# Effect of Ethanol Administration on Fatty Acid Desaturation

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## ABSTRACT

The effect of ethanol on the fatty acid desaturation by rat liver has been studied using liquid diets of different composition. Acute ethanol administration increased triacylglycerols of total liver lipids, but did not modify significantly the lipidic composition of microsomes. The Δ6 and Δ5 desaturases were inhibited by ethanol whereas the Δ9 desaturase and fatty acid synthetase were apparently modified only by diet composition. NADH-cytochrome (cyt.) c reductase was partially inhibited, whereas NADH-cyt. $b_5$ reductase remained practically unaltered and NADPH-cyt. c reductase activity was enhanced. Decreased electrons supplied by the microsomal cyt. $b_5$ electron transport chain would not be the reason for the inhibition of Δ6 and Δ5 desaturases by ethanol.

## INTRODUCTION

In animals, the desaturation of fatty acids occurs in the microsomal membrane. This reaction requires 3 amphipatic proteins embedded in the microsomal membrane: NADH cytochrome (cyt.) $b_5$ reductase, cyt. $b_5$ and the desaturase (1). There are different desaturases and their activity is related to dietary conditions. A diet high in carbohydrates increases the Δ9 desaturase, whereas a high-protein diet increases Δ5 and Δ6 desaturases and a fat-free diet increases both Δ9 and Δ6 desaturases (2).

Ethanol administration to rats produces fatty liver via accumulated fatty acids in hepatic triacylglycerols. The origin and extent of accumulation depend on dosage, period of administration and kind of diet (3,4). Liver triacylglycerols contain fatty acids provided by the fat depots, the diet or by endogenous synthesis (5).

Ethanol is oxidized to acetaldehyde and acetate through the cytosolic NADH dependent alcohol dehydrogenase (EC 1.1.1.1.) and aldehyde dehydrogenase (EC 1.2.1.3.). The oxidation of NADH has been considered the rate-limiting factor for this oxidation in vivo (6). However, the microsomal ethanol oxidizing system (MEOS) has been shown to contribute significantly to ethanol oxidation (7,8) and a role for catalase also has been suggested (9).

Since ethanol produces a fatty liver and microsomes are involved in both the biosynthesis of unsaturated fatty acids and alcohol oxidation, it is relevant to explore whether ethanol may modify (a) the lipid composition of liver and microsomes, and (b) the fatty acid desaturation reaction and some related enzymatic activities, such as NADH cyt. c reductase, NADPH cyt. c reductase, NADH ferricyanide reductase and fatty acid synthetase.

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## MATERIALS AND METHODS

[1-$^{14}$C]Palmitic acid (55 mCi/mmol), [1-$^{14}$C]linoleic acid (50 mCi/mmol) and [1-$^{14}$C]eicosa-8,11-14-trienoic acid (61 mCi/mmol) were provided by New England Nuclear, Boston, MA. Cyt. c was provided by Sigma Chem. Co., St. Louis, MO. Cofactors for desaturation reaction were provided by Boehringer Argentina, Buenos Aires, Argentina.

### Diet Treatment

In the first experiment, 15 female Wistar rats of 150-180 g body weight were divided into 3 lots: the first received Purina chow ad *libitum*, the second received a hyperlipidic diet and the third a hyperlipidic diet plus ethanol (see Table I). The second and third lots received isocaloric and force-fed diets, which were administered as 12.5 Kcal/100 g body weight every 12 hr for 48 hr.

In a second experiment, 20 female Wistar rats of 150-180 g body weight were divided into 4 lots. Each lot received the diet described in Table I. The isocaloric liquid diets were fed to the rats as described for the first experiment. Lot 2 received the same diet as lot 1, but 36% of dextrine calories were substituted by ethanol. Lot 3 received a diet without ethanol that maintained the same ratios of carbohydrates, lipids and proteins as lot 2; lot 4 received a diet with ethanol, but with the same ratios of carbohydrates, lipids and proteins as lot 1.

### Microsomes

After the treatment, the rats were killed by decapitation. Livers were immediately collected

and homogenized in a solution containing: 0.25 M sucrose, 0.15 M KCl, 62 mM phosphate buffer (pH 7) and 1.5 mM glutathione. The homogenate was centrifuged at 10,000 x g for 20 min, the pellet was discarded and the supernatant was centrifuged again at 110,000 x g for 60 min. The supernatant was discarded and the pellet containing the microsomes was suspended in the homogenizing solution.

