Selective Reduction of Δ6 and Δ5 Desaturase Activities but Not Δ9 Desaturase in Micropigs Chronically Fed Ethanol

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Abstract

This study investigated the mechanism by which chronic ethanol feeding reduces arachidonate and other highly unsaturated fatty acids in pig liver phospholipids. Five micropigs were fed a diet providing 89 kcal/kg body wt for 12 mo, with ethanol and fat as 40 and 34% of energy, respectively. Five control pigs were pairfed corn starch instead of ethanol. The activities of Δ6 and Δ5 desaturases (expressed as microsomal conversion of precursor to product) in liver from ethanol-fed pigs were reduced to less than half that of controls, whereas the activity of Δ9 desaturase was unaffected in the ethanol group. Δ5 Desaturase activity showed positive correlation with the abundance of its products in liver total phospholipids and microsomes in the ethanol group, but not in the controls. Correlation between Δ6 desaturase activity and its products showed similar pattern to that of Δ5 desaturase, but did not reach statistical significance. No difference was observed between the two groups in coenzyme A concentration in the liver. These results suggest that the selective reduction of Δ6 and Δ5 desaturase activities, not the microsomal electron transport system, are directly responsible for the altered profile of liver phospholipids. (*J. Clin. Invest.* 1994. 93:450–454.) Key words: pig • arachidonic acid • essential fatty acids • alcoholic liver disease

Introduction

In studies of chronic ethanol feeding using miniature pigs and micropigs, we have demonstrated that the proportion of arachidonic acid (20:4ω6)[1] and other highly unsaturated fatty acids are reduced in certain tissues and lipid fractions without a decrease in precursors (1, 2). This decrease in the ratio of 20:4ω6 to linoleic acid (18:2ω6) with ethanol was also observed in other studies with rats (3, 4) and humans (5, 6), although the effect of ethanol on fatty acid profile was variable among tissues and lipid fractions.

A high level of dietary fat has been shown to exacerbate alcoholic liver disease. The severity of fatty liver was correlated with the level of dietary fat in a rat liquid-diet model (7). An advanced stage of alcoholic liver disease (progression to fibrosis) has been observed only with the high fat diet in rats (8, 9), particularly with polyunsaturated fats (10). In concordance with this, we have reported that a low level of dietary fat (9% of energy from corn oil) given to pigs resulted in minimal liver damage (1, 11), whereas ethanol feeding with a diet high in corn oil showed necrosis and fibrosis (12).

The decrease in 20:4ω6 with ethanol intake occurred regardless of the level of dietary fat in our pig model (1, 2). However, a decrease in the ratio of (20:5ω3 + 22:6ω3)/20:4ω6 was observed only with ethanol feeding using a high fat diet (2). As this ω3/ω6 fatty acid ratio in phospholipids affects prostaglandin formation, we have proposed that the decrease in ω3/ω6 ratio in the pigs fed a high-fat plus ethanol diet may have contributed to the progress of alcoholic liver disease (2). If this hypothesis holds, the alteration in the fatty acid profile with ethanol feeding could offer an opportunity for therapeutic intervention to impede the development of alcoholic liver disease.

The mechanism leading to decreased desaturase products with ethanol feeding is not well understood. In rat studies, a uniform decrease in all three desaturases (Δ6, Δ5, and Δ9) has been reported with ethanol feeding (13, 14). This could be due to suppression of the microsomal electron transport system or CoA ligase by ethanol, as they are common components for all three desaturases. On the other hand, reports of the effect of ethanol on the microsomal electron transport system have been contradictory (13, 15, 16). Another study with rats reported no ethanol effect on Δ6 desaturase activity (17), although this study may have been confounded by essential fatty acid deficiency.

Another question, which is not yet answered, is whether altered activity of desaturases can account for the decreased products. Even if the activity is actually decreased, the substrate pool for the desaturases may be increased because of an alteration of lipid metabolism by ethanol intake, resulting in a compensatory increase in product output. Changes in the selectivity of phospholipid synthesis and hydrolysis also could alter the fatty acid profile in phospholipids. And finally, an increase in eicosanoid formation or peroxidation could cause decreased retention of Δ6 and Δ5 desaturase products in phospholipids.

