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Effect of Dietary Choline and Ethanol on Hepatic Triglycerides and Activity of Hepatic Ethanol Oxidizing Enzymes in Rats (37953)

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(Introduced by J. L. Rabinowitz)

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Studies involving both humans and laboratory animals have revealed the presence of ethanol oxidizing activity in the microsomal fraction of the liver (1–4). This enzyme system was first described by Orme-Johnson and Ziegler (5) and later confirmed by Lieber and DeCarli who named it the microsomal ethanol oxidizing system (MEOS) (3, 4). These investigators showed that MEOS activity was increased in rats fed a diet containing ethanol. This ethanol-containing diet has also been reported to produce fatty liver (1) and ultrastructural changes in hepatocytes such as proliferation of the smooth endoplasmic reticulum (3).

Chronic ethanol feeding also causes a decrease in phosphatidylcholine biosynthesis from choline (6). Since phosphatidylcholine is an essential component of the endoplasmic reticulum and other biological membranes, we decided to test for a dietary choline requirement for stimulation of microsomal ethanol oxidizing activity by chronic ethanol feeding.

Experimental animals. Female rats, 250–350 g (CD strain, obtained from Charles River Laboratories, North Wilmington, MA) were divided into 5 groups of 8 animals each. One group was given laboratory chow (Ralston-Purina Co., St. Louis, MO). The remaining four groups were fed a liquid diet. The liquid dietary base was 80% Similac Isomil (TM) (Ross Laboratories, Columbus, OH) plus supplements. Non-vitamin constituents (as wt% of the diet) included protein, 3.2; fat, 5.76; carbohydrates, 10.8; linoleic acid, 0.35; minerals, 0.64; and D,L-methionine, 0.016. Vitamins (per liter of diet) included vitamin A, 4000 USP U; vitamin D, 640 USP U; vitamin E, 8 IU; vitamin C, 80.0 mg; vitamin B_1, 0.64 mg; vitamin B_2, 0.96 mg; vitamin B_6, 0.64 mg; vitamin B_{12}, 4.8 μg; niacin, 9.6 mg; folic acid, 0.16 mg; pantothenic acid, 8.0 mg; and biotin, 0.24 mg.

The basal diet was varied in ethanol, choline, and sucrose for each group as follows: Group 2, designated control choline-deficient, was given sucrose, 12.5% wt of diet, and choline chloride, 0.008%. Group 3, designated control choline-supplemented, was given sucrose, 12.5%, and choline chloride, 0.120%. Group 4, designated ethanol-fed choline-deficient, was given ethanol, 7.3%, and choline chloride, 0.008%. Group 5, designated ethanol-fed choline-supplemented, was given ethanol, 7.3%, and choline chloride, 0.120%. Group 1 received laboratory chow. Thus, 4 groups received choline-deficient and choline-supplemented diets, with and without ethanol, containing, as a % of total calories, 31% sucrose or ethanol isocalorically substituted for the sucrose. All diets contained 165.2 cal/100 ml. Each group received its respective diet ad lib. for 21 days. Food consumption and body weight changes are given in Table I.

Preparation of microsomes. Animals were killed by decapitation after a 16-hr fast. Livers were quickly excised, a 2-g portion

TABLE I. Food Consumption and Change in Total Body Weight of Rats After 21 Days on Diets.

Diet group	No. of animals	Milliliters diet consumed per day	Body wt change (g) for 21-day feeding period
Laboratory chow	8	—	+23.0 ± 3.1
Control, choline-deficient	8	165.8 ± 11.5	+21.8 ± 2.2
Control, choline-supplemented	8	158.6 ± 18.3	+24.9 ± 3.8
Ethanol-fed, choline-deficient	8	111.7 ± 18.9	−14.1 ± 3.5
Ethanol-fed, choline-supplemented	8	104.8 ± 15.5	−13.1 ± 7.8

removed for triglyceride determination, and 4 g of tissue per rat from each experimental group pooled for MEOS and alcohol dehydrogenase (ADH) assays. The combined 4-g portions from each group were homogenized in cold 0.25 *M* sucrose. The homogenate was centrifuged three times at 9,000*g* for 10 min each time, and the microsomes were sedimented by centrifuging the resulting supernatant at 105,000*g* for 60 min. The microsomal pellet was washed once, resedimented, and then homogeneously suspended in 0.15 *M* sucrose at a protein concentration of about 20 mg/ml. Protein was determined by the method of Lowry *et al.* (7).