#### Fatty Acid Desaturase Assay

Fatty acid desaturase was assayed using 120 nmol of palmitic acid, 90 nmol of linoleic acid and 100 nmol o eicosa-8,11,14-trienoic acid. The acids were incubated with 5 mg of microsomal protein at 35 C for 20 min. Under these conditions, the enzymes were saturated by the substrate. The solution contained: 0.25 M sucrose, 0.15 M KCl, 0.04 M phosphate buffer (pH 7), 1.5 mM glutathione, 0.04 M KF, 1.3 mM ATP, 0.06 mM CoA, 0.87 mM NADH, 5 mM $MgCl_2$ and 0.33 mM nicotinamide in a final volume of 1.6 ml. Reactions were stopped by the addition of 2 ml of 10% methanolic KOH. Fatty acids were saponified for 45 min at 80 C under nitrogen, then acidified and extracted 3 times with 2 ml of petroleum ether (30-40 C bp). They were sterified 30 min with 3 N HCl in methanol. Conversion was measured by gas liquid radio chromatography in a Packard apparatus (with proportional counter) as described elsewhere (10).

#### NADH Cytochrome $B_5$–Ferricyanide Reducase Activity

Ferricyanide reductase activity was assayed at 25 C by measuring the NADH oxidation at 340 nm. The reaction mixture contained 30 nmol of NADH, 70 nmol of potassium ferricyanide and 2-10 μg of microsomal protein in a final volume of 0.27 ml of 0.05 M Tris acetate (pH 8.1) and 1 mM ethylenediaminetetraacetate (EDTA). The absorption decrease at 340 nm was followed as a function of time. An extinction coefficient of 6.22 $mM^{-1}$ x $cm^{-1}$ was used.

#### NADH Cytochrome c–Reductase Activity

The reduction of cyt. c was measured at 550 nm using 20 nmol of cyt. c, 30 nmol of NADH and 2-10 μg of microsomal protein in a final volume of 0.27 ml of 0.05 M Tris acetate (pH 8.1) and 1 mM EDTA. The absorption increase at 550 nm was followed as a function of time. An extinction coefficient of 18.5 $mM^{-1}$ x $cm^{-1}$ was used.

The activity of NADPH cyt. c reductase was assayed similarly using 20 nmol of cyt. c, 30 nmol of NADPH and 2-10 μg of microsomal protein.

#### Fatty Acid Synthetase Activity

Fatty acid synthetase activity was assayed by the Bruckdorfer et al. method (11), in which NADPH oxidation was measured at 340 nm.

#### Lipid Composition

Liver and microsomal lipids were extracted by the Folch et al. procedure (12). Complex lipids were separated in Silica Gel G (20 x 20 cm plates 0.5 mm thick), with chloroform/methanol/water (65:25:4, v/v), whereas the simple lipids were separated with petroleum ether/ethyl ether/acetic acid (90:10:1, v/v).

Spots were developed by spraying the plates with $H_2SO_4$ (70% saturated with $Cr_2O_7K_2$) and then heating at 200 C for 30 min. They were quantified by photodensitometry with adequate reference standards (13).

## RESULTS

When ethanol is included in the rat diet, an imbalance in fatty acid metabolism is produced

TABLE I

Percentage Caloric Composition of the Diets

| | Experiment 1 | | | Experiment 2 | | | |
|---|---|---|---|---|---|---|---|
| | Lot 1 | Lot 2 | Lot 3 | Lot 1 | Lot 2 | Lot 3 | Lot 4 |
| Carbohydrate (Dextrine) | | 40 | 4 | 53 | 17 | 26.6 | 34 |
| Fat (Oil mixture)[a] | Purina chow | 41 | 41 | 25 | 25 | 39 | 16 |
| Protein (Casein) | | 19 | 19 | 22 | 22 | 34.4 | 14 |
| Ethanol | --- | --- | 36 | --- | 36 | --- | 36 |

[a]16:0 = 11.8%; 16.1 = 0.3; 18:0 = 3.1; 18:1 = 27.6; 18:2 = 57.2.

and the animal develops a fatty liver. This can be produced either by repeated administration of low doses of ethanol or by a large single dose (3,4). Hyperlipidic diets contribute to this fatty liver production. In the first experiment, the effect of repeated doses of ethanol for 48 hr was studied on rats receiving a hyperlipidic diet (Table I). The compositions of liver lipids and liver microsomes were determined (Table II). The activities of Δ5, Δ6 and Δ9 desaturases, the NADH-cyt. c reductase, NADH-ferricyanide reductase and fatty acid synthetase were measured (Table III).