This work was presented in part at the Experimental Biology Meeting, March 1993, New Orleans, LA, and in abstract form (1993. *FASEB* [*Fed. Am. Soc. Exp. Biol.*] J. 7:A169.).

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Received for publication 14 May 1993 and in revised form 23 August 1993.

1. *Abbreviations used in this paper:* HOADH, hydroxyacyl dehydrogenase; 16:0, palmitic acid; 17:0, heptadecanoic acid; 18:2ω6, linoleic acid; 20:4ω6, arachidonic acid; 20:5ω3, eicosapentaenoic acid; 22:6ω3, docosahexaenoic acid.

J. Clin. Invest.

0021-9738/94/01/0450/05 $2.00
Volume 93, January 1994, 450–454

Therefore, the purpose of the current study was to investigate the mechanism by which chronic ethanol feeding alters essential fatty acid profiles by (*a*) analyzing activities of Δ6, Δ5, and Δ9 desaturases, and (*b*) the correlations between the activities of the desaturases and the abundance of their products in liver.

Methods

Animals and diets. The animals and diets used in this study have been described elsewhere (2, 12). Briefly, 10 Yucatan barrow micropigs (5 mo old) from Charles River Laboratories (Wilmington, MA) were paired by body weight after a 1- to 2-mo acclimation period. Five were fed ethanol together with a nutritionally adequate diet, Lab Mini-Pig Chow Starter 5080-2 (Purina Mills, Richmond, IN) and corn oil. Protein was given at the daily level of 2 g/kg body wt (10% of dietary energy). Ethanol, fat, and carbohydrate provided 40, 34, and 16% of daily energy intake (370 kJ/kg body wt), respectively. Corn oil, which contained 61% linoleic acid, supplied most of the dietary fat. Vitamins and minerals were supplied to meet the NRC requirement for swine (18). Five control pigs were pairfed to the ethanol pigs with the same diet, except that corn starch with equal metabolizable energy replaced ethanol. Consequently, carbohydrate provided 56% of dietary energy in the control diet. Three equal portions of the diet were given at 8:00, 12:00, and 17:00 h.

Biopsy procedure and body composition analysis. After 12 mo, the pigs were fasted overnight, pretreated with ketamine (20 mg/kg), acepromazine (0.2 mg/kg), and atropine sulfate (0.04 mg/kg), and anesthetized by isofluothane. Through an abdominal incision, a small piece of liver was excised, immediately freeze-clamped, and stored at −70°C for the analysis of CoA. A fresh piece of liver was taken for lipid analysis and the desaturase assay. Carcass composition was measured by underwater weighing described previously (2). The experimental protocol was approved by the Animal Use and Care Administrative Advisory Committee of the University of California at Davis.

Lipid extraction and quantitation. Fatty acid profile analysis of liver homogenate and subcellular fractions has been reported elsewhere (1, 11). Liver homogenate was centrifuged at 2,000 *g* for 15 min. The supernatant was then centrifuged at 17,000 *g* for 30 min to obtain mitochondrial fraction. The total amount of liver free fatty acids was quantitated using gas chromatography by adding 17:0 as an internal standard before extraction (1). The abundance of Δ6 desaturase products was calculated by summing up 18:3ω6, 20:3ω6, 20:4ω6, 22:4ω6, 22:5ω6, 18:4ω3, 20:4ω3, 20:5ω3, 22:5ω3, 22:6ω3, 20:2ω9, and 20:3ω9 fatty acids (mol%) in total fatty acids. Δ5 Desaturase products were the sum of 20:4ω6, 22:4ω6, 22:5ω6, 20:5ω3, 22:5ω3, 22:6ω3, and 20:3ω9 fatty acids (mol%) in total fatty acids.

