Dietary Oxidized Fat Prevents Ethanol-Induced Triacylglycerol Accumulation and Increases Expression of PPARα Target Genes in Rat Liver

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Abstract

Alcoholic fatty liver results from an impaired fatty acid catabolism due to blockade of PPARα and increased lipogenesis due to activation of sterol regulatory element-binding protein (SREBP)-1c. Because both oxidized fats (OF) and conjugated linoleic acids (CLA) have been demonstrated in rats to activate hepatic PPARα, we tested the hypothesis that these fats are able to prevent ethanol-induced triacylglycerol accumulation in the liver by upregulation of PPARα-responsive genes. Forty-eight male rats were assigned to 6 groups and fed isocaloric liquid diets containing either sunflower oil (SFO) as a control fat, OF prepared by heating of SFO, or CLA, in the presence and absence of ethanol, for 4 wk. Administration of ethanol lowered mRNA concentrations of PPARα and the PPARα-responsive genes medium chain acyl-CoA dehydrogenase (MCAD), long chain acyl-CoA oxidase (ACO), and carnitine palmitoyl-CoA transferase I and II, and cytochrome P450 4A1 and increased triacylglycerol concentrations in the liver (P < 0.05) whereas CLA did not. Rats fed OF with ethanol had similar mRNA concentrations of PPARα-responsive genes and lowered hepatic triacylglycerol concentrations compared with SFO (P < 0.05) whereas CLA did not. Rats fed SFO or CLA without ethanol. In contrast, hepatic mRNA concentrations of SREBP-1c and fatty acid synthase were not altered by OF or CLA compared with SFO. This study shows that OF prevents an alcohol-induced triacylglycerol accumulation in rats possibly by upregulation of hepatic PPARα-responsive genes involved in oxidation of fatty acids, whereas CLA does not exert such an effect. J. Nutr. 137: 77–83, 2007.

Introduction

PPARα is a ligand-activated transcription factor that is essentially required for the regulation of hepatic fatty acid metabolism. The important role of PPARα for normal liver function is underscored by the observation that PPARα knockout animals develop fatty liver when fasted (1). Fatty liver in humans most commonly develops in response to chronic alcohol abuse and is probably the result of both impaired fatty acid catabolism and increased lipogenesis in the liver (2). The latter is probably due to an increased mRNA expression of lipogenic genes such as fatty acid synthase (FAS) by activation of sterol regulatory element-binding protein (SREBP)-1 in response to ethanol feeding (3). The impaired fatty acid catabolism is presumably caused by the blockade of PPARα function by ethanol, because ethanol feeding decreased DNA binding of the transcriptionally active PPARα/RXR heterodimer in the promoter region of PPARα-regulated genes and consequently reduced or failed to induce hepatic mRNA levels of several PPARα-regulated genes involved in fatty acid catabolism [long-chain acyl-CoA dehydrogenase (LCAD), medium-chain acyl-CoA dehydrogenase (MCAD), acyl-CoA oxidase (ACO), and liver carnitine palmitoyl-CoA transferase (L-CPT) I] (4). The central role of the disturbed PPARα function in the pathogenesis of alcoholic fatty liver is evident by the observation that administration of pharmacological PPARα agonists to ethanol-fed animals prevented fatty liver by reversing PPARα dysfunction, inducing mRNA levels of several PPARα target genes, and stimulating the rate of fatty acid β-oxidation in the liver (5,6). In addition to pharmacological treatment of alcoholic liver disease, nutritional modulation of alcoholic fatty liver might also be an attractive approach. Recently, we and others demonstrated in rats that dietary oxidized fats activate hepatic PPARα and PPARα-regulated genes (7,8) and reduce hepatic triacylglycerol concentrations (8,9). Moreover, we demonstrated that dietary oxidized fats reduce mRNA concentrations and activities of lipogenic enzymes in the liver (9). This suggests that actions of dietary oxidized fat might be useful in the prevention of alcoholic fatty liver. However, to our knowledge, no studies are available from the literature in this regard.
Therefore, in this study we aimed to explore the effect of dietary oxidized fat (OF), prepared from sunflower oil by heating, on the development of alcoholic fatty liver by use of the well-established Lieb-DeCarli liquid diet for alcohol administration in rats (10). Although differences exist between alcoholic liver disease in rodents and humans, which must be considered when extrapolating the results to the human situation, rats are commonly used as a model for studying the effects of alcohol on hepatic lipid metabolism (11). We used unheated sunflower oil (SFO) as a control fat. Because conjugated linoleic acids (CLA) affect PPARα-dependent gene transcription and are beneficial with respect to prevention from several diseases, we used a CLA supplement containing 60% total fatty acids as CLA as another treatment fat. To estimate the extent of PPARα activation we determined the abundance of mRNA of typical PPARα-induced genes in the liver upon treatment with PPAR (M-CPT I) in the liver, because M-CPT I is also significantly measured mRNA abundance of the muscle form of CPT I such as MCAD, LCAD, CYP4A1, ACO, and L-CPT I. We also sive genes in the liver that are involved in fatty acid catabolism according to the Lieber-DeCarli low fat diet (10). Rats were assigned to 78 Ringseis et al.

