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Dietary Manipulation of Ethanol Preference in the Syrian Golden Hamster

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CUNNANE, S. C., Y.-S. HUANG AND D. F. HORROBIN. *Dietary manipulation of ethanol preference in the Syrian Golden hamster.* PHARMACOL BIOCHEM BEHAV **25**(6) 1285–1292, 1986.—Male Golden Syrian hamsters, in which ethanol preference was previously established, were fed a basal diet supplemented with essential fatty acid-rich oils (increased weekly from 10–160 g/kg diet), cholesterol (10 g/kg diet) or retinol palmitate (100 or 200 mg/kg diet), each in an independent study. Within 4–5 weeks, all three supplements were associated with significantly decreased ethanol preference. No consistent change in the fatty acid composition of liver or brain was associated with the decrease in ethanol preference but, in ethanol-fed hamsters, each of the supplements was associated with an increase in total cholesterol and the cholesterol/phospholipid ratio in liver. The essential fatty acid-induced reduction in ethanol preference was not associated with a significant change in blood ethanol elimination rate or time.

Cholesterol Essential fatty acids Ethanol Hamster retinol palmitate

THE Syrian Golden hamster prefers to drink a solution of 10% ethanol when offered it in a choice situation with water, e. g., the ethanol solution comprises <75% of the total fluid intake [1, 2, 5, 6, 9, 14, 19]. In spite of the fact that the preference for ethanol is sufficient to ensure the consumption of 8–10 g ethanol/kg b.wt./day, ethanol dependence or withdrawal apparently does not occur [11, 20, 25]. In comparison with the rat, the hamster exhibits rapid elimination of ethanol from the blood [28] and greater activity of the liver enzyme, alcohol dehydrogenase [15], factors which have been suggested to account for the hamster's tolerance to ethanol.

ABBREVIATIONS

18:2n-6	linoleic acid
20:3n-6	dihomo-gamma-linolenic acid
20:4n-6	arachidonic acid
20:5n-3	eicosapentaenoic acid
22:6n-3	docosahexaenoic acid
HBT	hydrogenated beef tallow
SFO	safflower oil
EPO	evening primrose oil
CLO	cod liver oil
EFA	essential fatty acid
CH	cholesterol
RP	retinol palmitate
PG	prostaglandin
PL	phospholipid

Dietary influences on ethanol metabolism and ethanol preference have been studied in humans and rats but not in the hamster. Total energy intake as well as the proportion of total energy as fat both appear to influence ethanol metabolism. In men, reduction of total energy intake to 1000 kcal/day with 900 kcal of the total as fat, significantly reduced the rate of ethanol metabolism [24]. Ethanol preference in the rat has been shown to be reduced by increasing the intake of fat [7, 8, 17, 18]. In most cases, the type of fat used has not been specified. Rats fed ethanol and offered a choice of diets containing low or high fat showed a preference for the low fat diet [16]. These data suggest a possible inverse relationship between dietary fat intake and ethanol consumption.

Since the Syrian Golden hamster has an acquired preference for ethanol solutions containing <30% absolute ethanol and, unlike the rat, does not need to be forcibly fed ethanol, it was considered worthwhile to assess the influence of supplemental essential fatty acids (EFA) on ethanol intake in hamsters with established ethanol preference. During the course of the studies concerning EFA effects on ethanol preference, it was observed that hamsters supplemented with EFA had higher liver total cholesterol than unsupplemented hamsters. Retinol also increases liver total cholesterol [23] and has been shown to reduce ethanol preference in ethanol-preferring female but not male rats [21]. It was therefore considered possible that decreased ethanol preference in the EFA-supplemented hamsters may have been related to increased liver total cholesterol. Hence, effects of both cholesterol and retinol palmitate supplementation on

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ethanol preference are reported in addition to the effects of supplemental EFA.

METHOD

Although the various dietary supplements were tested in different series of hamsters, each with its own control group, the same protocol was used for each study. Mature male Syrian Golden hamsters (starting weight 100–120 g) were purchased from Canadian Hybrid Farms (Halls Harbour, Nova Scotia) and housed individually in groups of six hamsters each. They were fed for 3 weeks on the basal diet (Purina rodent chow No. 5001, meal) and tap water ad lib. Subsequently, an ethanol solution (10% absolute ethanol in distilled water) was offerred in a second bottle in addition to the tap water. The positions of the two bottles were reversed daily to prevent position preference. Total consumption of both the water and ethanol solution were measured daily. Ethanol preference was measured on a daily basis and was calculated as the percentage that the ethanol solution represented of the total fluid intake.