In the first place, it was found that a change in the diet from Purina chow to an hyperlipidic diet evokes only minor changes in the lipid composition of the liver—an unimportant decrease in triacylglycerols. A decreased phosphatidylcholine (PC) and an increased phosphatidylethanolamine (PE) were shown in the microsomes. The Δ5 and Δ6 desaturases were not significantly altered, and the NADH-ferricyanide reductase and NADH-cyt. c reductase were only decreased to a minor extent, whereas the activities of the Δ9 desaturase and fatty acid synthetase were drastically

TABLE II

Percentage Lipid Composition in Experiment 1

| | | Liver | | | Microsome | | |
|---|---|---|---|---|---|---|---|
| | | Lot 1 | Lot 2 | Lot 3 | Lot 1 | Lot 2 | Lot 3 |
| Diacylglycerols | | 1.3 | 1.0 | 1.2 | 1.0 | 0.9 | 1.2 |
| Cholesterol | | 8.2 | 5.3 | 4.2 | 3.6 | 3.7 | 3.2 |
| Free fatty acids | | 1.1 | 3.1 | 2.0 | 2.1 | 1.6 | 2.2 |
| Triacylglycerols | | 19.0 | 12.9 | 35.5 | 5.5 | 6.0 | 7.2 |
| Cholesterol esters | | 5.5 | 7.5 | 7.1 | 1.4 | 3.7 | 3.6 |
| *Lysophospholipids* | | 5.8 | 10.1 | 4.1 | 13.5 | 12.3 | 10.2 |
| Phosphatidylcholine | | 37.6 | 36.2 | 23.7 | 52.9 | 40.9 | 42.1 |
| Phosphatidylethanolamine | | 21.7 | 23.9 | 22.2 | 20.1 | 30.8 | 30.4 |
| % Caloric dietary ratios[a] | C | Purina | 40 | 4 | Purina | 40 | 4 |
| | F | chow | 41 | 41 | chow | 41 | 41 |
| | P | | 19 | 19 | | 19 | 19 |
| | E | | --- | 36 | | --- | 36 |

[a]C = carbohydrate; F = fat; P = protein; E = ethanol.

TABLE III

Enzymatic Activities in Experiment 1

| Enzymes | | Lot 1 | Lot 2 | Lot 3 |
|---|---|---|---|---|
| Δ9 Desaturase (% conversion) | | 53.0 ± 3.2[a] | 8.2 ± 0.5 | 7.1 ± 0.3 |
| Δ6 Desaturase (% conversion) | | 23.3 ± 1.5 | 23.3 ± 1.9 | 14.1 ± 1.1 |
| Δ5 Desaturase (% conversion) | | 19.9 ± 3.3 | 17.2 ± 0.9 | 12.3 ± 1.8 |
| Fatty acid synthetase ($nmol/min^{-1}/mg^{-1}$) | | 37.6 ± 4.7 | 6.0 ± 0.4 | 5.8 ± 0.7 |
| NADH cyt.c reductase ($\mu mol/min^{-1}/mg^{-1}$) | | 0.87 ± 0.04 | 0.46 ± 0.01 | 0.30 ± 0.01 |
| NADH cyt.$b_5$ ferrireductase ($\mu mol/min^{-1}/mg^{-1}$) | | 2.51 ± 0.08 | 2.02 ± 0.08 | 1.86 ± 0.11 |
| % Caloric dietary ratios[b] | C | Purina | 40 | 4 |
| | F | chow | 41 | 41 |
| | P | | 19 | 19 |
| | E | | --- | 36 |

[a]Results represent the mean of 5 samples ± SEM.

[b]C = carbohydrate; F = fat; P = protein; E = ethanol.

diminished.

When ethanol replaced 36% of the calories provided by the carbohydrates of the hyperlipidic diet, the result was the typical increase of triacylglycerols in the total liver lipids. Triacylglycerols are minor components of the microsomes and were not modified (Table II). Δ6 Desaturase, Δ5 desaturase and the NADH-cyt. c reductase were significantly inhibited, but the activities of the other enzymes were not modified significantly.

These results would suggest ethanol decreases the activity of the Δ5 and Δ6 desaturation of fatty acids and the microsomal electron transport involved in the fatty acid desaturation, since the NADH-cyt. c reductase was inhibited. So, the steps that would be altered are the Δ5 and Δ6 desaturases and the electron transport from the cyt. $b_5$ reductase to the cyt. $b_5$ (Table III).

