were studied. The liver pathology score progressively worsened in rats fed alcohol, both in combination with fish oil and corn oil, but the severity of inflammation and focal fibrosis was greater in the ethanol fish oil fed rats as compared with the ethanol corn oil group, whereas the fatty change was greater in the ethanol corn oil fed rats. The alcohol treatment caused a 2-fold increase of the liver microsomal P450 content, and about a similar increase in the rate of microsomal NADPH oxidation. The amount of ethanol-inducible CYP2E1 was about 10-fold higher in alcohol-fed rats as compared with pair-fed controls. The NADPH-dependent lipid peroxidation in liver microsomes was about 10-fold higher in microsomes from alcohol-treated rats fed corn oil as compared with controls, but only 2- to 3-fold higher in alcohol-fed rats receiving fish oil than in pair-fed controls. This was due to a higher rate of NADPH-dependent lipid peroxidation in the control rats receiving fish oil. There was a pronounced correlation between the amount of CYP2E1 and the microsomal NADPH peroxidation in variously treated rats, and between the 2E1 levels and the pathology score. The data suggest that fish oil diet, like corn oil, supports ethanol-induced liver injury which is related to CYP2E1 induction and the presence of polyunsaturated fatty acids in the diet (i.e., either n-6 or n-3).**

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Preliminary data indicated that fish oil (20% menhaden oil) causes fatty liver and the induction of hepatic fibrogenic factors in the rat (1). When alcohol was added to the fish oil diet, many of the features of alcoholic liver disease (ALD), including fibrosis (2), were reproduced in rats. Recently we described some of the pathologic features of the liver in this experiment in a review of the role of nutrition in the pathogenesis of ALD (3). In the present report, we investigated the effect of ethanol and fish oil on liver cytochrome P450 2E1 (CYP2E1) as it relates to lipid peroxidation and liver pathology. This is the first publication which correlates the biochemical changes in liver microsomes with the liver pathology induced by fish oil and ethanol feeding in rats. It has already been established that ethanol ingestion induced CYP2E1 and lipid peroxidation (3–24) and fish oil ingestion increases CYP2E1 and lipid peroxidation in the liver (16, 25–31). The combination of ethanol and fish oil in the diet may enhance liver damage due to lipid peroxidation of polyunsaturated fatty acids.

The rationale for these studies, therefore, was to determine if a diet high in linoleic acid (18:2n-6) was essential for development of ALD in the intragastric tube feeding model. Previously, we showed that ALD was not produced in this model if the dietary fat was deficient in 18:2 (32). However, n-3 polyunsaturated fatty acids may substitute for 18:2n-6 to allow the development of ALD. Fish oil is ideal to test this hypoth-

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esis since it is very low in 18:2n-6 but is rich in polyunsaturated fatty acids (20:5n-3 and 22:6n-3) (3). Since the n-3 and n-6 fatty acids may be preferentially oxidized by different cellular compartments (i.e., microsomal or peroxisomal), some difference in the level of lipid peroxidation may be encountered when microsomal CYP2E1 is induced by ethanol when fish oil is substituted for corn oil in the diet.

Materials and Methods

Animals. The animal studies were conducted in compliance with applicable laws and regulations as well as principles expressed in the NIH, USPHS, Guide for the Care and Use of Laboratory Animals. The rats were male Wistar rats obtained from Charles River (Hollister, CA) weighing 210–240 g and were kept in 12-hr light:dark cycles in metabolic cages. They were divided into four groups: (i) fish oil plus ethanol; (ii) fish oil pair fed with isocaloric dextrose; (iii) corn oil plus ethanol; (iv) corn oil pair fed with isocaloric dextrose. There were five rats/group. The corn oil and fish oil (menhaden oil) each constitute 35% of dietary calories. The fish oil was obtained frozen from Biomedical Test Materials Program jointly sponsored by the National Institute of Health (NIH), the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA), and the National Oceanic and Atmospheric Administration (NOAA). It was vacuum extracted and analyzed for its fatty acid composition, and antioxidants were added before shipping (TBHQ 0.2 g/kg, α-tocopherol 1.1 g/kg and γ-tocopherol 0.9 g/kg). The fish oil was thawed and the diet mix containing antioxidants was added. It was made fresh daily and made bubble free by vacuum extraction. It was administered by syringe in a closed system which emptied directly into the stomach through an intragastric cannula. The diet was administered intragastric continuously over 24 hr. The intragastric cannula was implanted under thiopental sodium (2 mg/kg body wt ip) and ketamine hydrochloride (6 mg/kg body wt, im) anesthesia at which time a baseline liver biopsy was performed for morphology and baseline blood levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed on a clinical analyzer TDX.

Diet. The diets were prepared as described previously (17). The experimental liquid diet (1 kcal/ml) was fed continuously at the rate of 180 ml/kg body wt/day in order to achieve adequate weight gain. The fish oil was provided by the National Marine Fisheries Service.

Protein (25% of calories) was derived from lactalbumin hydrolysate. The diet was supplemented with the lipotropes choline bitartrate (0.53 g/liter) and DL methionine (1.05 g/liter). Vitamin mix was AIN-76A (Dyets 2.65 g/liter). Mineral mix was AIN-76 (Dyets 9.27 g/liter). The suspending agent from Bio-serve was used to prevent settling.

Alcohol (8 g/kg/day) was started to maintain high blood alcohol levels (BAL). As metabolic adaption developed the alcohol required to maintain a high BAL increased to about 14 g/kg/day. As the dose of alcohol increased, the calories derived from alcohol increased from 24% to 36%, and the amount of calories derived from fat and protein dropped to 30% and from 30% to 25%, respectively. Dextrose-fed isocaloric to controls increased as the ethanol was increased, so the decrease in fat and protein was controlled for. Carbohydrate in the diet was increased in the control but decreased in the ethanol-fed diet.

The fatty acid composition of the corn oil used was high in linoleic acid (18:2n-6) as previously published (32). Menhaden oil contained low levels of linoleic acid (1.8%) and high amounts of polyunsaturated fatty acids (3).

