Effects of Ethionine and Methionine on Serum Lipids and Lipoproteins. (27860)

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Feinberg *et al.*(1) and Furman *et al.*(2) have reported that administration of ethionine leads to a fall in serum lipid and lipoprotein concentrations in the dog. In rats fed a diet containing cholesterol, cholic acid and hydrogenated coconut oil administration of ethionine results in a marked reduction in serum cholesterol concentration within 40 hours(3,4). In rats fed this diet supplemented with ethionine for 3 weeks the rate of increase in serum cholesterol concentration is much less than that observed in rats fed the unsupplemented control diet. The effects of ethionine on serum cholesterol, phospholipid and triglyceride concentrations and on the distribution of these lipids in various lipoprotein fractions are reported here for rats fed on diets designed to produce hypercholesterolemia.

Methods. Male weanling rats of the Holtzman strain weighing 50 to 55 g were fed the experimental diets for 3 weeks. At the end of this period blood was obtained by cardiac puncture from animals under ether anesthesia. Blood samples were allowed to clot and were centrifuged at a low speed to separate serum. Serum from 8 to 20 rats, depending on the amount of blood obtainable from each rat and the serum lipid concentrations of various groups, were pooled.

Serum samples were centrifuged at 80,000 *g* for 20 to 22 hours in a sodium chloride solution of density 1.063 or 1.006 g per ml in a Spinco model L preparative centrifuge using a No. 40 rotor. The term "low-density lipoproteins" will be taken to mean lipoproteins flotated in saline of density 1.063 g per ml. Chylomicrons were obtained by flotation in a sodium chloride solution of density 1.006 g per ml for 1 hour at 14,000 *g*. Details of the method are given by de Lalla and Gofman (5). For lipid analyses the serum or lipoprotein preparation was extracted with a 2:1 (v/v) chloroform : methanol mixture and fractionated on silicic acid-super cell columns according to the method of Van Handel and Zilversmit(6). Cholesterol was determined by the method of Henly(7). Triglycerides were saponified and glycerol was determined by oxidation with periodate followed by development of color with chromotropic acid (4,5-dihydroxy-2,7-naphthalene disulfonic acid)(6). Lipid soluble phosphorus was determined by the method of Youngsberg(8) involving digestion with sulfuric acid and analysis of phosphate by a modification of the method of Fiske and SubbaRow(9). A factor of 25 was used to calculate phospholipid concentration.

The basal diet used in this work contained: casein, 10%; hydrogenated coconut oil, 25%; cholesterol, 1%; cholic acid, 0.5%; salts(10), 5%; choline chloride, 0.2%; water soluble vitamin mixture(11), 1%; and sucrose to make 100%. Fat soluble vitamins were administered weekly in ethanol(11) and all additions to the diet were made at the expense of sucrose.

Results and discussion. Rats fed the basal diet supplemented with 0.25% of DL-ethionine had lower serum cholesterol concentrations after 3 weeks than rats fed the unsupplemented basal diet (Table I). Ethionine appears to be less effective in reducing the amount of cholesterol associated with low-density lipoproteins than in reducing total serum cholesterol concentration. Results resembling those shown for Experiment 1 (Table I) were observed in 3 of 4 experiments but in the other, Exp. 2 (Table I), the concentration of cholesterol associated with the low-density lipoprotein fraction from rats fed the ethionine-supplemented diet was lower than in the other experiments. In all experiments, however, supplementing the diet with both ethionine and methionine greatly lowered the concentration of cholesterol in the whole serum and in the low-density lipoprotein fraction.

A series of 3 injections of ethionine over a

TABLE I. Effect of Ethionine and Methionine on Concentration and Distribution of Cholesterol in Serum.

