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Dietary Saturated Fatty Acids: A Novel Treatment for Alcoholic Liver Disease

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See editorial on page 617.

Background & Aims: **Lipid peroxidation may be important in the pathogenesis of alcoholic liver injury. The purpose of this study was to determine whether a saturated fatty acid–based therapy (palm oil) could decrease lipid peroxidation and alcoholic liver injury during ethanol withdrawal.** ***Methods:*** **Three groups of male Wistar rats (5 rats/group) were studied. Rats in group 1 were fed a fish oil–ethanol diet for 6 weeks; rats in groups 2 and 3 were fed a fish oil–ethanol diet for 6 weeks before treatment with fish oil–dextrose (group 2) or palm oil–dextrose (group 3) for 2 weeks. Liver samples were analyzed for histopathology, lipid peroxidation, fatty acid composition, cytochrome P450 2E1 activity, and tocopherol levels.** ***Results:*** **By 6 weeks, all rats had developed fatty liver, inflammation, and necrosis. Group 2 showed minimal histological improvement, whereas group 3 showed near normalization of the histology. The improvement in group 3 was associated with decreased lipid peroxidation and P450 2E1 activity. Higher levels of ω-3 fatty acids were detected in group 2 than group 3. Tocopherol levels were similar among the groups.** ***Conclusions:*** **A diet enriched in saturated but not unsaturated fatty acids reversed alcoholic liver injury. This effect may be explained by down-regulation of lipid peroxidation.**

Evidence shows that dietary lipid can be an important determinant of alcohol-induced liver injury. For example, epidemiological observations suggest that saturated fat is relatively protective against alcoholic liver disease.[1] Also, dietary lipid can modulate the severity of alcoholic liver injury in rats.[2,3] None of the histological features of alcoholic liver injury develop in rats fed ethanol and saturated lipid, whereas fatty liver, necrosis, inflammation, and fibrosis develop in rats fed ethanol and lipid enriched in polyunsaturated fatty acids.[2–4] Several investigators have postulated that polyunsaturated fatty acids potentiate alcohol-induced liver injury by inducing cytochrome P450 2E1 (*CYP2E1*).[5–9] The contribution of *CYP2E1* to microsomal reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent lipid peroxidation is believed to account, in part, for its importance in mediating liver injury.[10]

With these facts in mind, different strategies could be developed to decrease lipid peroxidation and treat alcoholic liver disease. One approach might be to directly inhibit *CYP2E1*. A second and potentially easier approach would be to use dietary saturated fatty acids as therapy, because *CYP2E1* levels[11,12] and the fatty acid composition of the liver[13–16] are sensitive to dietary manipulation. *CYP2E1* levels have been reported to be decreased in the livers of rats fed saturated vs. unsaturated lipids.[12] A diet containing saturated fatty acids would be expected to reduce the concentration of polyunsaturated fatty acids in the liver and diminish the availability of substrate for lipid peroxidation. Therefore, in the current study, rats with alcoholic liver injury were treated with a diet enriched in saturated fatty acids (palm oil) or a diet enriched in polyunsaturated ω-3 fatty acids (fish oil). In addition to being a rich source of saturated fatty acids, palm oil contains tocopherols and tocotrienols, which inhibit lipid peroxidation.[17] Palm oil feeding also modulates eicosanoid metabolism in a manner in which the ratio of prostacyclin to thromboxane in plasma is increased.[18] An increase in the prostacyclin/thromboxane ratio is associated with decreased liver injury in the intragastric feeding rat model.[19,20] Results from this study

Abbreviations used in this paper: FE group, group fed fish oil–ethanol; FE-FD group, group fed fish oil–ethanol plus fish oil–dextrose; FE-PD group, group fed fish oil–ethanol plus palm oil–dextrose; NADPH, reduced nicotinamide adenine dinucleotide phosphate; TBARS, thiobarbituric acid–reactive substances.

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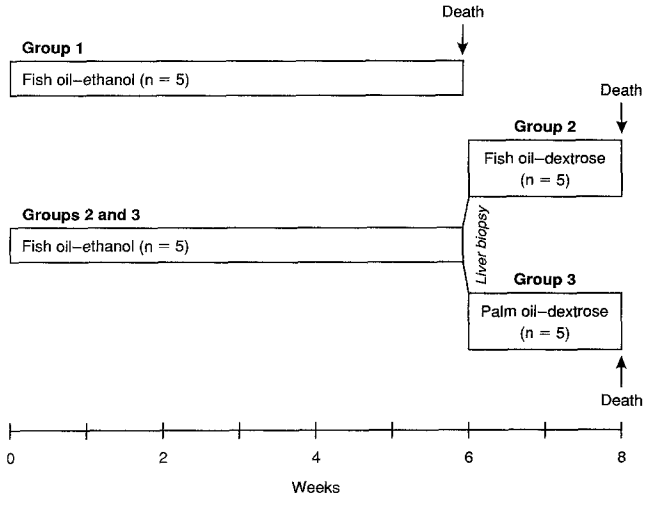

Figure 1. Schematic of the experimental design.

indicate that treatment with palm oil but not fish oil was associated with a marked improvement in liver pathology and a reduction in lipid peroxidation during ethanol withdrawal.