Materials and Methods

Animals and diets. Forty-eight male Sprague-Dawley rats, with an initial body weight of 251 ± 15 g, were obtained from Charles River. The animals were housed individually in Macrolon cages in a room with controlled temperature (23 ± 1°C), humidity (50–60%), and lighting (0600 to 1800 h). Nutritionally adequate liquid diets were formulated according to the Lieber-DeCarli low fat diet (10). Rats were assigned to 6 dietary groups (n = 8/group) and were fed liquid diets according to a 2-factorial design with 3 different dietary fats (SFO vs. CLA vs. OF), with or without ethanol (− ethanol vs. + ethanol) (Table 1). The ethanol-containing diets provided 4.184 MJ of energy per L and consisted of 18% of total energy as protein, 12% as fat, 35% as carbohydrate, and 35% as ethanol. All control rats were fed the same diet as the ethanol-fed rats except that ethanol was replaced isocalorically with maltose dextrin.

Both ethanol-fed and control rats ingested identical amounts of nutrients except carbohydrates.

The CLA oil (BASE) contained 60 g CLA isomers (29.3 g c9t11-CLA, 28.9 g t10c12-CLA, 1.4 g c10c12-CLA, 0.3 g 9t11-CLA) per 100 g CLA oil as analyzed by Ag-HPLC-DAD (14). The OF was prepared by heating SFO at 60°C for 25 d, and the extent of lipid peroxidation in the OF was estimated by assaying the peroxide value (POV), concentration of thiobarbituric acid substances (TBARS), concentration of conjugated dienes, concentration of total carbonyls, acid value, and percentage of polar compounds as described recently (15). The concentrations of lipid peroxidation products were (OF vs. fresh SFO): peroxide value (379 vs. 3 mEq O₂/kg), TBARS (13.1 vs. 1.1 mmol/kg), conjugated dienes (274 vs. <1 mmol/kg), total carbonyles (96.9 vs. 2.9 mmol/kg), acid value (5.8 vs. 0.4 g KOH/kg), and polar compounds (27.8 vs. 5.1).

Ethanol-fed rats were acclimated to their diets over a 5-d period by feeding graded ethanol at 12% of total energy on d 1 and 2, 24% on d 3 and 4, and 36% of energy on d 5 and thereafter. Diets were administered daily at 0800 h in graduated rat feeding tubes (Dyets) for 4 wk. The rats from all experimental groups received identical amounts of the isocaloric liquid diets. During the 24 h before sample collection, the liquid diets were given in 2 portions (70% at 0800 and the final 30% at 0600) to minimize variations in feeding patterns between the ethanol-fed rats and the control rats. All experimental procedures described followed established guidelines for the care and handling of laboratory animals (16) and were approved by the council of Saxony-Anhalt.