Assay of desaturase activity. Liver microsomes for desaturase assay were isolated by a modified combination of the methods of Reid (19) and Morre and Morre (20). About 10 g of fresh liver was rinsed with cold saline, blotted, and weighed. All subsequent steps were done under ice-cold conditions. The liver was minced into small pieces with scissors in 10 mM tris buffer (pH 7.5, 0.25 M sucrose), ground with a Polytron homogenizer (Brinkmann Inc., Westbury, NY) for 45 s, and then further homogenized with a Ten Broeck glass-glass homogenizer (Fisher Scientific, Santa Clara, CA). The final volume of the homogenate was adjusted to 15 times the original liver weight. The homogenate was centrifuged at 17,000 *g* for 30 min. The supernatant was then centrifuged at 110,000 *g* for 1 h. The microsomal pellet was resuspended with the incubation buffer (1 ml/g liver) described below. The recovery of microsomes, assessed by NADPH-cytochrome *c* reductase activity, was 48.9±5.2% and 45.1±4.1% (mean±SEM) for the control and alcohol groups, respectively, and was not statistically different between the two groups. The supernatant was saved to analyze cytosolic protein.

A modification of the method of Leikin and Brenner (21) was used for the desaturase assay. Activities of Δ6, Δ5, and Δ9 desaturases were estimated by measuring microsomal desaturation of linoleic (18:2ω6), dihomo-γ-linolenic (20:3ω6), and palmitic (16:0) acids, respectively. The incubation buffer contained 0.25 M sucrose, 0.15 M KCl, 0.04 M phosphate buffer, pH 7.4, and 0.70 mM *N*-acetylcysteine. The final concentrations of the cofactors were 0.04 M NaF, 1.3 mM ATP, 0.06 mM CoA, 0.87 mM NADH, 5 mM $MgCl_2$, and 0.33 mM nicotinamide dissolved in the incubation buffer. The cofactor mixture was prepared on the day of assay. 1-^{14}C-labeled 18:2ω6, 20:3ω6, and 16:0 fatty acids (~ 50 mCi/mmol in ethanol) were purchased from NEN (New England Nuclear, Boston, MA). The substrate suspension was also prepared on the day of incubation. ^{14}C-labeled fatty acid (1.9 nmol, or ~ 0.1 μCi per assay) was transferred into a glass vial. The ethanol was allowed to evaporate. 50 nmol unlabeled substrate (10 nmol/μl dissolved in propylene glycol) per assay was added to the vial. Then, 100 μl incubation buffer per assay was added, and the vial was vortexed. This substrate suspension provided 33 μM of substrate in the final volume of a 1.5-ml reaction mixture.

Incubation mixture (1.5 ml) consisted of 100 μl of substrate suspension, 50–400 μl of microsome suspension (1–8 mg protein), and the cofactors in the incubation buffer. Each sample was incubated for 15 and 30 min for Δ6 and Δ9 desaturase assays, and 10 and 20 min for Δ5 desaturase assay. The reaction was close to linear in the range above, i.e., the ratio, (activity at 20 or 30 min)/(activity at 10 or 15 min) was 0.89±0.03 (mean±SEM, $n = 27$). Thus, both time points were used for calculation of the reported activity. A blank without protein was always run simultaneously to check the background noise. The incubation was done in a 20-ml flat-bottom vial shaking at 100 cycle/min and 35°C in a Dubnoff shaker. The reaction was started by adding microsomal protein, and stopped by adding 2 ml of saponifying reagent (2N-KOH in methanol).

After the incubation was stopped, the incubation solution was transferred to a screw-cap tube, the vial was washed with 1 ml methanol, and the rinsed methanol was added to the tube. After adding 100 μl BHT (2 wt/vol% in methanol) and flushing with N_2, reaction products including acyl CoAs were saponified at 75°C in a water bath for 1 h. After acidifying with 1.0 ml 6N HCl, the fatty acids were extracted into 2 ml of petroleum ether.

The petroleum ether from the extract was blown down with N_2. After adding 100 μl 2% BHT and 3 ml methylating reagent (5 wt/vol% acetyl chloride in methanol), the extract was flushed with N_2, and methylated for 1 h at 75°C in the water bath. Methylated fatty acids were extracted after the addition of ~ 2 ml water and 2 ml petroleum ether.