Materials and Methods

Animal Model

Male Wistar rats weighing between 225 and 250 g were fed a liquid diet by continuous infusion through permanently implanted gastric tubes as described previously.[21,22] The rats were administered their total nutrient intake by intragastric infusion. The percentage of calories derived from fat was 35% of total calories. Vitamins and minerals were given as described previously.[21,22] The amount of ethanol given was modified to maintain high levels of blood ethanol (150–300 mg/dL) throughout the day. This amount was initially 8 g · kg^{-1} · day^{-1} and was increased up to 16 g · kg^{-1} · day^{-1} as tolerance developed. Each ethanol-fed rat had at least two measurements of blood alcohol level.

Three groups of rats (5 rats/group) were studied. The experimental design is shown schematically in Figure 1. Rats in group 1 were fed a fish oil–ethanol diet for 6 weeks (FE group), after which they were killed. Rats in groups 2 and 3 were fed the same fish oil–ethanol diet for 6 weeks, after which they were switched to a diet containing either fish oil with dextrose (FE-FD group) or palm oil with dextrose (FE-PD group) for 2 more weeks and then killed. A liver biopsy specimen was obtained for assessment of histopathology before switching the animals to the dextrose-containing diets. The percentage of calories derived from either fish oil or palm oil was 35% of total calories. The caloric intake was identical in all groups. The fatty acid composition of the palm oil and fish oil is shown in Table 1. When the animals were killed, a sample of the liver was obtained for histopathological analysis, and the remainder of the liver was rapidly excised, washed with ice-cold 1.15% (wt/vol) KCl, and cut into small pieces, which were transferred to plastic vials and placed in liquid nitrogen. The vials were stored at −80°C. The studies were conducted according to the guidelines on care and use of laboratory animals (National Institutes of Health).

Histological Analysis

A small sample of the liver was obtained by biopsy or when the rats were killed and formalin-fixed. H&E stain was used for light microscopy. The severity of liver pathology was assessed as follows[21]: steatosis (the percentage of liver cells containing fat) was scored 1+ with <25% of the cells containing fat; 2+, with 26%–50% fat; 3+, with 51%–75% fat; and 4+, with >75% fat. Necrosis was evaluated as the number of necrotic foci per square millimeter, and inflammation was scored as the number of inflammatory cells per square millimeter. At least three different sections were examined per sample of liver. The pathologist evaluating the sections was unaware of the treatment groups when assessing the histology.

Measurements of Blood Alcohol

Blood was collected from the tail vein, and ethanol concentration was measured using an alcohol dehydrogenase kit from Sigma Chemical Co. (St. Louis, MO).

Determination of Thiobarbituric Acid–Reactive Substances

Levels of liver thiobarbituric acid–reactive substances (TBARS) were measured according to the method of Ohkawa et al.[23] Briefly, 0.2 mL sodium dodecyl sulfate (8.1%), 1.5 mL 20% acetic acid, and 1.5 mL 0.8% thiobarbituric acid were added to 200 μL liver homogenate. After addition of distilled water, the tubes were vortexed and placed in boiling water for 1 hour. The reaction was stopped by immersion of tubes in a cold water bath. After addition of 15:1 (vol/vol) butanol-pyridine and centrifugation, the upper phase was removed, and

Table 1. Fatty Acid Composition of the Menhaden Fish Oil and Palm Oil Diets

	% of total fatty acids	
Fatty acid	Palm oil	Menhaden fish oil
Lauric ($C_{12:0}$)	0.4	ND
Myristic ($C_{14:0}$)	1.2	10.2
Palmitic ($C_{16:0}$)	44.8	20.0
Palmitoleic ($C_{16:1\ \omega-7}$)	ND	14.4
Stearic ($C_{18:0}$)	3.9	3.4
Oleic ($C_{18:1\ \omega-9}$)	40.1	10.6
Vaccenic ($C_{18:1\ \omega-7}$)	ND	3.2
Linoleic ($C_{18:2\ \omega-6}$)	8.4	2.0
Arachidonic ($C_{20:4\ \omega-6}$)	ND	0.7
Eicosapentaenoic ($C_{20:5\ \omega-3}$)	ND	9.8
Docosapentaenoic ($C_{22:5\ \omega-3}$)	ND	1.8
Docosahexaenoic acid ($C_{22:6\ \omega-3}$)	ND	6.1

ND, none detected.

absorbance at 532 nm was determined. Butylated hydroxytoluene (90 μmol/L) was added to prevent the formation of TBARS in vitro.

Measurement of Conjugated Dienes

Conjugated dienes in the total lipid extracted from liver homogenates were identified by their optical density of between 220 and 300 nm as described by Recknagel and Glende.[24]

Aniline Hydroxylase Assay

Aniline hydroxylase assays were performed according to the method of Imai et al.[25] with the following modification.[26] Liver microsomes were incubated for 60 minutes at 37°C in 0.45 mL of 0.1 mol/L KPi (pH 7.4) containing 8 mmol/L aniline and 1 mmol/L NADPH. Reactions were terminated with 90 μL of 40% trichloroacetic acid. Samples were then placed on ice for 10 minutes followed by 10 minutes of centrifugation. An aliquot of the supernatant (0.36 mL) was mixed with 10% Na_2CO_3 (0.24 mL) and 2% phenol (0.36 mL). A_{630} values were determined after incubation for 45 minutes in the dark. Specific activities were calculated from a standard curve prepared with the reaction product 4-aminophenol (Aldrich, Milwaukee, WI).