Sample collection. Four hours after the final portion had been administered, the rats were anesthetized with diethyl ether and killed by decapitation. The liver was excised immediately, and frozen with liquid nitrogen. Blood was collected from the opened neck into heparinized polyethylene tubes (Sarstedt) by the use of heparinized plastic funnels. Plasma was separated from blood by centrifugation (11000 × g; 10 min) at 4°C. For separation of VLDL the plasma density was adjusted to δ = 1006 g/L by adding 0.5 mL of a solution containing 0.195 mol/L sodium chloride and 2.44 mol/L sodium bromide to 1.0 mL of plasma, and centrifuged (19,000 × g; 20 h) at 4°C using a Discovery 90-Ultracentrifuge (Sorvall). Liver, plasma, and VLDL were stored at −80°C pending analysis.

Lipid analysis. Liver lipids were extracted with a mixture of hexane and isopropanol (3:2, v:v) (17). Total cholesterol and triacylglycerol concentrations of liver, plasma, and VLDL were determined using enzymatic

### TABLE 1 Composition of isocaloric liquid diets

<table>
<thead>
<tr>
<th>Component</th>
<th>SFO − ethanol</th>
<th>SFO + ethanol</th>
<th>CLA − ethanol</th>
<th>CLA + ethanol</th>
<th>OF − ethanol</th>
<th>OF + ethanol</th>
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<tr>
<td></td>
<td>g/L</td>
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<tr>
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<td>3.8</td>
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<tr>
<td>Oxidized fat</td>
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<tr>
<td>Dextrose maltose</td>
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</tr>
</tbody>
</table>

1. 1 L of diet contained 4,184 MJ.
2. Mineral mix (mg/L): calcium phosphate dibasic, 4,375; potassium citrate monohydrate, 1,925; sodium chloride, 648; potassium sulphate, 455; magnesium oxide, 210; ferrous sulphate heptahydrate, 43; manganese sulfate hydrate, 40; zinc carbonate, 14; chromium potassium sulphate, 4.82; cupric carbonate, 2.63; sodium fluoride, 0.52; potassium iodate, 0.09; and sodium selenite, 0.09.
3. Vitamin mix (per L): all-trans-retinol, 1.8 mg; cholecalciferol, 10 μg; all-rac tocopherol acetate, 30 mg; menadione sodium bisulfate, 200 μg; thiamine-HCl, 1.5 mg; riboflavin, 1.5 mg; pyridoxine-HCl, 1.75 mg; nicotinic acid, 7.5 mg; calcium pantothenate, 4 mg; folic acid, 0.5 mg; biotin, 50 μg; cyanocobalamin, 25 μg; p-amino benzoic acid, 12.5 mg; and inositol, 25 mg.
reagent kits obtained from Merck Eurolab (Refs. 157609990314 and 113009990314). For the measurement of liver total cholesterol and liver triacylglycerols, lipids of the extract were dissolved in Triton X-100 before enzymatic measurement as described by De Hoff et al. (18). 

**Plasma concentrations of β-hydroxybutyrate.** Plasma concentration of β-hydroxybutyrate was measured using a kit from R-Biopharm. (Ref. 10907979035).