Fatty acid methyl esters were separated on 10% silver nitrate-impregnated TLC plates, which were prepared by the method of Holloway (22). The developing solvent consisted of 60 ml petroleum ether and 40 ml diethyl ether for Δ6 desaturase (30 ml and 70 ml for Δ5 desaturase, and 90 ml and 10 ml for Δ9 desaturase). These respective solvent systems yielded clear resolution between 18:2ω6 and 18:3ω6, 20:3ω6 and 20:4ω6, and 16:0 and 16:1ω7 fatty acid methylesters. Standards were always run at the same time to identify each pair of fatty acid methyl esters. 2′,7′-Dichlorofluorescein (0.2% in 95% ethanol) was sprayed onto the plate, and the fatty acid methyl ester spots were located under ultraviolet light. Two spots corresponding to the substrate and product as well as the remaining area of the plate were scraped into separate scintillation vials. Methyl esters were eluted by adding xylene-based scintillation cocktail, and the radioactivity was counted with a scintillation counter. More than 97% of total radio activity recovered from the TLC plate was found in the substrate and product spots. Δ6 Desaturation of 18:2ω6 was calculated by dividing radioactivity in the 18:3ω6 spot by the total radioactivity recovered. Desaturation of Δ5 and Δ9 was obtained in the same way as the Δ6 desaturation.

Protein was assayed by the Coomassie blue binding method using a commercial kit (Bio-Rad Laboratories, Richmond, CA) with BSA as a standard.

Analysis of perchloric acid-soluble CoA and long-chain acyl CoA. Liver CoA and derivatives were quantitated by fluorimetric assay using hydroxyacyl dehydrogenase (HOADH). The method of Williamson

and Corkey (23) was used for the extraction of perchloric acid-soluble CoA and derivatives. Freeze-clamped frozen liver (~ 1 g) was powdered in liquid N_2 using a mortar and pestle. The powdered tissue was added into an ice-cold homogenizer (Ten Broeck glass-glass homogenizer) containing 4 ml 1.0 M $HClO_4$, and was homogenized quickly in ice. All subsequent processes were carried under ice-cold conditions. The homogenate was centrifuged at 10,000 *g* for 5 min at 4°C. The supernatant of the homogenate was transferred to a graduated conical centrifuge tube. The pellet was washed by resuspending with 4 ml of 1.0 M $HClO_4$ and repeating centrifugation. The supernatant was combined and used for the assay of perchloric acid-soluble CoA. The pellet was stored frozen for acyl CoA assay.

A modified method by Michal and Bergmeyer (24) was used for hydrolysis, derivatization, and fluorimetric assay of CoA. To the acid extract placed in ice, 0.01 ml 70% thioglycollic acid (Sigma Chemical Co., St. Louis, MO) was added, and the pH was adjusted to 9.0 with 2 M NaOH. The tube was left for 15 min in an ice bath and then pH was brought back to 7.3 with 1 M $HClO_4$. After adding 0.01 ml diketene (Sigma Chemical Co.), the tube was vortexed and left for 3 min at 0°C. CoA was assayed fluorimetrically by measuring disappearance of NADH. 10 μM NADH in 1% wt/vol $NaHCO_3$ solution was prepared just before the assay. To 0.1 M pyrophosphate buffer (pH 7.3 with 1.3 mM EDTA), the CoA derivative and NADH were added. The final volume was 3.0 ml. A light source of 340 nm and secondary radiation maximum 462 nm were used. The decrease in fluorescence was measured after 5 min at room temperature after adding HOADH (Sigma Chemical Co.).

Hydrolysis and derivatization of the precipitated long-chain acyl CoA for the fluorimetric assay was based on the method of Garland (25). The acid-insoluble precipitate was washed with 0.6% $HClO_4$ and centrifuged for 5 min at 10,000 *g* at 4°C. The precipitate was resuspended in 3.0 ml water, hydrolyzed by adding 0.1 ml of 0.15 M 2-mercaptoethanol at pH 12.5–13.0 adjusted with 2 M KOH. After cooling down to 0°C, 0.7 ml 1.0 M $HClO_4$ was added to bring the pH back to 1–2. The liberated CoA was extracted by $HClO_4$, derivatized, and assayed by the fluorimetric method described above.