Morphology. Liver biopsies were obtained under anesthesia after the baseline biopsy, after 1 month, 2 months, and at sacrifice at 72 days. Blood alcohol levels and AST and ALT levels were obtained at the time of these procedures. Biopsies were taken from different liver lobes and all five lobes at sacrifice. The liver samples were processed and stained by H&E and sirus red for collagen. A pathology score was calculated for each group according to a published method (32). Liver samples were also processed for examination by electron microscopy (17) and immunocytochemistry for CYP2E1 using the rabbit antibody to this isozyme and the avidin biotin method using diaminobenzene as the chromagen.

Biochemical Analysis. Microsomal NADPH-cytochrome P450 reductase activity, NADPH-oxidation, NADPH-dependent lipid peroxidation and CYP2E1 were quantitated by previously published methods (17).

Statistical Analysis. The means ± SD were calculated on all data. Comparisons between groups were done using the unpaired *t* test. For correlation between parameters we calculated Pearson's or Spearman's correlation coefficients where appropriate.

Results

The alcohol consumed, the blood alcohol levels achieved, and the liver weights achieved are given in Table I. All five rats in all four groups survived the 72 days, at which time they were sacrificed. There was no significant difference in BAL except where the rats were fed corn oil and alcohol for 72 days. The liver weights were increased in the ethanol-fed rats compared with controls. There was a significant increase in the liver weights of rats fed fish oil and ethanol compared with the corn oil-fed rats fed ethanol.

Weight gain was significantly reduced in the rats

Table I. Fish Oil Versus Corn Oil: Diet, Alcohol, and Liver Weights

	Time (days)	Fish oil		Corn oil		Units
		Alcohol	Control	Alcohol	Control	
Alcohol given	30	14.2 ± 0.2[a]		14.5 ± 0.0		g/kg/day
	60	16.6 ± 0.3		15.9 ± 0.3		
	72	14.5 ± 0.4		14.2 ± 0.4		
Blood alcohol level	30	153 ± 34		140 ± 46		mg/dl
	60	142 ± 27		274 ± 65		
	72	212 ± 36		346 ± 65[b]		
Liver weight	72	23 ± 3.1	16.1 ± 1.1[c]	18.5 ± 2.9[d]	13.4 ± 1.8[e]	g

[a] Mean ± SEM. $n = 5$ for each group.
[b] $P < 0.05$ when compared with fish oil rats fed alcohol.
[c] $P < 0.005$ when compared with fish oil rats fed alcohol.
[d] $P < 0.05$ (1 tail t test) when compared with fish oil rats fed alcohol.
[e] $P < 0.02$ when compared with corn oil rats fed alcohol.

fed ethanol compared with the controls at 60 and 72 days (Fig. 1 and 2). The serum ALT levels are shown in Figure 3–4. There was a significant increase in ALT levels at all time periods after baseline for the rats fed ethanol, but there was no significant difference between rats fed ethanol and fish oil compared with the rats that were fed ethanol and corn oil. Serum AST levels also increased in the rats fed ethanol and fish oil or corn oil but the difference was not significantly different when compared with controls.

The morphologic changes in the ethanol plus fish oil-fed rats appeared more extensive compared with the ethanol plus corn oil-fed rats in terms of inflammation, necrosis, and fibrosis but not fatty change. Since the changes were focal, they were difficult to quantitate. The baseline biopsy livers showed no pathology. The necrosis and inflammation seen in the rats fed fish oil and ethanol is illustrated in Figure 5. The liver cells contained micro- and macrovesicular fat. Foci of necrotic liver cells mixed with macrophages and mononuclear cells were frequently seen. In

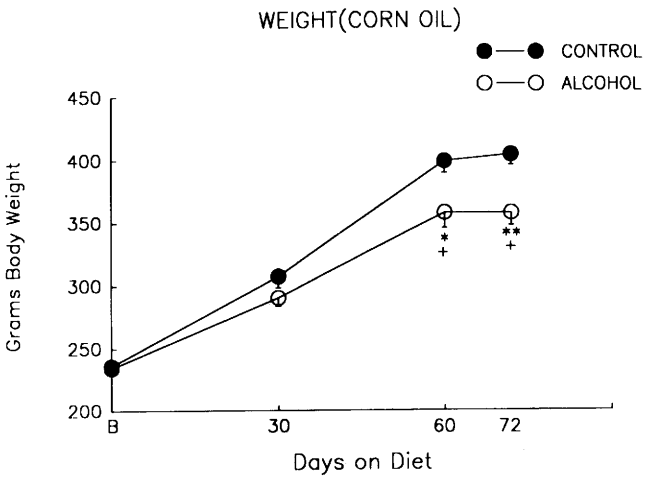

Figure 2. Weight gain of the rats fed corn oil. Note that the rats fed ethanol gained less than the controls (*$P < 0.01$; **$P < 0.005$; + = not significantly different compared with fish oil; n = 5/group; mean ± SEM).

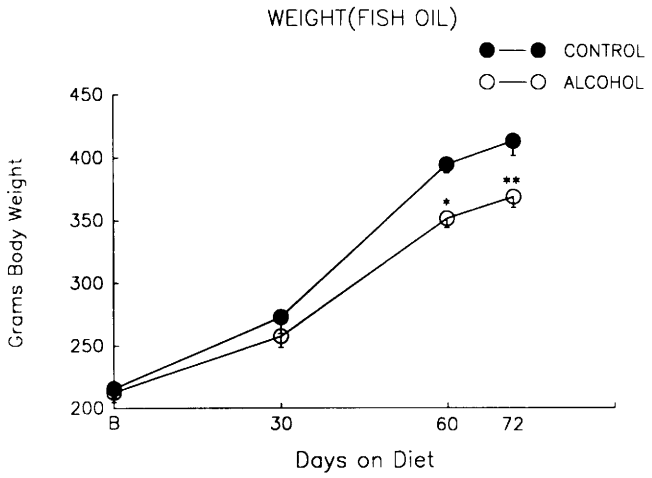

Figure 1. Weight gain of the rats fed fish oil. Note that the rats fed ethanol gained less weight than controls at 60 and 72 days (*$P < 0.005$; **$P < 0.05$; n = 5/group; mean ± SEM).