Essential Fatty Acid Supplementation

One week following the introduction of the choice between water and the ethanol solution (week 2), the basal diet was modified by the addition of hydrogenated beef tallow (HBT), safflower oil (SFO), evening primrose oil (EPO) or cod liver oil (CLO). SFO and EPO are of plant origin; SFO contains primarily linoleic acid (77%, 18:2n-6) and EPO contains linoleic acid (71%) and gamma-linolenic acid (9%, 18:3n-6). CLO is of marine origin and its EFA are mainly eicosapentaenoic acid (8%, 20:5n-3) and docosahexaenoic acid (8%, 22:6n-3). HBT was an energy control for the EFA supplements; it contains 60% 16:0, 29% 18:0 and <1% EFA.

The oils were added to the basal chow diet initially at 10 g/kg diet for one week. Each week for 6 weeks thereafter, the amount of supplemental oil in the diet was doubled; 20 g/kg—week 3, 40 g/kg—week 4, 80 g/kg—week 5, 160 g/kg—weeks 6 and 7 (see Fig. 1). The energy value of the oil supplements represented a maximum of 30% of the total energy in the diet when present at 160 g/kg of the diet. In the basal unsupplemented diet, the total fat represented 8% of total energy.

The reversibility of the effect of EFA supplementation on ethanol preference was determined in a separate study. Six hamsters housed individually in each of three groups were given the basal diet and offered a daily choice between drinking water or 10% ethanol. Once ethanol preference was well-established (1 week), the diet was supplemented with 100 g/kg SFP, EPO or CLO for an additional three weeks. Ethanol preference was monitored throughout. The basal diet (without the EFA supplements) was then re-introduced for an additional three weeks.

Cholesterol and Retinol Palmitate Supplementation

In separate studies, each with its own ethanol-consuming unsupplemented control group, the effects of cholesterol and retinol palmitate supplementation were studied. In each study, ethanol preference was previously well-established. For the cholesterol study, 10 g/kg diet cholesterol (Sigma) was added to the basal diet and ethanol preference was monitored for a period of 4 weeks. For the retinol palmitate study, two groups were supplemented with retinol palmitate (Sigma), one at 100 mg/kg diet and the other at 200 mg/kg diet. Ethanol preference was monitored daily for an additional 5 weeks.

Ethanol Metabolism

In the EFA-supplemented hamsters the rate of elimination of ethanol from the blood following a single IP injection of ethanol was assessed in each hamster by the method of Kulkowsky [14]. This was done at the end of the seven week period of EFA supplementation. After they had been fasted overnight, all the hamsters (both ethanol-naive and those previously consuming ethanol) were injected IP with 1.5 g/kg ethanol (absolute, 10% solution in saline). Thirty and 90 minutes later, 400 μl blood samples were obtained under light ether anesthesia by cardiac puncture using a 27 gauge needle and 1 ml syringe. Serum was assayed for ethanol using a Cobas-BIO centrifugal autoanalyser (Roche) and ethanol assay kit (Stat Pack Ethyl Alcohol Test, Calbiochem-Behring, Hoechst).

Lipid, Fatty Acid and Prostaglandin Analysis

In the EFA supplementation study, analyses were carried out to determine whether the EFA effects on ethanol preference were related to changes in liver or brain lipid, fatty acid or prostaglandin composition. In the hamsters fed the cholesterol or retinol palmitate supplements, only liver phospholipid fatty acids and total lipids were determined. The same protocol was used for all lipid analyses. After homogenization, total lipids were extracted with chloroform:methanol (2:1) and the total phospholipid and triacylglycerol fractions separated by thin layer chromatography [5]. The lipid extracts were assayed for total phospholipid by the spectrophotometric method of Stewart [27] and for total cholesterol by internal standardization using gas liquid chromatography [12]. In the EFA supplementation study, samples of brain were frozen in liquid nitrogen and stored at −70°C for assay of prostaglandin E [26].

Statistics

Statistical comparisons were made using one way ANOVA followed by Newman-Keuls test to determine individual differences.