**RNA isolation and real-time RT-PCR.** To determine mRNA expression levels of MCAD, LCAD, PPARα, CYP4A1, ACO, L-CPT I, M-CPT I, SREBP-1c, FAS, and glyceroldehyde-3-phosphate dehydrogenase (GAPDH) for normalization, total RNA was isolated from liver using Trizol reagent (Invitrogen) according to the manufacturer’s protocol. RNA concentration and purity were estimated from the optical density at 260 and 280 nm, respectively. Total RNA (1.2 μg) was subjected to cDNA synthesis using M-MuLV Reverse Transcriptase (MBI Fermentas). To determine mRNA expression levels, real-time detection RT-PCR using the Rotorgene 2000 system (Corbett Research) was applied. cDNA templates (2 μL) were amplified in 100 μL Rotorgene PCR tubes in a final volume of 20 μL containing 500 μmol/L dNTP (Roht), 3.5 mmol/L MgCl₂, 1.25 U GoTaq Flexi DNA Polymerase, 4 μL 5× buffer (all from Promega), 0.5 μL 10× Sybr Green I (Sigma-Aldrich), and 26.7 pmol of each primer pair. The PCR protocol comprised an initial denaturation at 95°C for 3 min and 20–35 cycles of amplification comprising denaturation at 95°C for 25 s, annealing at 60°C for 30 s and elongation at 72°C for 55 s. Subsequent melting-curve analysis was performed from 50°C to 99°C with a heating rate of 0.1°C/s and continuous fluorescence measurement. Identification of product length of the amplified product was confirmed using 2% agarose gel electrophoresis. Relative quantification was performed using the ΔΔct method (19). Ct-values of target genes and the reference gene were obtained using Rotorgene software, version 5.0. GAPDH served as an appropriate reference gene in this experiment because the Ct-values of GAPDH did not differ between treatment groups. Relative expression ratios are expressed as fold changes of mRNA abundance in the treatment group compared with the control group (SFO without ethanol). Sequences of gene-specific primers obtained from Oprean were as follows (forward, reverse): GAPDH (5′-GCA TGG CCT TTC GTG TTC C-3′, 5′-GGG TGG TCC AGG GTT TCT TAC TCC-3′), SREBP-1c (5′-GGA GCC ATG GAT TGG ACA ATC-3′, 5′-AGG AAG GCC TCC AGG GAA G-3′), FAS (5′-AGG TGC TAG AGG CTC TA-3′, 5′-GTG CAC AGA CAC CTT CCC AT-3′), MCAD (5′-CAA GAG AGC CTG GGA ACT TG-3′, 5′-CCC CAA AGA ATT TGC TCT AA-3′), LCAD (5′-AAG GAT TTA AGG AGA GCA AGC-3′, 5′-GGA AGC GGA GGC GGA GTC-3′), PPARα (5′-CCC TCT CTC CAG CCT TCA GCA CTC-3′, 5′-CCA CAA GAG GTT TCT CAG CAG TGC-3′), CYP4A1 (5′-GCA AAT GGA TGA TGG GGA TGG-3′, 5′-GGAG CAG GAA GTG GTG G-3′), ACO (5′-CCT TCT TGC CCT TCC TTC C-3′, 5′-GCC GTT TCA CCG CCT GTG A-3′), L-CPT I (5′-GGA GAC AGA CAG CAT CCA ACA ATA-3′, 5′-AGG TGA TGG ACT TGT CAA ACC-3′), and M-CPT I (5′-GCA AAC TGG ACC GAG AGA AG-3′, 5′-CCT TGA AGC GAC TTC TG-3′). 

**Statistical analysis.** Data were subjected to ANOVA using the Minitab Statistical Software. Classification factors were dietary fat, ethanol, and the interaction of both factors (fat × ethanol). In cases of large differences between variances and means, data were transformed to logarithms prior to ANOVA. For F-values, individual means of the treatment groups were compared by Fisher’s multiple range test. Means were considered significantly different at P < 0.05. Values in the text are means ± SD.

**Results** 

**Food intake and final body weights.** The food intake was the same for each rat due to the controlled feeding regimen used. The amount of liquid diet consumed daily by each rat was 61.4 mL, equivalent to 15.6 g of dry matter. Final body weights were not influenced by dietary treatments (P = 0.79; overall, 270 ± 13 g for all treatment groups, n = 48).

### Liver weights 

Liver weights were influenced by the type of fat. Rats fed the OF diets had higher liver weights than rats fed the SFO or the CLA diets (P < 0.05; Fig. 1A). Liver weights of rats fed the CLA diets did not differ from those fed the SFO diets. Ethanol did not influence liver weights of the rats, and there was no fat × ethanol interaction.