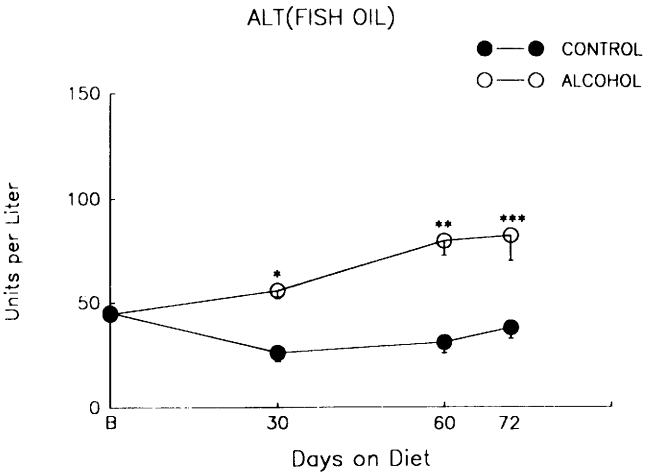

Figure 3. Serum ALT in the fish oil-fed rats. Note that ethanol feeding increased ALT to a significant extent at all time periods (*$P < 0.005$; **$P < 0.001$; ***$P < 0.01$; n = 5/group; mean ± SEM) partly because the levels fell in the controls.

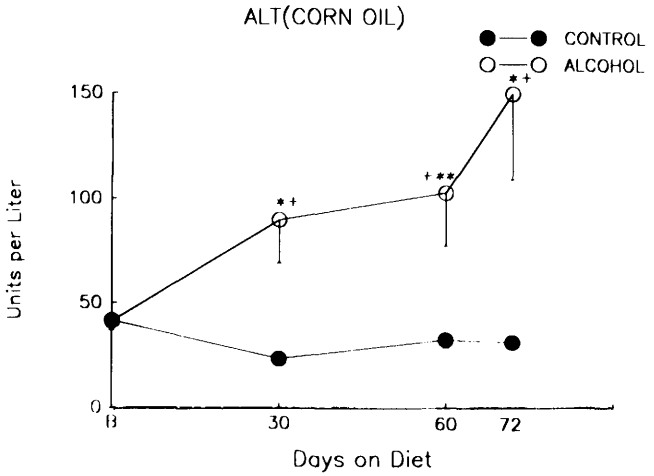

Figure 4. Serum ALT in the corn oil-fed rats. Note that all levels rose significantly at all time periods (*$P < 0.002$; **$P < 0.05$) but not greater than in the rats fed ethanol and fish oil (+ = $n = 5$; mean ± SEM).

contrast, the pair-fed fish oil controls appeared normal (Fig. 6) except for rare foci of central fibrosis. Minimal centrilobular fibrosis was also seen in the corn oil- and ethanol-fed rats. However, the focal central fibrosis seen in the rats fed fish oil and ethanol was more extensive (Fig. 7) with rare foci of portal-central bridging fibrosis. Necrosis, inflammation, and fibrosis were also seen by electron microscopy. Necrotic hepatocellular debris mixed with macrophages (Fig. 8) and regenerating capillaries were present early and activated Ito cells and extracellular matrix formation preceded fibrosis. Immunohistochemistry staining of CYP2E1 protein of the livers from the rats fed fish oil and ethanol was greater in the centrilobular area than pair-fed controls.

The biochemical data is summarized in Table II. Due to the technical difficulties in measuring CYP2E1 by Western blot, the data from four rats (one from each group) were not included (i.e., $n = 4$). The

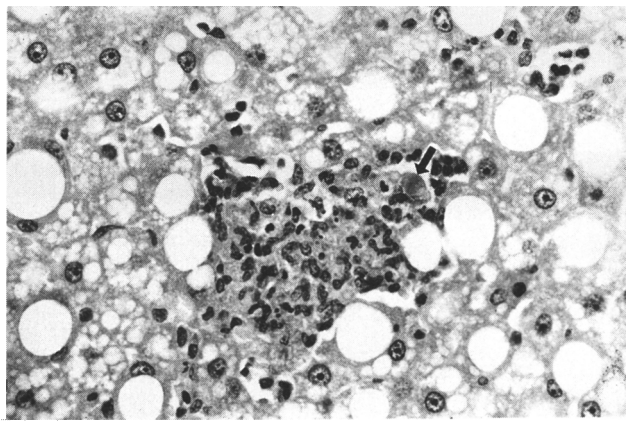

Figure 5. Photomicrograph showing focal necrosis and inflammation as well as fatty change in the liver of a rat fed fish oil and ethanol for 72 days. Note the necrotic liver cell (arrow) and the mononuclear infiltrate. H&E ×624.

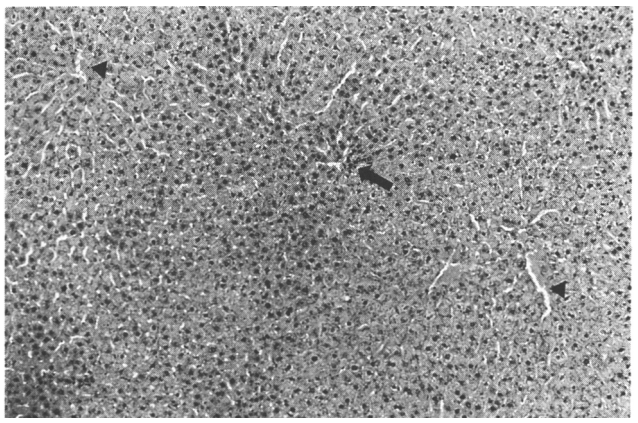

Figure 6. Photomicrograph of the liver from a control rat pair-fed fish oil and dextrose for 60 days. The normal liver parenchyma is shown including a small portal tract (arrow) and central veins (arrow heads). H&E ×156.