Analysis of Fatty Acid Composition

The composition of fatty acids (as methyl esters) of the liver was analyzed using the Microbial Identification System from MIDI (Newark, DE), which uses Hewlett-Packard hardware, including a 5890A gas chromatograph equipped with a hydrogen flame ionization detector, an automatic injector, a sample controller and sample tray, and an electronic integrator controlled by a minicomputer. The gas chromatograph used a fused silica capillary column with methylphenyl silicone (SE54) as the stationary phase. The computer-controlled operating parameters of the instrument were as follows: injector temperature, 250°C; detector temperature, 300°C; and oven temperature, programmed from 170°C to 270°C at 5°C/min. The fatty acid methyl esters were identified by comparing retention times with known standards and by computer calculation of equivalent chain lengths.

The peroxidizability index was calculated as an index of substrate availability for lipid peroxidation based on the greater likelihood for oxidative deterioration of fatty acids with two or more double bonds.[27] This index was determined from the percentage of monoenoic, dienoic, trienoic, tetraenoic, pentaenoic, and hexaenoic fatty acids in the liver as follows[28]: Peroxidizability Index = (Monoenoic % × 0.025) + (Dienoic % × 1) + (Trienoic % × 2) + (Pentaenoic % × 6) + (Hexaenoic % × 8). This formula is based on the maximal in vitro rates of oxidation reported by Holman[29] and has previously been used in relationship to fatty acid changes in ethanol-fed miniature pigs.[30]

Table 2. Blood Alcohol Levels and Weight Gain Before and After Switching to the Experimental Diets

Experimental group	Blood alcohol level (*mg/dL*)	Weight gain (*g*)	
		Before switching	After switching
FE	231 ± 59	47 ± 9	—
FE-FD	242 ± 68	51 ± 8	55 ± 6
FE-PD	221 ± 63	48 ± 13	53 ± 11

NOTE. Values represent means ± SD; n = 5.
FE, rats fed fish oil–ethanol for 6 weeks and then killed; FE-FD, rats fed fish oil–ethanol for 6 weeks and then switched to a fish oil–dextrose diet for 2 weeks; FE-PD, rats fed fish oil–ethanol for 6 weeks and then switched to a palm oil–dextrose diet for 2 weeks.

Determination of Tocopherol Levels in the Liver

Liver α- and γ-tocopherol levels were measured using a previously described method.[31] Briefly, 1 mL ethanol containing 1.2% pyrogallol was added to 1 mL tissue homogenate in phosphate buffer. After addition of 60% KOH, the mixture was heated at 70°C for 30 minutes in the presence of pyrogallol. After cooling, 1 mL distilled water and 1 mL hexane containing tocol as internal standard were added. After centrifugation, the hexane layer was dried under nitrogen, dissolved in methanol, and injected into the high-performance liquid chromatographic system. α- and γ-tocopherol were separated by 5-μm C18 reverse-phase column using 100% methanol as mobile phase. Eluted peaks were detected by fluorometry (Perkin–Elmer 650-15 Fluorometer; Norwalk, CT).

Statistical Analysis

Results are presented as mean ± SD. Comparison among groups was performed by one-way analysis of variance. The paired *t* test was used to evaluate differences in pathological changes in the same animal after switching to a dextrose-containing diet.

Results

Weight gains and blood alcohol levels in the three different groups are shown in Table 2. No differences were found in the amount of weight gained during the 6-week period of ethanol feeding or during the 2-week period after switching to the experimental diets. No significant differences were found in blood alcohol levels among the groups.

Effect of Experimental Diets on Liver Pathology

Feeding the fish oil–ethanol diet for 6 weeks resulted in fatty infiltration, inflammation, and necrosis (Figures 2 and 3) as previously reported.[5] Both the in-