Assay of MEOS. The standard assay medium for MEOS consisted of 50 m*M* Tris–maleate buffer with a pH of 7.4, 5 m*M* $MgCl_2$, 0.66 m*M* $NADP^+$, 40 m*M* ethanol, 30 m*M* glucose 6-phosphate, and 0.25 U of yeast glucose 6-phosphate dehydrogenase. The total reaction volume was 1.5 ml. Incubations were carried out at 25° in Conway diffusion dishes containing in the center wells 0.5 ml of 0.015 *M* semicarbazide hydrochloride in 0.16 *M* potassium phosphate buffer, pH 7.4. After a 10-min preincubation in which ethanol and microsomes were omitted from the medium, ethanol oxidation was begun by adding first ethanol and then microsomes. The reaction was allowed to proceed in the covered Conway dish for 10 min with gentle rotary shaking and was terminated by the addition of 0.2 ml of 70% $HClO_4$. Acetaldehyde was trapped in the center well as the semicarbazone by overnight diffusion at room temperature. The concentration of the acetaldehyde semicarbazone was determined spectrophotometrically as described by Gupta and Robinson (8). Reaction rate was linear up to 30 min and linear with protein concentration.

Determination of hepatic triglycerides. Hepatic triglyceride concentrations were measured according to the method of Butler *et al.* (9).

Alcohol dehydrogenase assay. ADH activity of the 105,000*g* liver supernatant was measured spectrophotometrically by the observed change in absorbance at 340 nm in a mixture containing 0.1 *M* Tris–HCl buffer, pH 10, 1 m*M* NAD^+, 75 m*M* semicarbazide hydrochloride, and 1.5 mg of the 105,000*g* supernatant protein. Initial rates up to 3 min reaction time were used. Reaction temperature was 25°.

Results. After 21 days, the choline-deficient ethanol-fed rats had a mean liver triglyceride content significantly greater than that of the choline-deficient controls ($P = 0.05$). In contrast, the choline-supplemented ethanol-fed rats had a mean liver triglyceride content not significantly different from the choline-supplemented controls or the rats fed laboratory chow (Table II).

Table III indicates that a significant increase in hepatic MEOS activity occurred only in the choline-supplemented ethanol-fed rats. Chronic administration of ethanol, however, did not produce an increase in hepatic alcohol dehydrogenase activity regardless of the choline content of the diet (Table IV).

Discussion. The results showing an increase in hepatic triglyceride content are consistent with those reported by DeCarli and Lieber who, after feeding rats for 24

TABLE II. Hepatic Triglyceride Content of Rats After 21 Days on Diets.[a]

Diet group	No. of animals	Triglyceride/G wet weight of liver (Mean ± SD)
1. Laboratory chow	8	13.8 ± 4.5
2. Control, choline-deficient	8	29.6 ± 15.0
3. Control, choline-supplemented	8	17.0 ± 5.9
4. Ethanol-fed, choline-deficient	8	41.2 ± 21.4
5. Ethanol-fed, choline-supplemented	8	12.7 ± 5.2

[a] Analysis of variance showed differences between animals in each group to be insignificant but differences between groups to be significant. The difference between groups 2 and 4 and 2 and 3 is significant ($P = .05$). The difference between groups 4 and 5 is significant ($P = .01$). The differences between groups 1 and 3 and 3 and 5 are not significant.