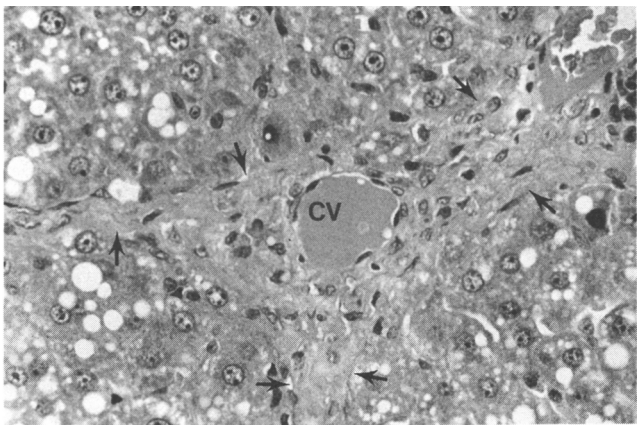

Figure 7. Photograph of central fibrosis in a liver from a rat fed fish oil and ethanol for 60 days. The borders of the scar around the central vein (cv) are outlined by arrows. H&E ×624.

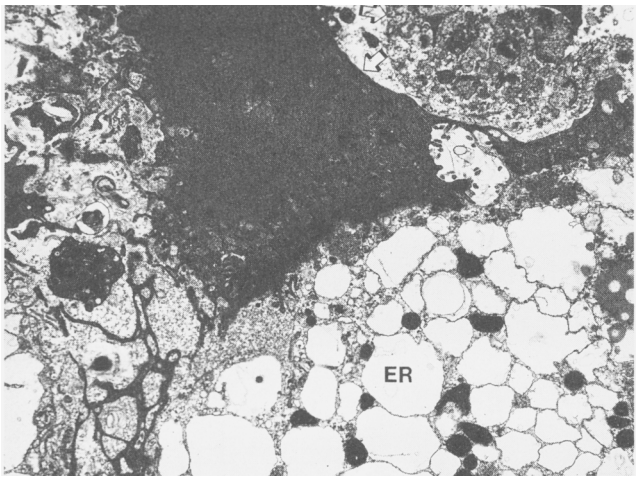

Figure 8. Electron micrographs of an area at the edge of a necrotic focus. Note the necrotic liver cells (arrows) and the viable liver cell showing dilated endoplasmic reticulum (ER). This corresponds to balloon degeneration by light microscopy. Note the canaliculus. ×10,200.

Table II. Liver Microsomal Data From Rats Fed Ethanol and Corn Oil Compared With Rats Fed Ethanol and Fish Oil

Assay	Units	Corn oil		Fish oil	
		A	C	A	C
P450	nmol/mg	1.02 ± 0.20*	0.59 ± 0.04[a]	1.24 ± 0.17	0.65 ± 0.07[b]
Reductase	μ/mg	110 ± 30	79 ± 12	146 ± 33	128 ± 28[c]
	μ/nmol[+]	104 ± 18	134 ± 18	118 ± 25	197 ± 36[d]
NADPH oxidation	nmol/mg/min	14.56 ± 2.88	6.65 ± 1.41[e]	20.59 ± 3.34[f]	9.54 ± 0.86[g]
	nmol/nmol/min	14.34 ± 0.31	11.20 ± 1.72[h]	16.58 ± 1.44[i]	14.70 ± 0.46[j]
Lipid peroxidation	nmol/mg/min	0.83 ± 0.41	0.06 ± 0.06[k]	0.91 ± 0.27	0.36 ± 0.41
	nmol/nmol/min	0.80 ± 0.39	0.10 ± 0.11[l]	0.88 ± 0.44	0.43 ± 0.37
CYP2E1	pmol/mg	521 ± 200	41 ± 27[m]	557 ± 149	56 ± 34[n]
	pmol/nmol P450	498 ± 100	70 ± 46[o]	452 ± 113	85 ± 42[n]
Pathology score	0–10	6.3 ± 0.5	1.0 ± 0.8[o]	6.8 ± 1.0	1.0 ± 1.4[n]

* Mean ± SD; $n = 4$ (rats/group); A = alcohol-fed; C = dextrose pair-fed control; [+]NADPH-dependent cytochrome P450 reductase in nmoles per nmoles total cytochrome P450.
[a] $P < 0.01$ when compared with CA.
[b] $P < 0.001$ when compared with FA.
[c] $P < 0.02$ when compared with CC.
[d] $P < 0.02$ when compared with FA or CC.
[e] $P < 0.01$ when compared with CA and <0.02 when compared with FC.
[f] $P < 0.04$ when compared with CA.
[g] $P < 0.001$ when compared with FA.
[h] $P < 0.02$ when compared with CA and <0.01 when compared with FC.
[i] $P < 0.03$ when compared with CA.
[j] $P < 0.05$ when compared with FA.
[k] $P < 0.03$ when compared with CA (the Wilcoxon Signed Rank test paired t test was used).
[l] $P < 0.02$ when compared with CA.
[m] $P < 0.005$ when compared with CA.
[n] $P < 0.001$ when compared with FA.
[o] $P < 0.001$ when compared with CA.

CYP2E1 measurements were repeated and the data from the rats whose results were not reproducible were omitted. Rats fed ethanol and corn oil had higher levels of total cytochrome P450 than corn oil-fed controls. Similarly ethanol and fish oil significantly increased P450 compared with the fish oil controls. NADPH oxidation was increased by ethanol and corn oil and fish oil compared with their respective controls, fish oil more than corn oil and ethanol.

Rats fed ethanol and fish oil or corn oil had higher levels of microsomal CYP2E1 than their respective controls (Table II). Lipid peroxidation was significantly increased by feeding ethanol and corn oil but not when fish oil and ethanol was fed partly because peroxidation was increased in one fish oil control rat and not increased in one rat fed corn oil and ethanol (see Fig. 14).