RESULTS

EFA Supplementation

Final body weights in the various groups of EFA-supplemented hamsters did not differ significantly from those in the ethanol-fed (128±8 g) or ethanol-free controls (130±14 g). In relation to final body weight, liver weight was different only in the ethanol-fed controls (2.8±0.2 vs. 3.6±0.4 in ethanol-free controls, $p<0.01$). Food intake was assessed at the end of the study and was not different between groups (mean, 6.1±1.0 g/100 g/day). Mean total fluid intake (water plus ethanol solution) varied from week to week by as much as 25% within a group. Nevertheless, the values were not significantly different between groups at any point (mean intake, 22±4 ml/day). Actual ethanol intake prior to EFA supplementation was 14–15 g/kg/day (Table 1). A random check of plasma ethanol levels in 10 hamsters in

TABLE 1

INFLUENCE OF SUPPLEMENTATION WITH VARIOUS OILS ON ETHANOL INTAKE AND ELIMINATION FROM THE BLOOD

	Ethanol Elimination		
	Rate (mg/kg/hr)	Time (hr)	Ethanol Intake (g/kg b.wt./day)
CTL(E+)[a]	540 ± 200†	2.2 ± 0.5	14.1 ± 1.9
HBT(E+)[b]	310 ± 70	3.4 ± 0.4*	15.2 ± 2.4
SFO(E+)[c]	280 ± 150	5.6 ± 3.0*	8.4 ± 0.9†
EPO(E+)[d]	470 ± 110	3.0 ± 0.8*	7.9 ± 1.3†
CLO(E+)[e]	490 ± 130	2.0 ± 0.1	9.2 ± 1.1†

[a]CTL(E+)—basal diet plus 10% ethanol in drinking water.
[b]HBT(E+)—ethanol plus 160 g/kg hydrogenated beef tallow in the diet.
[c]SFO(E+)—ethanol plus 160 g/kg safflower oil in the diet.
[d]EPO(E+)—ethanol plus 160 g/kg evening primrose oil in the diet.
[e]CLO(E+)—ethanol plus 160 g/kg cod liver oil in the diet.
[f]mean ± SD, n=6/group.
*$p<0.05$, compared to CTL(E+); ANOVA and Newman-Keuls test.
†$p<0.01$.

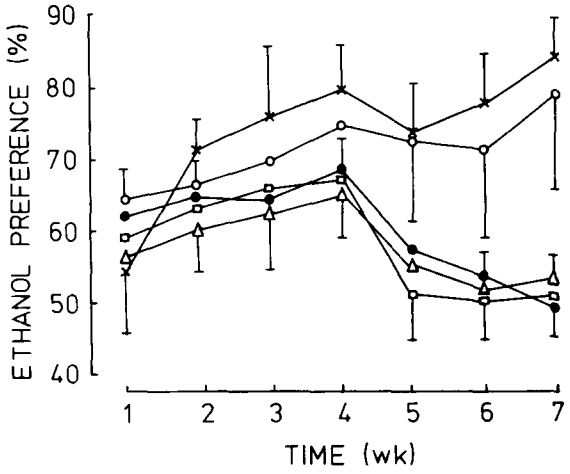

FIG. 1. Effect of supplemental oils on ethanol preference in the hamster. Unsupplemented hamsters fed 10% ethanol—CTL(E+) (×); ethanol-fed hamsters supplemented with hydrogenated beef tallow—HBT(E+) (○); safflower oil—SFO(E+) (△); evening primrose oil—EPO(E+) (□) or cod liver oil—CLO(E+) (●). Ethanol preference was defined as the percentage of intake of the ethanol solution over the total fluid intake (ethanol solution + water). The oils were added at (g/kg) 10 (week 2), 20 (week 3), 40 (week 4), 80 (week 5), and 160 (week 6–7). Values are mean and standard deviation for 6 measurements at each time point. Values for the SFO, EPO and CLO groups were significantly different from CTL(E+) at week 4 through week 7 and from HBT(E+) at week 6 and week 7 ($p<0.01$; ANOVA and Newman-Keuls test).

the ethanol-fed control group gave a value (mean±SD) of 0.12±0.10 mg/dl (range, 0.01 to 0.25 mg/dl).