**Figure 1** Liver weights (A), liver triacylglycerols (B), plasma triacylglycerols (C), VLDL triacylglycerols (D), and plasma β-hydroxybutyrate (E) in rats fed liquid diets containing SFO, CLA, or OF with or without ethanol. Bars represent means ± SD, n = 8/group. Means without a common letter differ, P < 0.05. Significant effects (P < 0.05) from 2-way ANOVA: liver weight: fat; liver triacylglycerols: fat, ethanol; plasma triacylglycerols: fat, fat × ethanol; VLDL triacylglycerols: fat, fat × ethanol; plasma β-hydroxybutyrate: fat, ethanol.
than those fed the control diets \((P < 0.05)\). Rats fed the OF diet with ethanol had a higher concentration of β-hydroxybutyrate than rats fed SFO with ethanol. Rats fed the CLA diets did not differ in the plasma concentration of β-hydroxybutyrate from rats fed the SFO diets.

**Relative mRNA concentrations of PPARα and PPARα-responsive genes in the liver.** The relative mRNA concentration of PPARα was influenced by ethanol but not by the type of dietary fat (Fig. 2). Relative mRNA concentrations of the PPARα-responsive genes MCAD, LCAD, CYP4A1, ACO, L-CPT I, and M-CPT I were influenced by the type of fat and by ethanol (Fig. 2). Rats fed the ethanol diets had lower relative mRNA concentrations of PPARα and of all PPARα-responsive genes than those fed the control diets \((P < 0.05)\). Rats fed the OF diets had higher relative mRNA concentrations of all PPARα-responsive genes than rats fed the SFO or CLA diets \((P < 0.05)\). Rats fed the OF diet with ethanol had similar relative mRNA concentrations of all PPARα-responsive genes as rats fed SFO without ethanol. Rats fed the CLA diets did not differ in the relative mRNA concentrations of all PPARα-responsive genes from rats fed the SFO diets.

**Relative mRNA concentrations of SREBP-1c and FAS in the liver.** Relative mRNA concentrations of SREBP-1c and FAS were influenced by ethanol, and there was a fat × ethanol interaction (Fig. 3). In rats fed CLA or OF diets, ethanol increased relative mRNA concentrations of SREBP-1c and FAS \((P < 0.05)\). In contrast, in rats fed the SFO diets, ethanol did not alter relative mRNA concentrations of SREBP-1c and FAS.

**Discussion**

In this study we investigated whether dietary OF and CLA could be useful in the prevention of alcoholic fatty liver. Alcoholic fatty liver is characterized by increased concentrations of triacylglycerols that are the result of an impaired fatty acid catabolism due to a blockage of PPARα and an increased lipogenesis in the liver due to activation of the SREBP-1 pathway (2).

The results of the present study clearly demonstrate that triacylglycerol accumulation in response to ethanol feeding could be markedly reduced in rats by simultaneous administration of dietary OF when compared with CLA or SFO. Moreover, the observation that dietary OF resulted in similar hepatic triacylglycerol levels during ethanol feeding as observed in rats fed SFO or CLA in the absence of ethanol suggests that dietary OF is indeed capable of preventing alcoholic fatty liver disease.

To elucidate the molecular mechanisms underlying these beneficial effects of OF, we investigated mRNA concentrations of fatty acid metabolism–related genes that are altered by ethanol administration. Based on these findings, we suggest that the potential mechanisms of action involved in the prevention of alcohol-induced fatty liver by dietary OF probably involve the restoration of disturbed PPARα function, whereas ethanol-induced SREBP-1c-dependent activation of lipogenesis was not affected by dietary OF. Although we did not measure the protein abundance of the transcriptionally active nuclear form of SREBP-1c, the observation that the SREBP-1c target gene FAS was also not altered by dietary OF supports our assumption that lipogenesis was not affected by dietary OF. However, because ethanol feeding in the SFO and OF group caused an increase in triacylglycerol concentrations only in the liver, and not in plasma and VLDL, it might be also possible that ethanol decreased the VLDL secretion rate (which was not addressed in the present study) from the liver of the SFO and OF group. Thus, future studies should also investigate whether the effect of OF on ethanol-induced fatty liver might be also related to alterations in the secretion rate of apoB-containing VLDL particles.