There was a significant positive correlation between the rate of NADPH oxidation and CYP2E1 levels in both the rats fed fish oil and corn oil (Fig. 9 and 10). There was a positive correlation between the rate of lipid peroxidation and CYP2E1 levels in the corn oil-fed rats (Fig. 11), but this correlation was not significant in the rats fed fish oil ($P < 0.20$). There was a significant positive correlation between the pathology score and CYP2E1 levels in both the corn oil- and the fish oil-fed rats (Fig. 12 and 13). Similarly, there was a positive correlation between the level of lipid peroxidation and the pathology score in both the rats fed corn oil and fish oil with ethanol (Fig. 14). On the other hand, there was no significant correlation between the reductase activity with NADPH oxidation, lipid peroxidation, CYP2E1, or the pathology score in the fish oil-fed rats. Thus, it is unlikely that the reductase was mechanistically involved in the ethanol-induced liver injury.

Discussion

The present experiment was designed to test whether fish oil n-3 PUFAs could substitute for corn oil 18:2n-6 to support the induction of the pathologic and biochemical changes characteristic of ethanol-induced liver injury. Our data was in good agreement with prior reports on the effect of corn oil and ethanol from our laboratories in terms of biochemical data and liver morphology (17, 33). Of the various biochemical changes noted in the microsomal system, CYP2E1 induction correlated best with the degree of liver injury observed. Recently, we studied the effect of corn oil and ethanol on lipid peroxidation and the pathology score using diallyl sulfide to reduce the induction of CYP2E1 by ethanol. When CYP2E1 was reduced to

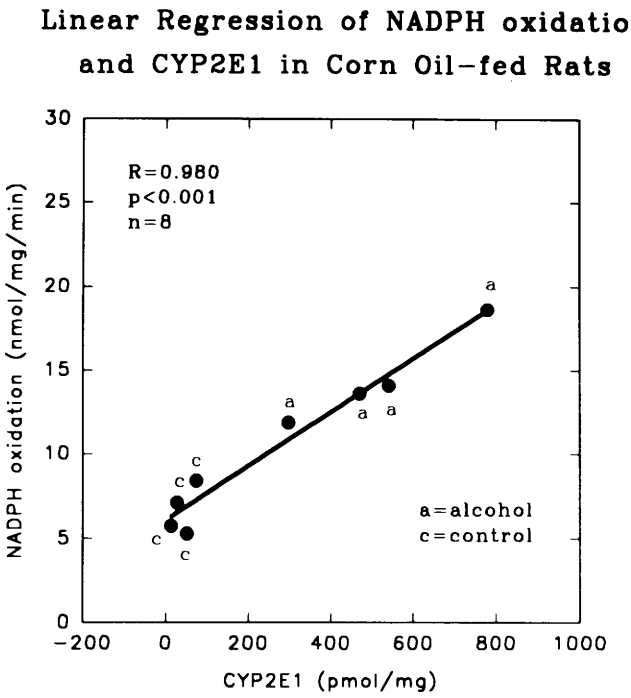

Figure 9. There was a significant positive correlation between the rate of NADPH oxidation and CYP2E1 levels in microsomes from the livers of rats fed corn oil (n = 4/group).

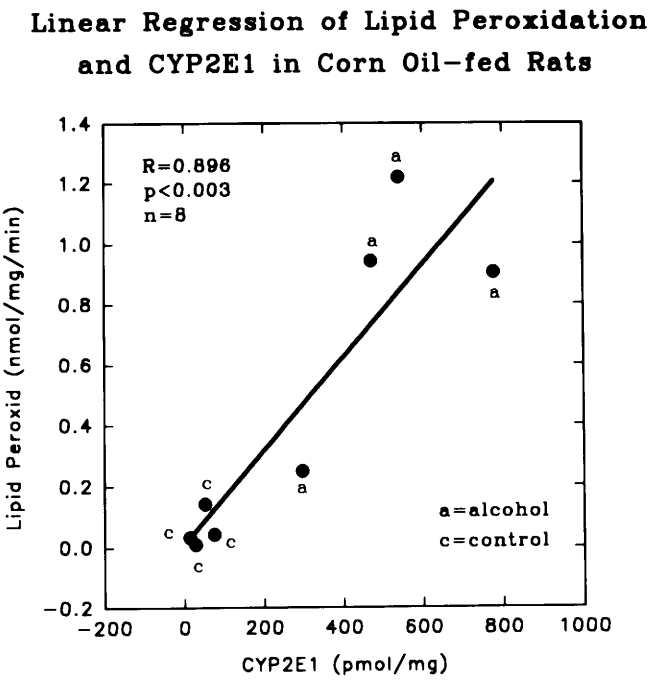

Figure 11. There was a significant positive correlation between the degree of lipid peroxidation and CYP2E1 induction in corn oil-fed rats (n = 4/group).

20% of the control ethanol-fed animals, lipid peroxidation dropped to 10% of controls and the liver pathology score was significantly reduced. This indicated that CYP2E1 was important in the mechanism of liver injury probably through its generation of free radicals during oxidation (18). Nanji *et al.* (21) also noted that the induction of CYP2E1 correlated with increased lipid peroxidation and the decrease in arachidonate using rats fed corn oil and ethanol by intragastric tube. They suggested that arachidonate was depleted because it was degraded as a target for free radicals generated by CYP2E1.