In the ethanol-fed controls, ethanol preference (% of total fluid intake as the ethanol solution) rose from 55% (week 1) to 80% by week 3 and remained stable thereafter (Fig. 1). Ethanol preference in the HBT-fed hamsters was not significantly different from the ethanol-fed control group at any point in the study. Ethanol preference in the EPO, SFO and CLO-fed hamsters was significantly lower than in the ethanol-fed controls or the HBT-supplemented ethanol-fed hamsters. In each of the three groups of EFA-supplemented hamsters (EPO, SFO, CLO), (a) ethanol preference did not rise above 68%, (b) was significantly less than in the ethanol-fed controls by week 4 (40 g/kg added oils), and (c) decreased to about 50% at the end of the 7 week period (160 g/kg added oils, Fig. 1). Actual ethanol intake was 8–10 g/kg/day in the EFA-supplemented groups (55–60% of that in the ethanol-fed controls, $p<0.01$, Table 1).

The reduction of ethanol preference caused by EFA was readily reversible. After 1 week of being offered the choice between 10% ethanol or water, hamsters fed 100 g/kg EPO, SFO and CLO had an ethanol preference of 49% compared with the ethanol-fed controls at 83% ($p<0.01$, Student's t-test). One week after discontinuing the supplemental oils, the ethanol preference in the previously EFA-fed groups increased to 75–80% (not significantly different from the ethanol-fed controls).

Assuming linear ethanol elimination from the blood [10], calculations were made of the rates of ethanol elimination and time points at which the ethanol dose would be totally eliminated ([14], Table 1). Blood ethanol elimination rates in the unsupplemented ethanol-fed hamsters were similar to those previously reported [15]. Ethanol elimination rate was fastest in the ethanol-fed controls, with intermediate values in the EPO and CLO-supplemented groups and was slowest in the SFO and HBT-fed groups. Due to wide variability, however, none of these differences were statistically significant. Time for total elimination of the dose (1.5 g/kg b.wt.) was shortest in the ethanol-fed controls and longest in the SFO group (Table 1). There was no significant correlation between reduction in ethanol preference and altered blood ethanol elimination.

Brain levels of PGE in the various ethanol-fed groups did not vary significantly from the ethanol-free control values (152±58 pg/g) although a trend to higher values in the SFO (236±75 pg/g), EPO (174±53 pg/g) and CLO-fed groups (173±25 pg/g) was observed.

Values for liver and brain levels of total cholesterol, total phospholipid and the ratio of cholesterol/phospholipid are shown in Table 2. In relation to the ethanol-free controls, liver total cholesterol was significantly higher in all the ethanol-fed hamsters, except those fed HBT. In relation to the HBT-fed hamsters (the energy equivalent for the EFA-fed groups), liver total cholesterol was significantly higher in the SFO, EPO and CLO-fed groups. Liver total phospholipid was significantly higher in all the ethanol-fed groups. Compared to the ethanol-free controls, the cholesterol/phospholipid ratio in liver was lower in the two groups with high ethanol preference (significant only in the HBT-fed group). Compared to the HBT-fed group, the cholesterol/phospholipid ratio was increased significantly in the SFO and CLO groups but not in the EPO-fed group (Table 2). In the brain, total cholesterol was only different from the ethanol-free controls in the CLO-fed group (decreased). Total phospholipids were not different between groups. In relation to both the ethanol-free and ethanol-fed controls, the cholesterol/phospholipid ratio was significantly lower in the three EFA-fed groups than in the other groups.

In the EFA-supplemented hamsters, the percentage com-

TABLE 2

TOTAL CHOLESTEROL, TOTAL PHOSPHOLIPIDS AND THE RATIO OF CHOLESTEROL/PHOSPHOLIPIDS IN LIVER AND BRAIN OF HAMSTERS GIVEN ETHANOL