Statistical analysis. Data are presented as mean±SEM. Data were analyzed by two-tailed *t* test. *P* values less than 0.1 were discussed as statistically significant. Coefficient of determination (r^2) was obtained by least-square linear regression.

Results

Animal characteristics and alteration of tissue fatty acid profiles. Animal characteristics and histology results have been reported elsewhere (12). Briefly, mean body wt of the control and ethanol groups at 12 mo were 44.4±3.3 vs. 34.6±3.5 kg. Differences in carcass fat but not lean mass accounted for most of the differences in body wt between the two groups (2). The diurnal peak of the blood ethanol level was 184±18 mg/dl. The histological examination of the liver from the ethanol group showed steatosis at 1 mo, varied degrees of steato-necrosis and focal inflammation at 5 mo, and increased pericentral and interstitial collagen infiltration in three of the five animals at 12 mo (12).

The tissue fatty acid profiles of this study have been published elsewhere (2, 26). Briefly, arachidonic acid and other Δ6 and Δ5 desaturase products were decreased in the ethanol group without a decrease in precursors. This tendency held in both ω6 and ω3 families, but the decrease in the ω3 family products was more severe over time than that of the ω6 family (2).

Liver proteins, liver/lean body ratio, and activity of desaturases. Neither the concentration of microsomal nor cytosolic protein in the liver differed between the control and ethanol groups (Table I). On the other hand, as the ethanol-fed pigs had larger livers and smaller lean body mass than the controls (2), the ratio of liver/lean body mass was significantly higher in the ethanol group than the control (Table I). Estimated in vitro activities of microsomal Δ6, Δ5, and Δ9 desaturases are also shown in Table I. In the ethanol-fed pigs, Δ6 desaturase activity was less than one third of the controls when the activity was expressed per milligram of microsomal protein, and remained significantly lower when the activity was expressed per kilogram of lean body wt. Activity of Δ5 desaturase in the ethanol group decreased to less than one half of the controls per milligram of microsomal protein. The decrease was not significant when the activity was expressed per kilogram of lean body wt. In contrast, the activity of Δ9 desaturase was not decreased in the ethanol group.

*Table I. Liver Proteins, Liver/Lean Body Ratio, and Desaturase Activity**

	Control	Alcohol	$P <$ ‡
Microsomal proteins, mg/g liver	20.8±0.9	21.4±1.8	
Cytosolic proteins, mg/g liver	103.7±4.5	121.1±9.5	
Liver/lean body mass	2.44%±0.15%	3.31%±0.13%	0.005
Desaturase activity, pmol/min per mg microsomal protein			
Δ6	17.2±4.1	4.9±1.5	0.03
Δ5	94.1±19.9	38.3±10.0	0.04
Δ9	5.5±2.7	9.3±3.9	
Desaturase activity, pmol/min per kg lean body mass			
Δ6	8.4±1.8	3.3±0.9	0.03
Δ5	47.8±10.3	25.9±5.5	
Δ9	2.7±1.3	6.6±2.8	

* Mean±SEM (n = 5 except n = 4 for desaturase activity in alcohol group. ‡ *P* value between the control and alcohol groups by two-tailed *t* test.

Concentration of free fatty acids and CoA in liver. Table II shows the liver content of free fatty acids and CoA. The concentration of liver free fatty acids was significantly lower in the ethanol group than the controls. Acid-soluble CoA, acyl CoA, or total CoA did not differ between two groups.

Correlations between desaturase activity and desaturase products. Table III shows the abundance of Δ6 and Δ5 desaturase products in liver phospholipids, microsomes, and mito-

*Table II. Liver Free Fatty Acids and Co A**

	Control	Alcohol	$P <$ ‡
Free fatty acids μmol/g liver	1.38±0.12	0.74±0.05	0.005
$HClO_4$-soluble CoA, nmol/g liver	73.0±7.2	76.8±9.9	
Long-chain acyl CoA, nmol/g liver	7.1±3.4	13.3±2.4	
Total CoA, nmol/g liver	80.1±9.1	90.1±8.3	

* Mean±SEM (n = 5). ‡ *P* value between the control and alcohol groups by two-tailed *t* test.