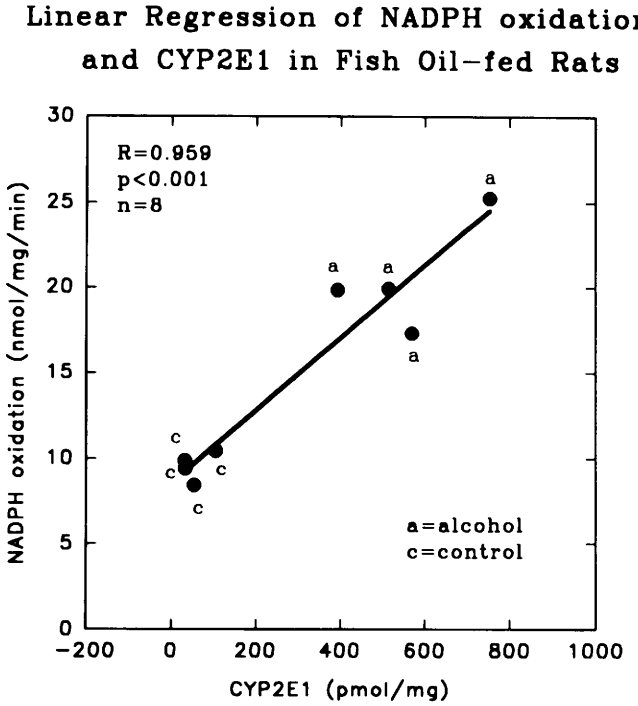

Figure 10. There was a significant positive correlation with the rate of NADPH oxidation and CYP2E1 levels in the fish oil-fed rats (n = 4/group).

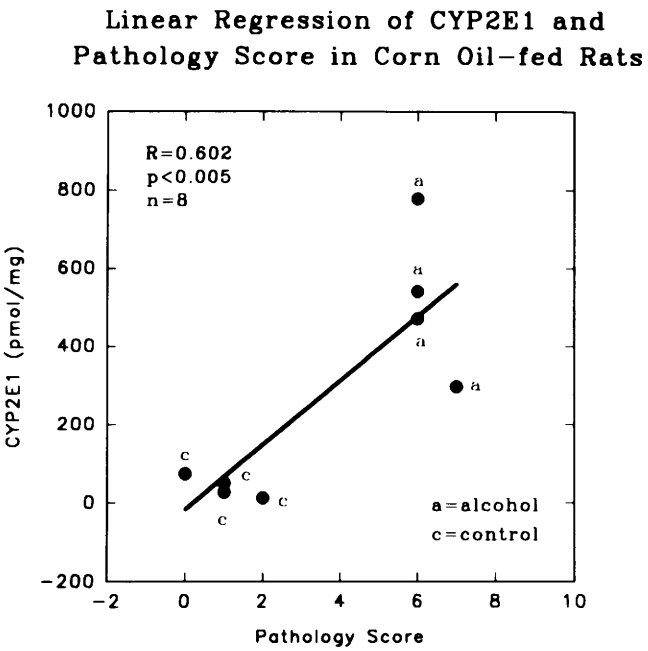

Figure 12. There was a significant positive correlation between the degree of liver injury and the CYP2E1 levels in the corn oil-fed rats (n = 4/group).

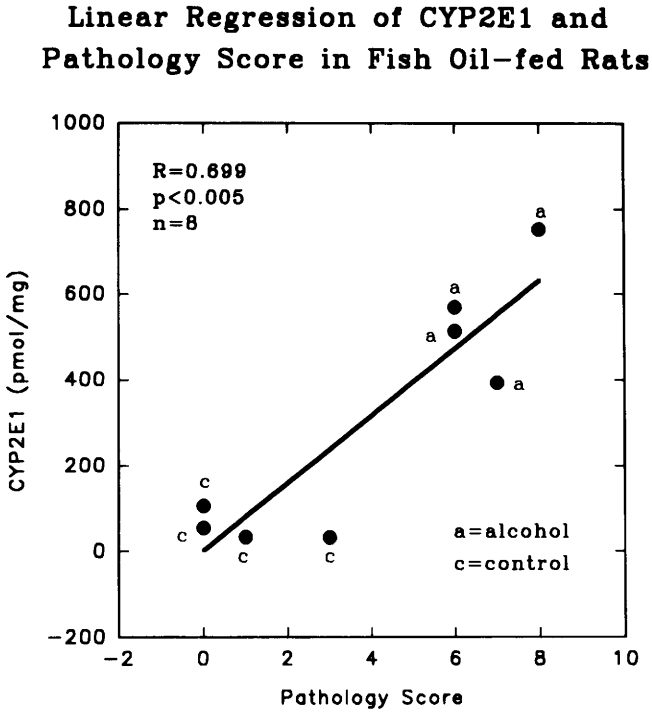

Figure 13. There was a significant positive correlation between the degree of liver injury and the CYP2E1 levels in the fish oil fed rats (n = 4/group).

When we fed rats beef fat instead of corn oil with ethanol, CYP2E1 was induced, but no liver damage resulted, presumably because the beef fat was very low in linoleic (0.7%) acid so that arachidonate could not be synthesized to act as a target for free radical attack (14, 32, 34). Although fish oil is low in linoleic acid (1.8%), it is rich in polyunsaturated fatty acids (20:5n-3 = 17.5%, 22:6n-3 = 19%) which may undergo free radical attack to account for the liver injury, especially the fibrosis. Free radicals have been shown to stimulate collagen synthesis of fibroblasts *in vitro* (35, 36). The data suggest that both CYP2E1 induction and the availability of polyunsaturated fatty acids for free radical attack are required for the development of alcohol-induced liver injury.

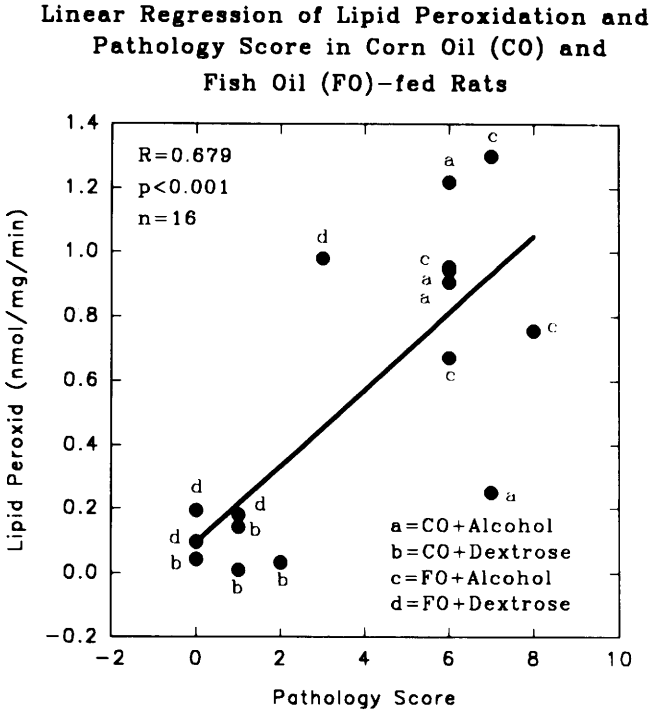

Figure 14. There was a significant positive correlation between lipid peroxidation and the pathology score in both the corn oil- and the fish oil-fed rats (n = 4/group).