However, since 36% of the calories provided by carbohydrates in the hyperlipidic diet (lot 2) were replaced by ethanol (lot 3) one might speculate that the decrease of the Δ6 desaturase and NADH-cyt. c reductase activities were evoked by a decreased percentage of dietary carbohydrates. In order to discount this possibility, a second experiment was programmed (Table I): a carbohydrate-rich diet was tested (lot 1) and compared to a similar diet in which 36% of the carbohydrate calories were replaced by ethanol (lot 2). Lot 3 received a diet without ethanol which maintained the same ratios of carbohydrates, lipids and proteins as lot 2; lot 4 received a diet with ethanol, but with the same ratios of carbohydrates, lipids and proteins as lot 1.

Total liver lipid composition showed variations in triacylglycerol content as follows: lot 1, 11.7%; lot 2, 26.5%; lot 3, 6.8%; and lot 4, 23.4%. The microsomal liver lipid composition showed no significant variation among the 4 lots and it was similar to the composition shown in Table II. The unsaturated fatty acid:saturated fatty acid ratio in microsomal fatty acid composition also showed no significant variation.

The effects of the diets on the enzyme activities is summarized in Table IV. The comparison of lot 1 with lot 2, where 36% of dextrine calories were replaced by ethanol, showed an inhibition of the 3 desaturases (Δ9, Δ6 and Δ5), the fatty acid synthetase and the NADH-cyt. c reductase, whereas the NADH-ferricyanide reductase remained unmodified. In this experiment, NADPH-cyt. c reductase, an enzyme involved in the transport of electrons to cyt. $P_{450}$, was also measured and we found the activity was enhanced.

However, when the effect of ethanol feeding (lot 2) was compared to rats fed on ethanol-free diet (lot 3) in which the proportion of carbohydrates, lipids and proteins remained constant, ethanol only inhibited the Δ6 and Δ5 desaturases; the Δ9 desaturase and the fatty acid synthetase were unmodified. For the reductases tested, only the NADH-cyt. c and the NADH-cyt. c reductase were changed by ethanol.

TABLE IV

Enzymatic Activities in Experiment 2

| Enzymes | | Lot 1 | Lot 2 | Lot 3 | Lot 4 |
|---|---|---|---|---|---|
| Δ9 Desaturase (% conversion) | | 16.2 ± 1.1[a] | 9.6 ± 0.9 | 9.7 ± 0.8 | 44.5 ± 4.1 |
| Δ6 Desaturase (% conversion) | | 18.0 ± 1.2 | 9.9 ± 0.8 | 18.3 ± 0.6 | 11.1 ± 1.0 |
| Δ5 Desaturase (% conversion) | | 27.6 ± 0.9 | 16.7 ± 2.4 | 32.0 ± 0.6 | 17.5 ± 1.5 |
| Fatty acid synthetase ($nmol/min^{-1}/mg^{-1}$) | | 40.7 ± 2.4 | 24.8 ± 3.6 | 23.6 ± 1.9 | 71.0 ± 3.2 |
| NADH cyt.c reductase ($\mu mol/min^{-1}/mg^{-1}$) | | 0.6 ± 0.06 | 0.36 ± 0.01 | 0.60 ± 0.06 | 0.43 ± 0.03 |
| NADH cyt.$b_5$ ferrireductase ($\mu mol/min^{-1}/mg^{-1}$) | | 2.5 ± 0.1 | 2.4 ± 0.1 | 2.3 ± 0.1 | 2.4 ± 0.1 |
| NADPH cyt.c reductase ($nmol/min^{-1}/mg^{-1}$) | | 52.6 ± 0.3 | 77.3 ± 1.5 | 52.9 ± 4.1 | 76.4 ± 11.9 |
| Percent caloric dietary ratios[b] | C | 53 | 17 | 26.6 | 34 |
| | F | 25 | 25 | 39 | 16 |
| | P | 22 | 22 | 34.4 | 14 |
| | E | --- | 36 | --- | 36 |

[a]Results represent the mean of 5 samples ± SEM.

[b]C = carbohydrate; F = fat; P = protein; E = ethanol.

Apparently, the specific effect of ethanol is produced only in the Δ6 and Δ5 fatty acid desaturases and the NADPH-cyt. c and NADH-cyt. c reductase. The comparison of the decreased fatty acid synthetase and Δ9 desaturase activity of lot 2 with lot 1 indicates there is no specific ethanol effect, since the activity of both enzymes shows a similar decay when lots 3 and 1 are compared. Ethanol was not given to the rats in lots 1 and 3, but the diet composition was changed. The decreased Δ9 desaturase and fatty acid synthetase could result from decreased carbohydrates in the diet (14) or from an increased polyunsaturated lipid content (15), or from a combined effect.

The comparison of lot 2 with lot 4 in Table IV supports this last interpretation. Both groups of rats received, in this case, the same caloric intake of alcohol, but lot 4 was fed a diet richer in carbohydrates and lower in polyunsaturated lipid than the lot 2 diet.