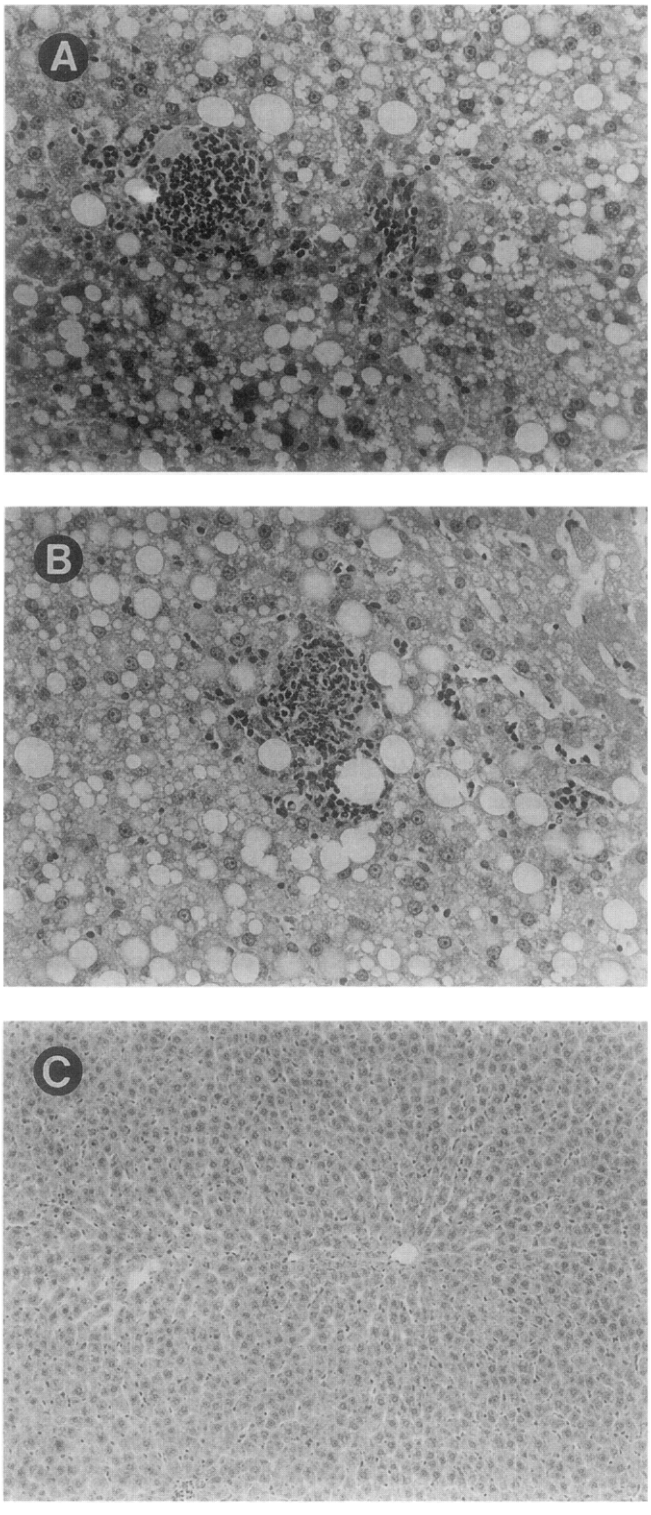

Figure 2. (*A*) Liver section from a rat fed fish oil–ethanol for 6 weeks showing evidence of fatty infiltration and a large focus of necrosis and inflammation. (*B*) Liver section from a rat treated with fish oil–dextrose for 2 weeks after 6 weeks of fish oil–ethanol (group 2). The pathological changes (fatty liver and necrotic and inflammatory foci) are still present after the fish oil–dextrose treatment. (*C*) Liver section from a rat treated with palm oil–dextrose for 2 weeks after 6 weeks of fish oil–ethanol (group 3). There is no evidence of pathological changes (H&E; original magnifications: *A*, 400×; *B*, 400×; *C*, 155×).

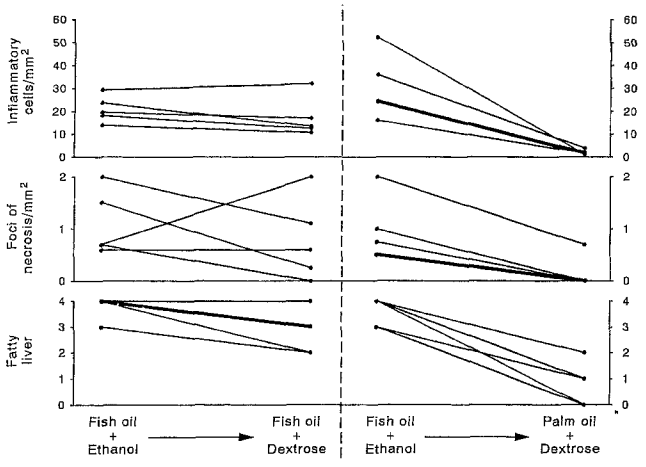

Figure 3. Changes in severity of fatty liver, necrosis, and inflammation in rats fed fish oil–ethanol for 6 weeks followed by either fish oil–dextrose (group 2) or palm oil–dextrose (group 3) for 2 weeks. All animals underwent liver biopsy after completing 6 weeks of fish oil–ethanol feeding. The results of these initial biopsies served as a baseline for comparison with the pathological changes after treatment with the two experimental diets. In the FE-FD group (group 2), there was a trend toward histological improvement after 2 weeks of treatment with fish oil–dextrose. No single difference reached statistical significance. In this group, results were as follows: fatty liver, 3.8 ± 0.4 to 2.8 ± 0.8; necrosis, 1.1 ± 0.6 to 0.8 ± 0.6 foci/mm^2; and inflammation, 21.1 ± 5.3 to 17.3 ± 7.8 cells/mm^2. The severity of all pathological changes was significantly reduced ($P < 0.01$) after 2 weeks of treatment with palm oil–dextrose (group 3). In the FE-PD group (group 3), the results were as follows: fatty liver, 3.6 ± 0.5 to 0.8 ± 0.7 ($P < 0.01$); necrosis, 1.0 ± 0.6 to 0.2 ± 0.1 foci/mm^2 ($P < 0.01$); and inflammation, 30.6 ± 12.6 to 2.0 ± 1.0 cells/mm^2 ($P < 0.01$). The severity of fatty liver, necrosis, and inflammation in the FE-PD group (group 3) was also significantly less severe ($P < 0.01$) than in the FE-FD group (group 2) at the end of 2 weeks of treatment.

flammation and necrosis were focal in nature. There was minimal improvement when the administration of ethanol was stopped and the rats were switched to a fish oil–dextrose diet (group 2) for 2 weeks (Figures 2*B* and 3). Rats fed a lipid enriched in polyunsaturated ω-3 fatty acids (fish oil) continued to have evidence of significant fatty infiltration, inflammation, and necrosis. In contrast, the degrees of fatty liver, inflammation, and necrosis were markedly improved ($P < 0.01$) when the administration of ethanol was stopped and the rats were switched to a palm oil–dextrose diet (group 3) for 2 weeks (Figures 2*C* and 3). In fact, treatment with a lipid enriched in saturated fatty acids (palm oil) led to almost complete normalization of the liver histology.