Lipid peroxidation in the rats fed ethanol and fish oil was not significantly increased over controls fed fish oil alone. However, this lack of significance can be explained by the high variability in the results and also by the increase in lipid peroxidation observed in the fish oil-fed controls. The fact that n-3 fatty acids are preferentially oxidized by peroxisomes rather than the microsomal pathway might explain why CYP2E1 induction in microsomes by ethanol did not enhance the lipid peroxidation in the rats fed fish oil. Others have reported that fish oil in the diet increases lipid peroxidation (25, 27–31). Thus, we have shown for the first time that not only dietary 18:2n-6 but also dietary n-3 polyunsaturated fatty acids can support the development of ALD in the rat provided that CYP2E1 is also induced to several fold. High blood alcohol levels are also required probably because CYP2E1 has a high K_m and a high blood alcohol level is needed in order for ethanol to be oxidized by it and consequently generate free radicals (3).

Fish oil n-3 PUFAs inhibit the formation of n-6 fatty acid eicosanoid metabolites which originate from 20:4 n-6 PUFA (37). The eicosanoid products of the n-3 pathway exert less intense agonist actions at eicosanoid receptors compared with n-6-derived eicosanoids (38). Thus, LTB_4- and thromboxane A_2-mediated reactions which might induce liver inflammation in ALD would be diminished by a diet rich in fish oil (39). Thus, fish oil might be expected to reduce the inflammation caused by ethanol, but we noted the opposite in that the liver of the rats fed ethanol and fish oil showed an increased inflammation.

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1. Annoni G, Giambrone MA, Biempica L, Scharschmidt L, Zern MA. A diet rich in omega 3 fatty acids (ω-3FA) induced liver injury in rats. Hepatology **8**:1380, 1988.