Table III. Δ6 and Δ5 Desaturase Products and Correlations with Desaturase Activity

	Abundance of desaturase products*			Correlations between enzyme activity and products§		
	Control	Alcohol	$P <$ ‡	Control	Alcohol	P
Liver homogenate phospholipids						
Δ6	20.4±0.6	16.1±0.9	0.005	0.21	0.70+	
Δ5	19.8±0.6	15.2±0.9	0.005	0.02	0.94+	0.032
Liver microsomes						
Δ6	23.3±1.0	16.5±1.2	0.005	0.04	0.52+	
Δ5	22.3±1.0	15.3±1.2	0.005	0.00	0.82+	0.095
Liver mitochondria						
Δ6	21.5±0.9	15.3±0.6	0.001	0.17	0.42+	
Δ5	20.3±1.0	14.2±0.5	0.001	0.00	0.57+	

* Mol % in total fatty acids. Mean±SEM (n = 5). ‡ P value between the control and alcohol groups by two-tailed t test. § n = 5 for control group and n = 4 for alcohol group. r^2 value followed by direction of correlation (when $r^2 > 0.4$) and P value.

chondria. Decreases in both Δ6 and Δ5 products were highly significant in the ethanol group. Table III also shows the correlations of Δ6 and Δ5 desaturase activities with the abundance of their corresponding products in the liver. The activity per gram of liver was used for the correlations with liver phospholipids and liver organelles. The correlations between desaturase activities and their products were very different between the control and the ethanol animals. In the control group, neither Δ6 nor Δ5 desaturase activity correlated with the respective products. On the other hand, the ethanol group showed much higher r^2 values than the controls. The correlation of Δ5 desaturase with its products reached statistical significance in liver total phospholipids and a borderline value in microsomes.

Discussion

This is the first report of hepatic fatty acid desaturase activities in a pig model of alcoholism, and the first report of a positive correlation between the estimated activity of Δ5 desaturase and the hepatic abundance of its products in the ethanol-fed animals. These results provide new information on possible mechanisms for both the ethanol effects on essential fatty acid metabolism and its effect on hepatic function.

The activity of Δ6 desaturase per milligram of microsomal protein in the control micropigs (Table I) was about one-tenth that of the activity in rats (13, 14). The activity of Δ5 desaturase in the pigs was much higher than that of Δ6 desaturase (Table I), and it was fairly close to the value for rats (13, 14). It is unknown at present whether this difference in Δ6 desaturase activity between the two species has any effect on tissue essential fatty acid profiles.

As mentioned in the introduction, studies in which the activities of Δ6, Δ5, and Δ9 desaturases were measured with rat liver (13, 14) have shown that ethanol feeding reduced the activities of all three desaturases. In our pig model, chronic ethanol feeding decreased Δ6 and Δ5 desaturase activity significantly, whereas there was no significant effect on Δ9 desaturase activity (Table I). This differential effect of ethanol on the activities of individual desaturases indicates that the reduced activity of Δ6 and Δ5 desaturases is not due to changes in common components of all three desaturases, such as the microsomal electron transport system of CoA ligase activity. This conclusion is in agreement with the studies that showed no decrease in the microsomal electron transport system by ethanol in rats (15, 16). Thus, a likely mechanism for the effect of ethanol in our study is a selective reduction in Δ6 and Δ5 desaturase activities. An alternative mechanism would be alterations in the activities of other enzymes involved in the turnover of ester lipids such as phospholipases and lysophospholipid acyl transferases.