Dietary Modulation of Lipid Peroxidation

Our hypothesis that feeding saturated fatty acids should result in decreased levels of lipid peroxidation was supported by measurements of TBARS and conjugated

Table 3. Evaluation of Lipid Peroxidation in the Experimental Groups

	FE (n = 5)	FE-FD (n = 5)	FE-PD (n = 5)
Conjugated dienes (mean ± SD) (A_{232})	0.56 ± 0.11	0.33 ± 0.06[a]	0.14 ± 0.01[b]
TBARS (*nmol/mg protein*)	1.54 ± 0.29	0.79 ± 0.20[c]	0.28 ± 0.08[b]
Aniline hydroxylase activity ($nmol \cdot mg^{-1} \cdot min^{-1}$)	1.08 ± 0.1 (4)	0.43 ± 0.04 (4)[c]	0.32 ± 0.04 (4)[b]

NOTE. Numbers in parentheses indicate numbers of animals in each group in whom measurements of aniline hydroxylase activity were performed.
[a]$P < 0.05$ compared with FE.
[b]$P < 0.01$ compared with FE and FE-FD.
[c]$P < 0.01$ compared with FE.

dienes in the different groups (Table 3). The levels of both TBARS and conjugated dienes were significantly lower after administration of ethanol was discontinued (FE-FD group vs. FE group). Feeding palm oil (group 3) instead of fish oil (group 2) led to a further reduction in lipid peroxidation. Thus, the levels of both conjugated dienes and TBARS were about 60% lower in the FE-PD group than in the FE-FD group.

As stated above, differences in lipid peroxidation between the various groups could reflect changes in the activity of *CYP2E1*, fatty acid composition, or both. The activity of aniline hydroxylase, which reflects the activity of *CYP2E1*, in the various groups is shown in Table 3. Discontinuation of ethanol administration in fish oil–fed rats resulted in about a 2.5-fold decrease in aniline hydroxylase activity ($P < 0.01$). The activity of aniline hydroxylase in the palm oil treatment group was significantly lower than in the fish oil treatment group ($P < 0.01$). The fatty acid composition of the liver in the different experimental groups is shown in Table 4. The higher concentration of polyunsaturated ω-3 fatty acids found in the FE-FD group probably contributed to the increased lipid peroxidation observed in this group. In fact, the peroxidizability index was significantly higher in the FE-FD group than in the FE-PD group. Because palm oil contains higher levels of α- and γ-tocopherol than fish oil, we determined whether differences in dietary tocopherols contributed to the effects of diet on pathology and lipid peroxidation. This was performed by measuring the concentrations of α- and γ-tocopherols in livers from the different groups. As shown in Table 5, no significant differences in either α- or γ-tocopherol levels were found in any of the groups.

Table 4. Fatty Acid Composition of Liver in the Experimental Groups

	FE	FE-FD	FE-PD
14:0	1.8 ± 0.6	2.0 ± 0.4	1.0 ± 0.6
16:0	22.3 ± 2.3	22.1 ± 1.1	25.6 ± 2.4
16:1 ω-7	6.7 ± 3.4	6.4 ± 0.8	3.2 ± 0.4[a]
18:0	5.0 ± 0.8	6.4 ± 1.4	7.6 ± 2.1
18:1 ω-9	14.4 ± 1.1	9.7 ± 1.2[b]	15.9 ± 0.8
18:2 ω-6	12.7 ± 5.8	4.2 ± 0.5	10.8 ± 0.7[a]
20:4 ω-6	3.5 ± 0.5	4.8 ± 1.0	6.5 ± 2.4
20:5 ω-3	8.7 ± 1.8	14.2 ± 1.3[b]	8.8 ± 1.8
22:5 ω-3	5.1 ± 2.1	4.8 ± 0.8	2.9 ± 1.6
22:6 ω-3	11.1 ± 1.5	15.2 ± 0.4[c]	10.8 ± 2.9
PI	202 ± 42	250 ± 19	181 ± 49[a]

NOTE. Values are means ± SD; n = 4.
PI, peroxidizability index (see Materials and Methods for details on calculation of PI).
[a]$P < 0.01$ compared with FE-FD.
[b]$P < 0.01$ compared with FE and FE-PD.
[c]$P < 0.05$ compared with FE and FE-PD.