	Cholesterol	Phospholipids	Cholesterol/ Phospholipids ($\times 10^{-1}$)
Liver			
CTL(E−)[a]	1.3 ± 0.1[g]	15.5 ± 1.5[g]	0.82 ± 0.13
CTL(E+)[b]	1.5 ± 0.2	22.4 ± 1.3*	0.67 ± 0.12
HBT(E+)[c]	1.3 ± 0.1	21.6 ± 1.3*	0.62 ± 0.11
SFO(E+)[d]	1.9 ± 0.6	20.8 ± 1.3*	0.86 ± 0.12†
EPO(E+)[e]	1.7 ± 0.3†	20.1 ± 1.9*	0.74 ± 0.20
CLO(E+)[f]	2.1 ± 0.3†	19.6 ± 0.5*	1.05 ± 0.19†
Brain			
CTL(E−)	14.1 ± 1.8	23.5 ± 2.6	0.60 ± 0.06
CTL(E+)	13.0 ± 2.5	20.4 ± 2.4	0.65 ± 0.07
HBT(E+)	12.4 ± 1.9	24.7 ± 2.7	0.55 ± 0.09
SFO(E+)	12.1 ± 1.1	23.9 ± 1.8	0.51 ± 0.02*
EPO(E+)	12.0 ± 2.2	23.6 ± 2.8	0.46 ± 0.07*
CLO(E+)	10.4 ± 1.7*	21.9 ± 3.1	0.48 ± 0.01*

[a]CTL(E−)—ethanol-free.
[b]CTL(E+)—10% ethanol in drinking water.
[c]HBT(E+)—ethanol plus 160 g/kg hydrogenated beef tallow in the diet.
[d]SFO(E+)—ethanol plus 160 g/kg safflower oil in the diet.
[e]EPO(E+)—ethanol plus 160 g/kg evening primrose oil in the diet.
[f]CLO(E+)—ethanol plus 160 g/kg cod liver oil in the diet.
[g]mean ± SD, n=6/group, mg/g.
*$p<0.01$, compared to CTL(E+); ANOVA and Newman-Keuls test.
†$p<0.01$, compared to HBT(E+).

TABLE 3

FATTY ACID COMPOSITION OF LIVER TOTAL PHOSPHOLIPIDS IN HAMSTERS GIVEN ETHANOL

CTL(E−)[a]	CTL(E+)[b]	HBT(E+)[c]	SFO(E+)[d]	EPO(E+)[e]	CLO(E+)[f]
18:2n-6:[g]					
19.2 ± 0.6	18.0 ± 0.5	19.0 ± 0.8	24.5 ± 0.8	19.0 ± 1.3	14.0 ± 1.5
20:3n-6:					
0.9 ± 0.5	0.6 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	3.2 ± 1.0	0.6 ± 0.1
20:4n-6:					
11.8 ± 1.1	13.2 ± 0.7	13.3 ± 0.8	12.8 ± 1.0	16.8 ± 0.4	7.5 ± 0.5
20:5n-3:					
2.5 ± 0.6	1.7 ± 0.5	1.0 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	7.4 ± 1.6
22:6n-3:					
15.9 ± 2.6	16.1 ± 1.0	16.6 ± 1.2	14.8 ± 1.8	13.8 ± 1.7	23.1 ± 2.6

[a]CTL(E−)—ethanol-free.
[b]CTL(E+)—10% ethanol in drinking water.
[c]HBT(E+)—ethanol plus 160 g/kg hydrogenated beef tallow in the diet.
[d]SFO(E+)—ethanol plus 160 g/kg safflower oil in the diet.
[e]EPO(E+)—ethanol plus 160 g/kg evening primrose oil in the diet.
[f]CLO(E+)—ethanol plus 160 g/kg cod liver oil in the diet.
[g]mean ± SD, n=6/group, mg%.
*$p<0.01$, compared to CTL(E+); ANOVA and Newman-Keuls test.
†$p<0.01$, compared to HBT(E+).

TABLE 4

EFFECTS OF CHOLESTEROL ON ETHANOL PREFERENCE, LIVER TOTAL CHOLESTEROL, LIVER TOTAL PHOSPHOLIPIDS AND THE RATIO OF CHOLESTEROL/PHOSPHOLIPID (×10) HAMSTERS GIVEN ETHANOL

Ethanol[e] Preference	Ethanol[f] Intake	Cholesterol[g]	Phospholipids[g]	Cholesterol/ Phospholipids ($\times 10^{-1}$)
CTL(E−):[a]				
—	—	1.36 ± 0.16	23.4 ± 2.7	0.64 ± 0.09
CTL(E+):[b]				
76 ± 7	12.9 ± 1.2	1.41 ± 0.17	25.0 ± 1.6	0.65 ± 0.08
CH(E−):[c]				
—	—	2.73 ± 0.39*	19.2 ± 0.8*	1.66 ± 0.32*
CH(E+):[d]				
49 ± 6†	8.5 ± 3.9	3.39 ± 0.68	19.8 ± 2.4†	2.00 ± 0.37†

[a]CTL(E−)—ethanol, cholesterol-free.
[b]CTL(E+)—10% ethanol in the drinking water.
[c]CH(E−)—10 g/kg cholesterol in the diet.
[d]CH(E+)—ethanol plus 10 g/kg cholesterol in the diet.
[e]percent of total fluid intake as ethanol solution.
[f]mean ± SD, n=6/group, g/kg/day.
[g]mg/g.
*$p<0.01$ compared to CTL(E−); ANOVA and Newman-Keuls test.
†$p<0.01$ compared to CTL(E+).