PPARα activation and function has been shown to be inhibited by ethanol feeding (4,20) as also evidenced in the present study by decreased mRNA levels of PPARα and PPARα-responsive genes such as MCAD, LCAD, ACO, CYP4A1, L-CPT I, and M-CPT I. However, although mRNA expression levels of PPARα-responsive genes were reduced in all ethanol-treated groups when compared with their respective control...
groups, administration of OF during ethanol feeding resulted in activation of PPARα-responsive genes such as MCAD, LCAD, and CYP4A1 compared with SFO or CLA. In addition, mRNA abundance of ACO and L-CPT-I was increased by administration of OF during ethanol feeding. Interestingly, the mRNA concentration of M-CPT-I, which is normally virtually absent from the liver (21), also tended to be increased (P = 0.08) in the liver by treatment with OF during ethanol feeding. Because M-CPT-I is also induced in the liver upon treatment with PPARα activators (12), this finding also supports our assumption that OF caused a PPARα response even in the presence of ethanol.

Insofar as these enzymes are involved in fatty acid catabolism, we suggest that the increased mRNA expression of these enzymes increased the capacity of the liver to oxidize fatty acids and thus counteracted the elevated levels of triacylglycerols and the diminished PPARα function during ethanol feeding. Similar observations have been made using synthetic PPARα agonists such as WY14,643 and fibrates, respectively (4,5,22). Treatment with WY14,643 restored the ability of the PPARα/RXR heterodimer to bind its specific PPAR response element and induce mRNA levels of many PPARα target genes resulting in a higher rate of fatty acid β-oxidation (4). Consequently, excessive accumulation of triacylglycerols in the livers during ethanol feeding was prevented by these agents (4,5,22). In connection with our aforementioned findings, this suggests that dietary OF prevents fatty liver development by similar mechanisms as reported for synthetic PPARα activators.

As a further parameter indicating a PPARα response, we measured the plasma concentration of the ketone body β-hydroxybutyrate, because it has been shown that production of ketone bodies is also induced upon PPARα activation (2,4) and mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase, the key enzyme in ketone body formation, is also a PPARα target gene (13). Thus, the observation that treatment with OF and, to a slight extent, with CLA increased plasma concentration of β-hydroxybutyrate, is also indicative of the induction of a PPARα response. The finding that plasma concentration of β-hydroxybutyrate was increased during ethanol feeding as observed herein is consistent with findings from others (4,23) and reflects stimulation of ketogenesis as a consequence of ethanol administration, similar to the pathogenesis of alcoholic ketoacidosis (24).

Treatment with CLA did not influence ethanol-induced triacylglycerol accumulation compared with SFO. In addition, mRNA concentrations of hepatic PPARα-responsive genes were not altered by CLA compared with SFO. This suggests that CLA has no beneficial effect on alcoholic liver disease, although the ability of CLA to bind and activate PPARα has been well established and the induction of a PPARα response in the liver of rats fed CLA has been demonstrated (25–28). Nevertheless, the effects of CLA on hepatic lipid metabolism in rats reported in the literature are very inconsistent. Whereas some authors demonstrated an induction of a PPARα response in the liver of rats after feeding CLA (27,28), others did not (29), which is similar to the present study. Moreover, different observations have been made with respect to the species investigated. In mice, feeding CLA, predominantly the t10c12-CLA isomer, resulted in a significant liver enlargement and steatosis in several independent studies (30–32), whereas, in studies with rats, these effects have not been documented. Thus, the lack of effect of CLA in this study cannot be explained currently. However, we assume that the failure of effect of treatment with CLA is not explained by an insufficient dose of CLA because the concentration of CLA in the diet was similar (33) or even higher (26) compared with studies demonstrating transcriptional modulation of PPAR-responsive genes by CLA. It has been suggested that weaker PPAR-ligands like polyunsaturated fatty acids (e.g., linoleic acid or CLA) may not bind to this ligand-activated transcription factor with sufficient affinity to prevent the actions of ethanol compared with high affinity ligands like WY14,643 (4). For instance, 30 μmol/L of linoleic acid induced PPAR/RXR complex formation only to 30% of that observed with 5 μmol/L WY14,643 (34). In contrast, oxidized metabolites of linoleic acid such as 9- and 13-hydroxyoctadecadienoic acid (9-HODE and 13-HODE), which are present in oxidized fats due to formation from PUFA during heat treatment, are considered to be very potent activators of PPARα (35). Thus, this could explain the difference between the effects of OF and CLA on ethanol-induced fatty liver in the present study. In addition, 9-HODE and 13-HODE were also identified as potent PPARγ activators (36). This is noteworthy with respect to fatty liver development, because the pharmacological PPARγ-ligand pioglitazone was also recently reported to prevent alcoholic fatty liver by mechanisms involving restoration of the ethanol-induced downregulation of c-Met and upregulation of SREBP-1c (35).