Discussion

The problem of treating alcoholic liver injury remains intractable. Although nutrition is a principal component of supportive care, little effort has been directed toward developing a dietary strategy that treats the underlying disorder in addition to providing calories. Recently, it was suggested that the amount and type of fatty acid in the diet can influence a wide variety of metabolic processes including eicosanoid synthesis and xenobiotic metabolism.[32] Thus, it is recognized that dietary fatty acids may have pharmacological effects in addition to being a source of calories. With this in mind, we attempted to develop a lipid-based therapy to decrease the severity of alcohol-induced liver injury. Our study

Table 5. Liver α- and γ-Tocopherol Levels in the Experimental Groups

	Dietary groups[a]		
	FE	FE-FD	FE-PD
α-Tocopherol (*ng/mg*)	44.1 ± 7.7	32.2 ± 12.7	33.9 ± 6.0
γ-Tocopherol (*ng/mg*)	0.65 ± 0.12	0.36 ± 0.19	0.46 ± 0.14

NOTE. Values are means ± SD; n = 5.
[a]Palm oil contained 300 mg/L α-tocopherol and 316 mg/L γ-tocopherol; neither tocopherols were detected in fish oil.

clearly shows that a diet enriched in saturated fatty acids, i.e., palm oil, reverses the pathological changes induced by ethanol. In contrast, treatment with a diet enriched in ω-3 fatty acids, i.e., fish oil, did not result in substantial improvement in the severity of alcohol-induced liver injury. It should be noted that administration of fish oil in the absence of ethanol does not cause any of the pathological changes evaluated in the present study.[5,33]

The observed differences in liver pathology in rats fed palm oil or fish oil can be explained in part by differences in lipid peroxidation. For example, the most severe liver pathology and the highest level of lipid peroxidation were detected in rats fed fish oil–ethanol. Moderately severe liver pathology and an intermediate level of lipid peroxidation were detected in rats treated with fish oil–dextrose after discontinuing ethanol. Animals treated with palm oil–dextrose had near normal liver histology and the lowest level of lipid peroxidation after discontinuing ethanol. The data indicate that two separate but related effects of palm oil could have decreased the extent of lipid peroxidation. Thus, the activity of *CYP2E1* was about 2.5-fold higher in animals fed ethanol than in animals fed fish oil–dextrose. The activity of *CYP2E1* was lower still in rats fed palm oil. This decrease in the activity of *CYP2E1* is consistent with the previously observed relationship between fatty acid saturation and *CYP2E1* activity,[12] which showed induction of *CYP2E1* by polyunsaturated fatty acids. We also have shown that dietary fat is important in modifying ethanol-dependent induction of *CYP2E1*.[34] In addition to the changes in the activity of *CYP2E1*, differences in lipid peroxidation between the palm oil and fish oil treatment groups can be explained by differences in the fatty acid composition of the liver. Evidence shows that the extent of lipid radical formation in response to oxidative stress can be influenced by changes in the polyunsaturated fatty acid composition of cellular lipids.[35] As shown in Table 4, higher levels of long-chain polyunsaturated ω-3 fatty acids were found in rats fed fish oil than in rats fed palm oil. Although palm oil contains a higher level of linoleic acid than fish oil and although we have shown that linoleic acid promotes ethanol-induced liver injury,[3] fish oil is highly enriched in long-chain polyunsaturated fatty acids, which in the presence of ethanol cause much more severe lipid peroxidation in liver tissue than does corn oil, which is rich in linoleic acid.[16] Thus, it is the overall content of polyunsaturated fatty acids in the diet rather than the level of linoleic acid that is important in promoting alcohol-induced liver injury. These higher levels of polyunsaturated fatty acids were also reflected in the higher peroxidizability index of liver tissue in the fish oil–fed group. It is noteworthy that higher levels of 22:5 ω-3 and 22:6 ω-3 were detected in the livers of rats fed the fish oil diets than in the levels of the same fatty acids in the diet itself. The most likely explanation for this observation is that the increased synthesis and preferential incorporation of 22:5 ω-3 and 22:6 ω-3 into liver phospholipids and triglycerides in rats fed fish oil is a compensatory response to the decrease in ω-6 fatty acid synthesis observed in the group.[36,37]

Another possibility that should be considered in attempting to explain the therapeutic benefits of treatment with palm oil is its effect on eicosanoid metabolism. Palm oil has been reported to increase prostacyclin levels and decrease thromboxane B_2 levels in plasma.[18] We have recently shown that the severity of alcoholic liver injury correlates with thromboxane B_2 levels in plasma.[38] Moreover, we showed that decreased production of prostacyclin by nonparenchymal cells in the liver preceded the development of pathological liver injury.[39] Also, treatment with cimetidine, which prevents alcoholic liver injury in the rat, results in a decreased ratio of thromboxane B_2 to prostacyclin.[19] Thus, an increase in the ratio of prostacyclin to thromboxane in rats fed palm oil may contribute to its therapeutic efficacy. A final possibility that we considered in evaluating the effect of palm oil–dextrose in markedly reducing the severity of established liver injury was the role of vitamin E. Palm oil contains both tocopherols and tocotrienols and is unusually rich in γ-tocotrienol.[17] α- and γ-tocopherol levels are markedly decreased in the livers of ethanol-fed rats.[31,40,41] Although palm oil contains much higher levels of tocopherols than fish oil,[17] we did not find differences in the hepatic concentrations of these tocopherols in the different groups. The absence of a difference in tocopherol levels despite significant differences in lipid peroxidation in the fish oil and palm oil groups may be related to the relatively short interval (2 weeks) during which the dextrose-containing diets were fed. Some studies have shown that a much longer period of vitamin E supplementation is necessary to increase hepatic vitamin E levels even when the stimulus for lipid peroxidation is discontinued.[42] Another possible explanation for the lower level of lipid peroxidation in the FE-PD group than in the FE-FD group is the higher ratio of α-tocopherol to unsaturated fatty acids in the former group. We did not measure concentrations of tocotrienol; in some studies, tocotrienols show more potent antioxidant activity than tocopherols.[43]