Alterations in lipid metabolism with ethanol include a decrease in fatty acid oxidation (27) and an increase in fatty acid binding protein (28), both of which suggest an increased free fatty acid concentration in the liver with ethanol intake. This increased free fatty acid concentration in turn could increase the substrate pool for desaturases (acyl CoA). If this is the case, the decrease in terminal enzyme activity of Δ6 and Δ5 desaturases in the ethanol-fed group could be due to downregulation by increased substrate level (29). However, our study showed that liver free fatty acids decreased in the ethanol group, and there were no significant changes in the $HClO_4$-soluble, acyl, or total CoA pools (Table II). This suggests that a mass change in the substrate pool is not responsible for the decreased activity of Δ6 and Δ5 desaturases in the ethanol group. A reservation is warranted in the interpretation of these data, as our liver biopsy was performed after an overnight fast. Conceivably, the free fatty acid pool or acyl CoA pool in the fed state may vary from that in the fasted state shown above.

Our observations of reduced hepatic essential fatty acid products plus reduced hepatic free fatty acids raise the possibility that the two are causally linked. Both 20:4ω6 and 20:5ω3 have been shown to reduce hepatic triglyceride synthesis (30). As the decrease in hepatic phospholipid 20:4ω6 and 20:5ω3 with ethanol intake would allow enhanced triglyceride synthesis, this could explain the low concentration of hepatic free fatty acids, and also provide a mechanism for the increased hepatic and plasma triglycerides seen with chronic ethanol use.

Our previous study with miniature pigs (1), in contrast to the current study, showed higher liver free fatty acids in both control and ethanol-fed animals (9.6 and 18.0 μmol/g liver, respectively), with the value in the ethanol-fed animals being higher than that in the controls. As the same general biopsy procedure was employed, the major differences between the

two studies were animal breed and dietary lipid level (three times higher in the present study). However, it is unclear whether and how these differences in protocol are related to the differences in hepatic free fatty acid pool between the two studies.

In the controls in the present study, no correlation was observed between the desaturase activities and the abundance of products of Δ6 and Δ5 desaturases. This suggests that, under normal conditions, the activities of both Δ6 and Δ5 desaturases are more than sufficient to maintain their product levels in a defined range in liver phospholipids. In this condition, factors other than desaturase activity, such as acylation and deacylation of phospholipids, would participate in regulating the levels of Δ6 and Δ5 desaturase products in liver phospholipids. With chronic ethanol feeding, however, the estimated activities of Δ6 and Δ5 desaturases were positively correlated with their products in the liver, although only the correlation between Δ5 desaturase activity and its products in liver phospholipids and microsomes reached statistical significance. This may be due in part to the small sample numbers of the ethanol group ($n = 4$). This positive correlation between desaturase activities and their products implies that ethanol reduced the in vivo activities of the Δ6 and Δ5 desaturases to the level that their products in liver phospholipids became directly dependent upon the residual enzyme activity. This hypothesis is also supported by our observation that the fatty acid profile of liver (and to some degree other tissues) in the ethanol group showed decreased Δ6 and Δ5 desaturase products of both ω6 and ω3 families without a decrease in either substrate (2). This decrease in the Δ6 and Δ5 desaturase pathway, together with increased dietary 18:2ω6, may also explain the decline in the ω3/ω6 product ratio in tissue phospholipids with the high-fat diet plus ethanol feeding observed in this pig model (2).

In summary, ethanol intake selectively decreased Δ6 and Δ5 desaturase activities, but not Δ9 desaturase in an in vitro assay. Thus, ethanol is not likely to act by affecting components common to all three desaturases. The positive correlation between the in vitro activities of the Δ5 desaturase and the proportion of its products in liver phospholipids suggests that decreased activities of the Δ6 and Δ5 desaturases in vivo are directly responsible for the altered fatty acid profile in liver phospholipids with chronic ethanol feeding.

Acknowledgments

The authors thank Dr. Richard A. Freedland for help with the CoA assay, Katherine Olin for preparing some subcellular components, and Jacquelin Lai, Henry Huang, Steven Young, and Sumeet Sharma for technical assistance.

This work was supported by the National Institutes of Health under the auspices of National Institute of Alcoholism and Alcohol Abuse grant 06938 and National Institute of Diabetes, Digestive and Kidney Diseases grant 35747, and by a grant from the Alcoholic Beverage Medical Research Foundation.

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