TABLE 5

EFFECTS OF CHOLESTEROL ON FATTY ACID COMPOSITION IN LIVER TOTAL PHOSPHOLIPIDS OF HAMSTERS GIVEN ETHANOL

	CTL(E−)[a]	CTL(E+)[b]	CH(E−)[c]	CH(E+)[d]
18:2n-6:[e]	18.3 ± 0.7	17.7 ± 1.2	20.2 ± 0.9*	21.1 ± 2.0†
20:3n-6:	1.0 ± 0.1	1.1 ± 0.2	1.4 ± 0.2*	1.5 ± 0.2*
20:4n-6:	12.1 ± 0.4	11.8 ± 0.8	10.8 ± 0.7*	10.9 ± 0.7
20:5n-3:	1.3 ± 0.3	1.3 ± 0.1	1.5 ± 0.2	1.3 ± 0.3
22:6n-3:	16.4 ± 0.9	15.2 ± 0.9	11.9 ± 0.9*	11.6 ± 11†

[a]CTL(E−)—ethanol, cholesterol-free.
[b]CTL(E+)—10% ethanol in the drinking water.
[c]CH(E−)—ethanol-free with 10 g/kg cholesterol in the diet.
[d]CH(E+)—ethanol plus 10 g/kg cholesterol in the diet.
[e]mean ± SD, n=6/group, mg%.
*$p<0.01$ compared to CTL(E−); ANOVA and Newman-Keuls test.
†$p<0.01$ compared to CTL(E+).

position of the EFA in the liver total PL tended to reflect that of the dietary fatty acids; hamsters supplemented with SFO had higher 18:2n-6, those supplemented with EPO had higher 20:3n-6 and 20:4n-6, the CLO-supplemented hamsters had higher n-3 EFA (20:5n-3, 22:6n-3) in the liver (Table 3). There was no significant correlation between the composition of the EFA in the liver total PL and altered ethanol preference in any of the groups of EFA-supplemented hamsters. The fatty acid composition of the brain total PL varied only slightly betwen groups; 22:4n-6 was increased in the EPO-fed hamsters and 22:6n-3 was increased in the CLO-fed hamsters (results not shown).

Cholesterol Supplementation

Effects of cholesterol supplementation on ethanol preference, actual ethanol intake and liver cholesterol, phospholipid and the cholesterol/phospholipid ratio are shown in Table 4. Compared to the ethanol-fed control hamsters, ethanol preference was significantly decreased in the hamsters fed the basal diet supplemented for 4 weeks with 10 g/kg cholesterol. Food intake did not differ between groups (mean±SD, 7.1±0.7 g/100 g body weight/day). Actual ethanol intake was decreased by 33% in the cholesterol-fed, ethanol-fed group (Table 4).

TABLE 6

ETHANOL PREFERENCE, ETHANOL INTAKE, LIVER TOTAL CHOLESTEROL, LIVER TOTAL PHOSPHOLIPIDS AND CHOLESTEROL/PHOSPHOLIPID RATIO IN HAMSTERS FED RETINOL PALMITATE

Ethanol[e] Preference	Ethanol[f] Intake	Cholesterol[g]	Phospholipids[g]	Cholesterol/ Phospholipids ($\times 10^{-1}$)
CTL(E−):[a]				
—	—	1.44 ± 0.11	21.5 ± 1.8	0.67 ± 0.03
CTL(E+):[b]				
85 ± 8	17.4 ± 1.9	1.61 ± 0.09	20.1 ± 1.3	0.80 ± 0.04*
RP100(E+):[c]				
47 ± 16	10.9 ± 1.1	1.45 ± 0.07	20.7 ± 2.2	0.71 ± 0.08
RP200(E+):[d]				
37 ± 11	6.1 ± 1.8	1.77 ± 0.08†	18.1 ± 1.5*	0.99 ± 0.10†