We should also point out that Lieber et al., using another lipid-based strategy, were able to protect baboons against alcoholic fibrosis and cirrhosis.[44] They used poly-

unsaturated lecithin extracted from soybeans. The active species of polyunsaturated lecithin is dilinoleoylphosphatidylcholine, which attenuates the transformation of lipocytes into collagen-producing transitional cells.[45]

Finally, it is important to stress that regardless of the mechanism of action of palm oil, feeding palm oil represents a simple and effective treatment of alcoholic liver injury. If the efficacy of this treatment reflects down-regulation of lipid peroxidation, it will be important to determine whether other saturated lipids are equally effective. Based on the results of this study, we believe that it is important to determine whether a lipid-based strategy is effective in treating patients with alcoholic liver injury.

References

1. Nanji AA, French SW. Dietary factors and alcoholic cirrhosis. Alcohol Clin Exp Res 1986;10:271–273.
2. Nanji AA, Mendenhall CL, French SW. Beef fat prevents alcoholic liver disease in the rat. Alcohol Clin Exp Res 1989;13:15–19.
3. Nanji AA, French SW. Dietary linoleic acid is required for development of experimentally induced alcoholic liver injury. Life Sci 1989;44:223–227.
4. Nanji AA, Khwaja S, Tahan SR, Sadrzadeh SMH. Plasma levels of a novel noncyclooxygenase-derived prostanoid (8-isoprostane) correlate with severity of liver injury in experimental alcoholic liver disease. J Pharmacol Exp Ther 1994;269:1280–1285.
5. Nanji AA, Zhao S, Sadrzadeh SMH, Dannenberg AJ, Tahan SR, Waxman DJ. Markedly enhanced cytochrome P450 2E1 induction and lipid peroxidation is associated with severe liver injury in fish oil-ethanol fed rats. Alcohol Clin Exp Res 1994;18:1280–1285.
6. Takahashi H, Johansson I, French SW, Ingelman-Sundberg M. Effects of dietary fat composition on activities of the microsomal ethanol oxidizing system and ethanol-inducible cytochrome P450 (CYP2E1) in the liver of rats chronically fed ethanol. Pharmacol Toxicol 1992;70:347–352.
7. 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 P4502E1 induction and conjugated diene formation. Alcohol Clin Exp Res 1993;17:598–603.
8. Ronis MJJ, Lumpkin CK, Ingelman-Sundberg M, Badger TM. Effects of short-term ethanol and nutrition on the hepatic microsomal oxygenase system in a model utilizing total enteral nutrition in the rat. Alcohol Clin Exp Res 1991;15:693–699.
9. Morimoto M, Hagbjork AL, Nanji AA, Ingelman-Sundberg M, Lindros KO, Fu PC, Albano E, French SW. Role of cytochrome P450 2E1 in alcoholic liver disease pathogenesis. Alcohol 1993;10:459–464.
10. Ekstrom G, Ingelman-Sundberg M. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P450. Biochem Pharmacol 1989;38:1331–1319.
11. Yoo JH, Hong JY, Ning SM, Yang CS. Roles of dietary corn oil in the regulation of cytochromes P450 and glutathione-S-transferases in rat liver. J Nutr 1990;120:1718–1726.
12. Yoo JH, Ning SM, Pantuck CB, Pantuck EJ, Yang CS. Regulation of hepatic microsomal cytochrome P450 II E1 level by dietary lipids and carbohydrates in rats. J Nutr 1991;121:959–966.
13. Dannenberg AJ, Zakim D. Dietary lipid regulates the amount and functional state of UDP-glucuronosyltransferase in rat liver. J Nutr 1992;122:1607–1613.
14. Dannenberg AJ, Yang EK. Effect of dietary lipids on levels of UDP-glucuronosyltransferase in liver. Biochem Pharmacol 1992;44:335–340.
15. Yang EK, Radominska A, Winder BS, Dannenberg AJ. Dietary lipids coinduce multiple xenobiotic metabolizing enzymes in rat liver. Biochem Biophys Acta 1993;1168:62–58.
16. Nanji AA, Sadrzadeh SMH, Dannenberg AJ. Liver microsomal fatty acid composition in ethanol-fed rats: effect of different dietary fats and relationship to liver injury. Alcohol Clin Exp Res 1994;18:1024–1028.
17. Cottrell RC. Introduction: nutritional aspects of palm oil. Am J Clin Nutr 1991;53:989S–1009S.
18. Sugano M, Imaizumi K. Effect of palm oil on lipid and lipoprotein metabolism and eicosanoid production in rats. Am J Clin Nutr 1991;53:1034S–1038S.
19. Nanji AA, Zhao S, Khwaja S, Sadrzadeh SMH, Waxman DJ. Cimetidine prevents alcoholic hepatic injury in the intragastric feeding rat model. J Pharmacol Exp Ther 1994;269:832–837.
20. Nanji AA, Khwaja S, Sadrzadeh SMH. Eicosanoid production in experimental alcoholic liver disease is related to vitamin E levels and lipid peroxidation. Mol Cell Biochem 1994;140:85–89.
21. French SW, Miyamoto K, Tsukamoto H. Ethanol-induced fibrosis in the rat: role of the amount of dietary fat. Alcohol Clin Exp Res 1986;10:13S–19S.
22. Tsukamoto H, Gaal K, French SW. Insights into the pathogenesis of alcoholic liver necrosis and fibrosis: status report. Hepatology 1990;12:599–608.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–358.
24. Recknagel RO, Glende EA. Spectrophotometric detection of lipid conjugated dienes. Methods Enzymol 1984;105:331–337.
25. Imai Y, Ito A, Sato R. Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. J Biochem 1966;60:417–423.
26. Waxman DJ, Morrissey JJ, LeBlanc GA. Female predominant rat hepatic P450 forms j (IIE1) and 3 (IIA1) are under hormonal regulatory controls distinct from those of the sex-specific P450 forms. Endocrinology 1989;124:2954–2966.
27. Sagai M, Arakawa K, Ichinose T, Shimojo N. Biochemical effects on combined gases of nitrogen dioxide and ozone. I. Species differences of lipid peroxides and phospholipids in lungs. Toxicology 1988;46:251–255.
28. Witting LA, Horwitt MK. Effect of degree of fatty acid unsaturation in tocopherol deficiency induced creatinuria. J Nutr 1964;82:19–25.
29. Holman RT. Autooxidation of fats and related substances, in Holman RT, Lundberg WO, Malkin T, eds. Progress in the chemistry of fats and other lipids. New York: Academic, 1954;2:51–98.
30. Zindenberg-Cherr S, Olin KL, Villanueva J, Tang A, Phinney SD, Halsted CH, Keen CL. Ethanol-induced changes in hepatic free radical defense mechanisms and fatty acid composition in the miniature pig. Hepatology 1991;13:1185–1192.
31. Sadrzadeh SMH, Nanji AA, Meydani M. Effect of chronic ethanol feeding on plasma and liver α and γ-tocopherol levels in normal and vitamin E deficient rats: relationship to lipid peroxidation. Biochem Pharmacol 1994;47:2005–2010.
32. Dannenberg AJ, Reidenberg MM. Dietary fatty acids are also drugs. Clin Pharmacol Ther 1994;55:5–9.
33. French SW. Nutrition in the pathogenesis of alcoholic liver disease. Alcohol Alcoholism 1993;28:97–109.
34. Nanji AA, Zhao S, Lamb RG, Dannenberg AJ, Sadrzadeh SMH, Waxman DJ. Changes in cytochromes P450 2E1, 2B1 and 4A