	Total cholesterol concentration					
	Exp 1 (3 wk)			Exp 2 (3 wk)		
		Lipoproteins			Lipoproteins	
Diet	Whole serum	<1.063 g/ml*	Chylomicrons	Whole serum	<1.063 g/ml*	<1.006 g/ml*
	(mg/100 ml)					
Basal	2030	1390	90	2210	1620	1200
+.25% DL-Ethionine	1400	1160	80	720	470	190
+.25% DL-Ethionine & .25% DL-Methionine	140	40	—	110	40	30
Basal + 75 mg DL-Ethionine†				210	80	—
+ .6% DL-Methionine	830			680	480	180

* Hydrated density.
† Rats were fed the basal diet for 3 wk and given 3 injections of 25 mg of ethionine at 12-hr intervals, then fasted for 16 hr before blood samples were taken.

24-hour period lowered serum cholesterol concentrations to less than 10% of control values in whole serum and in the low-density lipoprotein fraction.

Inclusion of 0.25% of ethionine in the basal diet for 3 weeks had no consistent effect on serum phospholipid concentrations in several experiments, thus the slight rise observed in the experiment shown in Table II cannot be considered significant. However, addition of both ethionine and methionine to the diet lowered the phospholipid concentration of whole serum and of the low-density lipoprotein fraction.

TABLE II. Effect of Ethionine and Methionine on Phospholipid* Concentration in Serum and in Low Density Lipoproteins.

	Phospholipid concentration	
Diet	Whole serum	Lipoproteins, <1.063 g/ml
	(mg/100 ml)	
Basal	250	180
+.25% DL-Ethionine	320	185
+.25% DL-Ethionine and .25% DL-Methionine	80	20
Basal + 75 mg DL-Ethionine†	120	40
.6% DL-Methionine	150	115

* Lipid soluble phosphorus in mg/100 ml × 25.
† Injected on final day of experiment, conditions described in Table I.

Serum triglyceride concentrations of rats fed the ethionine-supplemented diet increased 7- to 15-fold over those of rats fed the unsupplemented diet (Table III). This rise in serum triglyceride concentration was largely confined to triglycerides associated with the low-density lipoproteins. Only a small fraction of the total serum triglycerides of ethionine-fed rats was found in the chylomicrons separated by centrifugation at 14,000 *g* for 1 hour in saline of density 1.006 g per ml. This treatment also flotates some very low density lipoproteins(12). However, these results suggest that the largest effect of ethionine on serum triglycerides was in the lipoprotein fraction of hydrated density less than 1.063 but greater than that of the chylomicrons.

The rise in serum triglyceride concentration produced by ethionine was prevented by the presence of an equal amount of methionine in the diet. Methionine produced similar reductions in both triglyceride and cholesterol concentrations when included in a diet containing ethionine (Tables I and III).

The injection of 75 mg of ethionine over a 24-hour period had little effect on the serum triglyceride concentration.

It has been shown that the serum cholesterol concentration of rats fed this basal diet supplemented with 0.25% of ethionine in-

TABLE III. Effects of Ethionine and Methionine on Triglyceride Concentration and Distribution in Serum.

Diet	Triglyceride concentration, Exp 1: Whole serum	Exp 1 Lipoproteins: <1.063 g/ml	Exp 1 Lipoproteins: Chylomicrons	Exp 2: Whole serum	Exp 2 Lipoproteins: <1.063 g/ml	Exp 2 Lipoproteins: <1.006 g/ml
	(mg/100 ml)					
Basal	90	—	40	120	110	120
+.25% DL-Ethionine	1340	1060	180	870	670	350
+.25% DL-Ethionine & .25% DL-Methionine	130	100	50	220	130	90
Basal + 75 mg DL-Ethionine*				160	120	—
.6% DL-Methionine	60			90	70	—

* Injected on final day of experiment, conditions described in Table I.

creases with time but at a slower rate than that of rats fed the unsupplemented basal diet(4). This increase can be prevented by methionine but not by choline or cystine, suggesting that ethionine is acting specifically as a methionine antagonist.

The increase in serum cholesterol concentration with time in rats fed the ethionine-supplemented diet may be related to the concomitant rise in serum triglyceride concentration. This is suggested by the observation that the concentrations and distributions among lipoprotein fractions were similar for both cholesterol and triglycerides in rats fed the diet supplemented with 0.25% of ethionine. Also, addition of methionine to the ethionine-containing diet reduced the concentrations of both cholesterol and triglycerides in the whole serum and in each lipoprotein fraction tested. Such a relationship might account for the observation that a large dose of ethionine given over a 24-hour period lowered serum cholesterol concentration more than did feeding ethionine as 0.25% of the diet (Table I). Under the former conditions little or no rise in serum triglyceride concentration was observed while under the latter a marked increase in serum triglyceride concentration occurred.