[a]CTL(E−)—ethanol free.
[b]CTL(E+)—10% ethanol in the drinking water.
[c]RP100(E+)—ethanol plus 100 mg/kg retinol palmitate in the diet.
[d]RP200(E+)—ethanol plus 200 mg/kg retinol palmitate in the diet.
[e]percent of total fluid intake as ethanol solution.
[f]mean ± SD, n=6/group, g/kg b.wt./day.
[g]mg/g.
*$p<0.01$ compared to CTL(E−); ANOVA and Newman-Keuls test.
†$p<0.01$ compared to CTL(E+).

TABLE 7

EFFECT OF RETINOL PALMITATE ON FATTY ACID COMPOSITION IN LIVER TOTAL PHOSPHOLIPIDS OF HAMSTERS GIVEN ETHANOL

	CTL(E−)[a]	CTL(E+)[b]	RP100(E+)[c]	RP200(E+)[d]
18:2n-6	17.0 ± 0.6[e]	16.5 ± 1.8	16.9 ± 0.8	18.6 ± 0.4*
20:3n-6	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.04	1.1 ± 0.1*
20:4n-6	12.2 ± 0.7	11.4 ± 0.6	12.6 ± 0.3†	12.1 ± 0.2
20:5n-3	1.2 ± 0.1	2.5 ± 1.0*	1.8 ± 0.1	1.9 ± 0.3
22:6n-3	16.5 ± 0.8	14.4 ± 0.3*	14.8 ± 0.6*	14.3 ± 0.5*

[a]CTL(E−)—ethanol free.
[b]CTL(E+)—10% ethanol in the drinking water.
[c]RP100(E+)—ethanol plus 100 mg/kg retinol palmitate in the diet.
[d]RP200(E+)—ethanol plus 200 mg/kg retinol palmitate in the diet.
[e]mean ± SD, n=6/group, mg%.
*$p<0.01$ compared to CTL(E−); ANOVA and Newman-Keuls test.
†$p<0.01$ compared to CTL(E+).

Liver total cholesterol was significantly increased in hamsters fed cholesterol or cholesterol plus ethanol but not ethanol alone (Table 4). Total phospholipids were decreased in the cholesterol-fed hamsters and the cholesterol/phospholipid values were increased in both cholesterol-fed groups (Table 4).

In liver phospholipid fatty acids, ethanol alone had no effect on the EFA composition. Cholesterol alone increased 18:2n-6 and 20:3n-6 and decreased 20:4n-6 and 22:6-3. Compared to ethanol alone, cholesterol and ethanol together further increased 18:2n-6 and 20:3n-6 (Table 5). In brain phospholipids, the only fatty acid different from the ethanol-naive controls was lower 22:6n-3 ($p<0.01$) in the hamsters fed both cholesterol and ethanol (results not shown).

Retinol Palmitate Supplementation

Effects of supplementation with retinol palmitate at 100 or 200 mg/kg diet on ethanol preference and actual ethanol intake are shown in Table 6. After supplementation for 5 weeks, ethanol preference was 47% in those hamsters receiving 100 mg/kg and 37% in those receiving 200 mg/kg retinol palmitate as compared to 85% in the ethanol-fed controls (Table 6). Actual ethanol intake was decreased 40–60% in the retinol palmitate-supplemented groups (Table 6).

Also shown in Table 6 are the total cholesterol, total phospholipid and cholesterol/phospholipid values in liver. Total cholesterol was increased only in the hamsters fed ethanol plus 200 mg/kg retinol palmitate. Total phospholipids were lower only in the hamsters supplemented with retinol

palmitate at 200 mg/kg diet. The cholesterol/phospholipid ratio was increased in the hamsters fed ethanol only or ethanol plus 200 mg/kg retinol palmitate (Table 6).

With respect to the EFA composition of the liver phospholipids, ethanol feeding decreased 22:6n-3 and 20:5n-3. Compared to the ethanol-fed hamsters, 100 mg/kg retinol palmitate increased 20:4n-6 and 200 mg/kg retinol palmitate increased 18:2n-6 and 20:3n-6 (Table 7).