Although dietary treatment with ethanol and OF is not possible in humans due to ethical reasons, we, like many others (3,5,37–39), used rats as an animal model for the current investigation. The Lieber-DeCarli liquid for administering alcohol orally was an excellent way to reproduce early lesions of alcoholic liver disease such as steatosis (11). Moreover, some of the treatment approaches, when applied to nonhuman primates, prevented signs of alcoholic liver disease in a similar manner as shown in rodent models (11). In the discussion of the results with respect to human nutrition, it must be considered, however, that there are differences between rats and humans with respect to activation of PPARα by treatment with PPARα agonists. In rats, treatment with PPARα agonists cause peroxisome proliferation and a strong upregulation of PPARα target genes (40–43). In contrast, humans, which belong to the “nonproliferating” species such as guinea pigs, swine, or monkeys, have a lower expression of PPARα in the liver and show a weaker response of most of the PPARα target genes to treatment with PPARα agonists (44). Therefore, we expect that the upregulation of hepatic PPARα target genes involved in fatty acid oxidation by treatment with OF in humans may be weaker than in rats. Nevertheless, as PPARα agonists retain a triacylglycerol-lowering effect also in nonproliferating species (45), we assume that OF could also counteract the development of an alcoholic fatty liver in humans (5). This pilot study revealed that treatment with 200 mg fenofibrate/d for 4 wk significantly

Figure 3 Relative mRNA concentrations of SREBP-1c (A) and FAS (B) in the liver of rats fed liquid diets containing SFO, CLA, or OF with or without ethanol. SFO without ethanol is considered as control (control = 1.00). Bars represent means ± SD, n = 8/group. Means without a common letter differ, P < 0.05. Significant effects (P < 0.05) from 2-way ANOVA: SREBP-1c: ethanol, fat × ethanol; FAS: ethanol, fat × ethanol.
decreased serum triacylglycerol levels in 8 patients with alcoholic fatty liver, although all patients continued to drink alcohol (\(>80\) g/d) during fenofibrate administration. Serum levels of aspartate aminotransferase, alanine aminotransferase, and \(\gamma\)-glutamyl transpeptidase did not differ before and after treatment with fenofibrate, indicating that liver function was not impaired during short-term treatment with fenofibrate. Although the extent of fatty liver after treatment with fenofibrate has not been reported in that study and a controlled study on a larger number of patients will be necessary to obtain definitive results, it suggests that PPAR\(\alpha\) fibrate has not been reported in that study and a controlled study although the extent of fatty liver after treatment with fenofibrate decreased serum triacylglycerol levels in 8 patients with alcoholic fatty liver and, thus, provides a nutritional approach to modulate this most common liver disease. However, because the uptake of OF has unfavorable effects, such as the impairment of the antioxidant defense system, the uptake of OF for the prevention of human diseases such as alcoholic fatty liver must be considered with caution. Nevertheless, as triacylglycerol accumulation makes the liver more prone to injury by various agents such as drugs and toxins (46), which are presumably involved in the pathogenesis of alcoholic hepatitis and fibrosis (47,48), approaches to prevent or even treat fatty liver are of great importance.

**Literature Cited**