and phospholipases A and C in the intragastric feeding rat model for alcoholic liver disease: relationship to dietary fats and pathologic liver injury. Alcohol Clin Exp Res 1994;18:902–908.
35. North JA, Spector AA, Buettner GR. Cell fatty acid composition affects free radical formation during lipid peroxidation. Am J Physiol 1994;267:C177–C188.
36. Ulmann L, Blond JP, Poisson JP, Bezard J. Incorporation of Δ6 and Δ5 desaturation fatty acids in liver microsomal lipid classes of obese Zucker rats fed n-6 or n-3 fatty acids. Biochem Biophys Acta 1994;1214:73–78.
37. Kawashima Y, Mizuguchi H, Kozuka H. Modulation by dietary oils and clofibric acid of arachidonic acid content in phosphatidylcholine in liver and kidney of rat: effects on prostaglandin formation in kidney. Biochem Biophys Acta 1994;1210:187–194.
38. Nanji AA, Khettry U, Sadrzadeh SMH, Yamanaka T. Severity of liver injury in experimental alcoholic liver disease: correlation with plasma endotoxin, prostaglandin E_2, leukotriene B_4 and thromboxane B_2. Am J Pathol 1993;142:367–373.
39. Nanji AA, Khwaja S. Sadrzadeh SMH. Decreased prostacyclin production by liver non-parenchymal cells precedes liver injury in experimental alcoholic liver disease. Life Sci 1994;54:455–461.
40. Meydani M, Seitz H, Blumberg JB, Russell RM. Effect of chronic ethanol feeding on hepatic and extrahepatic distribution of vitamin E in rats. Alcohol Clin Exp Res 1991;15:771–774.
41. Kawase T, Kato S, Lieber CS. Lipid peroxidation and antioxidant defense systems in rat liver after chronic ethanol feeding. Hepatology 1989;10:815–821.
42. Cho SH, Choi YS. Lipid peroxidation and antioxidant status is affected by different vitamin E levels when feeding fish oil. Lipids 1994;29:47–52.
43. Watkins T, Lenz P, Gapor A, Struck M, Tomeo A, Bierenbaum M. γ-Tocotrienol as a hypocholesterolemic and antioxidant agent in rats fed atherogenic diets. Lipids 1993; 28:1113–1118.
44. Lieber CS, DeCarli LM, Mak KM, Kim CI, Leo MA. Attenuation of alcohol-induced hepatic fibrosis by polyunsaturated lecithin. Hepatology 1990;12:1390–1398.
45. Lieber CS, Robins SJ, Li J, DeCarli LM, Mak KM, Fasulo JM, Leo MA. Phosphatidylcholine protects against fibrosis and cirrhosis in the baboon. Gastroenterology 1994;106:152–159.

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