DISCUSSION

Our results indicate that the ethanol preference acquired by the hamster when offered 10% ethanol in a choice situation with water can be significantly reduced by feeding supplemental EFA, cholesterol or retinol palmitate. Four aspects of the effect of EFA on ethanol preference are of significance: (a) non-specificity, (b) reversibility, (c) it was not an effect of increased dietary energy intake, and (d) its possible relation to increased liver total cholesterol. The EFA effect on ethanol preference appears to have been non-specific with regard to the EFA source; oils of plant origin (SFO, EPO) containing mainly n-6 EFA had qualitatively and quantitatively similar effects to an oil of marine origin (CLO) containing mainly n-3 EFA. This suggests that these oils have a non-specific metabolic effect in common on ethanol preference, e.g., one which is not dependent on the n-6 or n-3 fatty acid composition of the oils. The effect of all three EFA-rich oils was rapidly reversible; within one week of discontinuing the EFA supplements, ethanol preference returned to the pre-supplementation preference values (approximately 80%). The effect of the EFA in reducing ethanol preference was not an effect of increased dietary energy intake because an oil with a total energy content equivalent to the EFA-rich oils (HBT) did not reduce ethanol preference (Fig. 1). All groups consuming ethanol also had equivalent food intake.

The ethanol-preference lowering effect of the EFA sources appears to have been related in part to the effects of EFA on cholesterol metabolism. In cholesterol-fed rats, both SFO and EPO have been shown to lower plasma cholesterol but only EPO was associated with higher liver cholesterol after cholesterol-feeding [12]. In ethanol-fed hamsters whose diets were supplemented with SFO, EPO or CLO, plasma total cholesterol values were unrelated to reduced ethanol preference (results not shown). However, in relation to the ethanol-free controls and energy controls (ethanol plus HBT), *liver* total cholesterol was significantly increased in the EFA-fed groups (Table 3). Thus, higher liver total cholesterol and a higher cholesterol/phospholipid ratio were noted in the groups of ethanol-fed hamsters which were fed supplemental EFA and had lower ethanol preference.

Other observations support the possibility that a reduction in ethanol preference in hamsters is accompanied by an increase in liver total cholesterol: (a) liver total cholesterol was increased and ethanol preference decreased in hamsters fed 10 g/kg cholesterol (Table 4), (b) liver total cholesterol was increased and ethanol preference was decreased in hamsters fed 200 mg/kg retinol palmitate in the diet (Table 6), and (c) the rat, which has significantly higher liver cholesterol than the hamster [12], has lower ethanol preference than the hamster [30]. Membrane content of cholesterol and the cholesterol/phospholipid ratio are frequently increased in rodents chronically consuming ethanol [3,29]. *In vitro*, the increase in cholesterol and the cholesterol/phospholipid ratio in membranes appears to be a stabilizing response to the lipid membrane-disordering effects of ethanol [4]. Therefore, our data are consistent with the concept that dietary agents which increase total cholesterol and the cholesterol/phospholipid ratio in liver (EFA, cholesterol and retinol palmitate) lower ethanol preference in the hamster possibly by competing with the membrane-fluidizing effects of ethanol.

Elimination of a standard dose of ethanol from the blood was tested in the same animals in which effects of EFA supplementation on ethanol preference were tested. In the CTL(E+) group, ethanol elimination values were similar to those published previously [15]. Methodological difficulties (primarily the need for anesthesia with hamsters, hence limiting us to two data points) may preclude anything but tentative conclusions. Nevertheless, there did not appear to be any relation between reduction of ethanol preference by EFA (Table 1) or cholesterol (results not shown) and alteration of blood ethanol elimination. Therefore, if increased liver total cholesterol also alters (decreases) ethanol metabolism thereby causing an aversion to ethanol, this was not evident from our limited blood ethanol elimination data (Table 1).

An increase in dihomo-gamma-linolenic (20:3n-6) in liver phospholipids has been previously shown to occur in rats supplemented with SFO or EPO [12] or cholesterol [12] and has also been reported in hamsters supplemented with 200 mg/kg retinol palmitate [13]. In the present study, except for EPO, the EFA did not significantly increase 20:3n-6 (Table 3) but both cholesterol (Table 5) and 200 mg/kg retinol palmitate (Table 7) did significantly increase liver phospholipid content of 20:3n-6. A specific increase in the liver phospholipid content of 20:3n-6 may also be relevant to decreased ethanol preference in the hamster.

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