2. Degli Esposito S, Frizell O, He Q, Abraham A, French SW, Zern MA. Improved rat model of alcoholic liver disease:

Omega-3 fatty acids (ω3 FAs) plus ethanol administration. Hepatology **14**:132A, 1991.
3. French SW. Nutrition in the pathogenesis of alcoholic liver disease. Alcohol Alcoholism **28**:97–109, 1993.
4. Rouach H, Clement M, Orfanelli M-T, Janvier B, Nordmann J, Nordmann R. Hepatic lipid peroxidation and mitochondrial susceptibility to peroxidative attacks during ethanol inhalation and withdrawal. Biochim Biophys Acta **753**:439–444, 1983.
5. Harata J, Nagata M, Sasaki E, Ishiguro I, Ohta Y, Murakami Y. Effect of prolonged alcohol administration on activities of various enzymes scavenging activated oxygen radicals and lipid peroxide level in the liver of rats. Biochem Pharmacol **32**:1798–1800, 1983.
6. Ingelman-Sundberg M, Johansson I. Mechanisms of hydroxyl radical formation and ethanol oxidation by ethanol-inducible and other forms of rabbit liver microsomal cytochrome P-450. J Biol Chem **259**:6447–6458, 1984.
7. Ingelman-Sundberg M, Johansson I, Penttila KE, Glaumann H, Lindros KO. Centrilobular expression of ethanol-inducible cytochrome P450 (IIE1) in rat liver. Biochem Biophys Res Commun **157**:55–60, 1988.
8. Ingelman-Sundberg M, Ekström G, Tindberg N. Lipid peroxidation dependent on ethanol-inducible cytochrome P-450 from rat liver. Adv Biosci **71**:43–47, 1988.
9. Kawase T, Kato S, Lieber CS. Lipid peroxidation and antioxidant defense systems in rat liver after chronic ethanol feeding. Hepatology **10**:815–821, 1989.
10. Tsutsumi M, Lasker JM, Shimizu M, Rosman AS, Lieber CS. The intralobular distribution of ethanol-inducible P450 IIE1 in rat and human liver. Hepatology **10**:437–446, 1989.
11. Ekström G, Ingelman-Sundberg M. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450 IIE1). Biochem Pharmacol **38**:1313–1319, 1989.
12. Yamada S, Yamada M, Murawaki Y, Hirayama C. Increase in lipoperoxides and prolyl hydroxylase activity in rat liver following chronic ethanol feeding. Biochem Pharmacol **40**:1015–1019, 1990.
13. Albano E, Tomasi A, Persson J-O, Terelius Y, Goria-Gatti L, Ingelman-Sundberg M, Dianzani MU. Role of ethanol-inducible cytochrome P-450 (P450IIE1) in catalyzing the free radical activation of aliphatic alcohols. Biochem Pharmacol **41**:1895–1902, 1991.
14. Takahashi H, Johansson I, French SW, Ingelman-Sundberg M. Effects of dietary fat composition on activities of the microsomal ethanol-inducible cytochrome P450 (CYP2E1) in the liver of rats chronically fed ethanol. Pharmacol Toxicol **70**:347–352, 1992.
15. Kamimura S, Gaal K, Britton RS, Bacon BR, Triadafilopoulos G, Tsukamoto H. Increased 4-hydroxynonenal levels in experimental alcoholic liver disease: association of lipid peroxidation with liver fibrogenesis. Hepatology **16**:448–453, 1992.
16. Yang CS, Brady JF, Hong J-Y. Dietary effects on cytochromes P450, xenobiotic metabolism, and toxicity. FASEB J **6**:737–744, 1992.
17. French SW, Wong K, Jui L, Albano E, Hagbjörk A-L, Ingelman-Sundberg M. Effect of ethanol on cytochrome P450 2E1 (CYP2E1), lipid peroxidation, and serum protein adduct formation in relation to liver pathology pathogenesis. Exp Mol Pathol **58**:61–75, 1993.
18. Morimoto M, Hagbjörk A-L, Nanji AA, Ingelman-Sundberg M, Lindros KO, Fu PC, Albano E, French SW. Role of cytochrome P450 2E1 in alcoholic liver disease pathogenesis. Alcohol **10**:459–464, 1993.
19. Tsutsumi M, Lasker JM, Takahashi T, Lieber CS. *In vivo* induction of heptic P450 2E1 by ethanol: Role of increased enzyme synthesis. Arch Biochem Biophys **304**:209–218, 1993.
20. Badger TM, Ronis MJJ, Lumpkin CK, Valentine CR, Shahare M, Irby D, Huang J, Mercado C, Thomas P, Ingelman-Sundberg M, Crouch J. Effects of chronic ethanol on growth hormone secretion and hepatic cytochrome P450 isozymes of the rat. J Pharmacol Exp Ther **264**:438–447, 1993.
21. Nanji AA, Zhao S, Lamb RG, Sadrzadeh SMH, Dannenberg AJ, Waxman DJ. Changes in microsomal phospholipases and arachidonic acid in experimental alcoholic liver injury: Relationship to cytochrome P-450 2E1 induction and conjugated diene formation. Alcoholism Clin Exp Res **17**:598–603, 1993.
22. Badger TM, Huang J, Ronis M, Lumpkin CK. Induction of cytochrome P450 2E1 during chronic ethanol exposure occurs via transcription of the CYP 2E1 gene when blood alcohol concentrations are high. Biochem Biophys Res Commun **190**:780–785, 1993.
23. Ronis MJJ, Huang J, Crouch J, Mercado C, Irby D, Valentine CR, Lumpkin CK, Ingelman-Sundberg M, Badger TM. Cytochrome P450 CYP 2E1 induction during chronic alcohol exposure occurs by a two-step mechanism associated with blood alcohol concentrations in rats. J Pharmacol Exp Ther **264**:944–950, 1993.
24. Takahashi T, Lasker JM, Rosman AS, Lieber CS. Induction of cytochrome P-450 2E1 in the human liver by ethanol is caused by a corresponding increase in encoding messenger RNA. Hepatology **17**:236–245, 1993.
25. Hu M-L, Frankel EN, Leibovitz BE, Tappel AL. Effect of dietary lipids and vitamin E on *in vitro* lipid peroxidation in rat liver and kidney homogenates. J Nutr **119**:1574–1582, 1989.
26. Yoo J-S H, Ning SM, Pantuck CB, Pantuck EJ, Yang CS. Regulation of hepatic microsomal cytochrome P450 IIE1 level by dietary lipids and carbohydrates in rats. J Nutr **121**:959–965, 1991.
27. Meydani M, Natiello F, Goldin B, Free N, Woods M, Schaefer E, Blumberg JB, Gorbach SL. Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. J Nutr **121**:484–491, 1991.
28. Haglund O, Luostarinen R, Wallin R, Wibell L, Saldeen T. The effects of fish oil on triglycerides, cholesterol, fibrogen and malondialdehyde in humans supplemented with vitamin E. J Nutr **121**:165–169, 1991.
29. L'Abbe MR, Trick KD, Beare-Rogers JL. Dietary (n-3) fatty acids affect rat heart, liver and aorta protective enzyme activities and lipid peroxidation. J Nutr **121**:1331–1340, 1991.
30. Gonzalez MJ, Gray JI, Schemmel RA, Dugan L Jr., Welsch CW. Lipid peroxidation products are elevated in fish oil diets even in the presence of added antioxidants. J Nutr **122**:2190–2195, 1992.
31. Meydani M, Meydani SN. (n-3) Fatty acids, tissue lipid peroxidation and tocopherol status. J Nutr **122**:1748, 1992.
32. Nanji AA, Mendenhall CL, French SW. Beef fat prevents alcoholic liver disease in the rat. Alcoholism Clin Exp Res **13**:15–19, 1989.
33. French SW, Miyamoto K, Tsukamoto H. Ethanol-induced hepatic fibrosis in the rat: Role of the amount of dietary fat. Alcoholism Clin Exp Res **10**:135–195, 1986.
34. Arbuckle LD, Rioux FM, Mackinnon MJ, Hrboticky N, Innis SM. Response of (n-3) and (n-6) fatty-acids in piglet brain, liver and plasma to increasing, but low, fish oil supplementation of formula. J Nutr **121**:1536–1547, 1991.
35. Geesin JC, Gordon JS, Berg RA. Retinoids affect collagen syn-

thesis through inhibition of ascorbate-induced lipid peroxidation in cultured human dermal fibroblasts. Arch Biochem Biophys **278**:250–355, 1990.
36. Houghlum K, Brenner DA, Chojkler M. d-α-Tocopherol inhibits collagen α(1) gene expression in cultured human fibroblasts. Modulation of constitutive gene expression by lipid peroxidation. J Clin Invest **87**:2230–2235, 1991.
37. Blackburn GL. Nutrition and inflammatory events: Highly unsaturated fatty acids (ω-3 vs ω-6) in surgical injury. Proc Soc Exp Biol Med **700**:183–188, 1992.
38. Lands WE. Biochemistry and physiology of n-3 fatty acids. FASEB J **6**:2530–2536, 1992.
39. Kim DN, Schmee J, Lee CS, Solis O, Thomas WA. Reductions in serum thromboxane, prostacyclin and leukotriene B_4 levels in swine fed a fish oil supplement to an atherogenic diet. Exp Mol Pathol **55**:1–12, 1991.