## DISCUSSION

The results of the experiments described show the modification of lipid metabolism produced by ethanol addition. Ethanol also increases liver triacylglycerols.

When rats are given a low-fat diet and ethanol is included, the liver fatty acid biosynthesis is enhanced (4,5). However, it has been shown that fatty acid synthetase and Δ9 desaturase activities respond to diet changes and that carbohydrates activate both enzymes (2,14-16). Therefore, the results of Table IV must be analyzed considering the lower lipid and higher carbohydrate content of the diet in lot 4 which cause increased activities of fatty acid synthetase and Δ9 desaturase when compared to lot 2. Also, administering ethanol, fewer carbohydrates and the same lipid and protein content to lot 2 results in lower synthetase and Δ9 desaturase activities than in lot 1. Under our experimental conditions than the activities of these enzymes are probably more dependent on the diet components than on ethanol ingestion.

Ethanol decreases the biosynthesis of polyunsaturated fatty acids. This effect is produced by (a) a decreases Δ6 desaturase activity that converts linoleic acid to γ-linolenic acid, and (b) a decreased Δ5 fatty acid desaturation that converts eicosa-8,11-14-trienoic acid to arachidonic acid. The decreased Δ6 and Δ5 desaturase activity is apparently caused by ethanol and not by a different composition of the diet for the following reasons: first, it is known that carbohydrates decrease the Δ6 desaturase (17) and that they may not modify the Δ5 desaturase (10). However, if we compare lots 2 and 1 of Table IV, we see the decreased carbohydrates (lot 2) neither, in this case evoke, an increased Δ6 desaturase nor change the Δ5 desaturase. On the contrary, we found that both enzyme levels decreased and suggest this effect results from the ethanol administration to lot 2. Second, proteins are known to increase the Δ6 desaturase level (17). In spite of the dietary proteins provided consistently to lots 1 and 2 decreases in Δ5 and Δ6 desaturases resulted.

The decreased Δ6 and Δ5 desaturase activities are probably not caused by a change in the lipid composition of the microsomal membrane. (The fatty acid desaturases and the electron transport system involved in the fatty acid desaturation are embedded in the membrane). (Table II).

Ethanol feeding induces a small but significant decrease in the microsomal electron transport activity involved in fatty acid desaturation. The electron transport from NADH to cyt. $b_5$ is significantly reduced; the specific decrease is not, however, produced by the cytochrome $b_5$ reductase activity but by the flux of electrons from the flavoprotein to the cyt. $b_5$. Moreover, the NADPH-cyt. c reductase activity of the microsomes was enhanced by the alcohol (Table IV) and this enzyme is linked mainly to the cytochrome $P_{450}$ and MEOS systems which oxidize ethanol (7,8,18). Therefore, we see (a) a decreased electron flux to the cytochrome $b_5$ and polyunsaturated fatty acid desaturation and (b) an increased electron flux to the microsomal system involved in the oxidation of alcohol.

It is improbable that the decreased electron flux to the cytochrome $b_5$, shown in Tables III and IV, might induce a decreased Δ6 and Δ5 desaturation of fatty acid for the following reasons: first, the same electron transport system is used for the Δ9, Δ6 and Δ5 desaturation (19) and the Δ9 desaturation of fatty acids was not diminished by ethanol (Tables III and IV); Second, the supply of electrons by the NADH-cyt. $b_5$ system is so high that, provided sufficient NADH is present, it can easily fulfill the requirements of fatty acid desaturation (20-22). It has been experimentally shown that even after 99% inhibition of NADH-cyt. $b_5$ reductase activity, there are enough electrons to account for 80% of the desaturase activity (20,21). Finally, NADPH may provide electrons to cyt. $b_5$ and fatty acid desaturase by a link through the NADPH-cyt. c reductase (18). The fatty acid desaturase is generally the rate-limiting step of the fatty acid desaturation system, since it has the lowest turnover number

(20,22). These results would therefore suggest that alcohol evokes a specific effect on the Δ6 and Δ5 desaturation of fatty acids.

These experiments demonstrate again that the Δ9 desaturase follows a pattern of activity control different from the Δ6 and Δ5 desaturases—a thesis already stated by us in previous works (2). Moreover, the Δ9 desaturase and fatty acid synthesis would not be modified by ethanol under the conditions of this experiment; they follow a behavior similar sequence to that caused by changes in the diet. We suggest they may be linked as a unit by a common mechanism, a theory already proposed by Jeffcoat and James (23).

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