TABLE III. Microsomal MEOS Activities of Pooled Liver Samples from Rats After 21 Days on Diets.[a]

Diet group	Specific activity	Stimulation ratio
1. Laboratory chow	21.2	—
2. Control, choline-deficient	22.4	—
3. Control, choline-supplemented	24.7	—
4. Ethanol-fed, choline-deficient	25.8	1.15
5. Ethanol-fed, choline-supplemented	42.4	1.72

[a] Specific activity is expressed as nmoles of acetaldehyde produced per minute per milligram of microsomal protein at 25°. Stimulation ratio is the ratio of the specific activity of ethanol-fed animals to the specific activity of the control animals.

days a liquid diet containing ethanol and a choline concentration of 25 mg/100 ml, found that hepatic triglycerides increased (10) whereas increasing the choline concentration to 250 or 500 mg/100 ml resulted in a 70–80% reduction in the accumulated triglycerides (11).

The failure of chronic ethanol feeding to produce an increase in the activity of hepatic alcohol dehydrogenase activity (Table IV) has been reported by other laboratories (3, 12, 13). The specific activity of alcohol dehydrogenase in the microsomal fraction of each of the five groups of rats was less than 1 mole $\times$ min^{-1} $\times$ mg^{-1}, even when the 3-acetyl pyridyl analogue of NAD (3APDPN) was used as coenzyme in place of NAD. The absence of alcohol dehydrogenase activity in the microsomal fraction indicates no significant contamination with cytosol.

TABLE IV. Liver ADH Activities of Pooled 105,000*g* Supernatants from Rats After 21 Days on Liquid Diets.[a]

Diet group	Specific activity
1. Laboratory chow	12.1
2. Control, choline-deficient	11.2
3. Control, choline-supplemented	12.5
4. Ethanol-fed, choline-deficient	13.8
5. Ethanol-fed, choline-supplemented	13.2

[a] Specific activity is expressed as nmoles of acetaldehyde produced per minute per milligram of soluble protein at 25°.

It has been suggested that phospholipids play an important role in microsomal oxidizing systems (14–16). Klatskin *et al.* (17) have shown that consumption of alcohol increases the choline requirement of the rat, and Skurdal and Cornatzer have reported that ethanol feeding causes a decrease in phosphatidylcholine biosynthesis from choline (6). In addition, Tuma *et al.* (18) have suggested that ethanol metabolism stimulates oxidative degradation of choline in isolated perfused rat liver. It

appears that under the conditions of this study a choline concentration of 8 mg/100 ml of diet was insufficient for ethanol-induced stimulation of MEOS activity. This concentration falls far below reported maintenance requirements of 75 mg/100 ml diet (19). The failure in stimulation of MEOS activity in the ethanol-fed choline-deficient group could have resulted from a deficiency in phospholipid biosynthesis due to choline deficiency since increasing the choline content of the diet to 125 mg/100 ml resulted in significant stimulation (Tables I and III).

Consistent with our results are those of Dobbins *et al.* (20) who have reported an increase in hepatic aniline hydroxylase activity in rats treated with ethanol and and choline. Both Carter and Isselbacher (21) and Lieber and DeCarli (3, 4) have reported increases in hepatic MEOS activity in rats after 2 or 3 weeks of feeding an ethanol-supplemented liquid diet, but the diets they used contained 25 mg choline/100 ml.

The results of these experiments suggest that in rats supplementation of the diet with choline not only protects against alcoholic fatty liver but also enhances the stimulation of MEOS activity by chronic ethanol feeding.

Summary. Female rats were maintained for 21 days on a liquid diet with and without choline supplementation. Ethanol was given to half of the rats as an isocaloric substitution for dietary sucrose. Choline supplementation inhibited the accumulation of hepatic triglycerides and was required for maximum stimulation of the hepatic microsomal ethanol oxidizing system (MEOS) activity. Increase in hepatic alcohol dehydrogenase (ADH) activity did not occur.

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