The increase in serum triglyceride concentration in ethionine-fed rats is contrary to results obtained by Feinberg *et al.*(1) in the dog. However the use of a diet containing cholesterol and cholic acid and a large amount of saturated fat in the rat study may have contributed to the divergence of these results. Species difference should also be considered.

Harris and Robinson(13) have observed in the rat that ethionine reduces the rate of removal of radioactivity from the blood when chyle containing C^{14}-labelled fatty acids is injected. However there is not sufficient evidence to relate the rise in serum triglyceride concentration observed in these experiments with a reduced rate of removal of fatty acids from the blood.

It appears from this and other work(14) that the serum concentration of low-density lipoprotein bound cholesterol and the ratio of β-lipoproteins to α-lipoproteins of rats fed a diet containing cholesterol and cholic acid are elevated above the normal level for this species. It has been suggested that this may be the result of an abnormal condition in which the low-density (beta) lipoproteins "store" cholesterol(15) rather than use it solely as a component of a transport vehicle. A relatively high cholesterol to triglyceride ratio in the serum (23.5) was observed in these experiments in rats fed our basal diet as compared to a ratio of 3.0 reported for rats fed a stock ration(16). The ratio of cholesterol to triglyceride in the low-density lipoprotein fraction was also high for rats fed the basal diet (9.3) although, as would

TABLE IV. Effect of Adding Saline or Water to Cholesterol Standard on Optical Density.

Vol of saline (D = 1.063 g/ml)	Vol of water	Optical density	% error
0	0	.40 (.39 -.425)	
0	.2	.43 (.425-.44)	8
.1	.1	.44 (.425-.445)	10
.2	0	.455 (.435-.47)	14

be expected from the high triglyceride content of this fraction, it was lower than that of whole serum. The cholesterol to phospholipid ratio in the serum of rats fed our basal diet was 3.4 as compared to 0.65 observed for rats fed a stock ration(16).

Thus some caution is necessary in interpreting these results in terms of schemes of lipid transport based on the results of studies in man and animals with markedly different distribution of serum lipids than those of rats used in this work.

Results shown in Tables I-III indicate that addition of methionine alone to the basal diet while markedly reducing the serum cholesterol concentration(17) only slightly reduced the concentration of phospholipids and triglycerides in the serum. The basal diet used in this work however produced considerably smaller per cent increases in the concentrations of serum phospholipids and triglycerides than in the concentration of cholesterol.

Since this work was begun Boyle *et al.*(18) reported that the use of NaCl in the flotation of low-density lipoproteins introduces an error in the analysis of these preparations for cholesterol by the method used in this work. The results shown in Table IV support this observation. Addition of an amount of NaCl solution of density 1.063 g per ml equal to the maximum amount of lipoprotein preparation used in the analysis of cholesterol introduced an error of 14%; however, addition of this amount of distilled water introduced an error of 8%. Presumably this latter error is also present when serum is added and thus the addition of NaCl of a density of 1.063 presumably introduces an error of approximately 6% in the cholesterol determination. Because of the large differences observed in these experiments an error even as large as 14% would not affect the conclusions drawn from the results.

Summary. Prolonged feeding of ethionine to rats receiving a diet containing cholesterol, cholic acid and hydrogenated coconut oil leads to about a 10-fold rise in serum triglyceride concentration which is primarily confined to triglycerides bound to low-density lipoproteins. This increase is prevented if an equal amount of methionine is included in the diet. Serum cholesterol concentrations of rats fed ethionine are lower than those of unsupplemented rats. Addition of methionine with ethionine further lowers the cholesterol concentration. Methionine lowers the concentrations of serum triglycerides and phospholipids only slightly but substantially lowers serum cholesterol concentration.

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