Hypocholesteremic Effect of Glutamic Acid in the Mongolian Gerbil* (34133)

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The hypocholesterolemic effect of glutamic acid in humans fed a chemically defined formula diet has been reported from our laboratory (1–4). These subjects were fed an amino acid formula diet containing the eight essential amino acids required by the adult human subject plus glutamic acid as the sole source of nonessential amino nitrogen. The caloric intake was constant and the dietary lipid maintained at 36% calories with 4% polyunsaturated fatty acids (PUFA) as triglycerides. When the nonessential nitrogen was supplied as ammonium acetate (or citrate) and glycine, no hypocholesterolemic effect was observed.

While the effect of glutamic acid, under the above conditions, is well established, the mechanism of this interesting phenomenon remains to be determined. For the elucidation of the above mechanism, an animal model was sought. It is shown here that under experimental conditions comparable to those used in the human investigations the Mongolian gerbil responds to the high glutamate formula with a fall in serum cholesterol.

Materials and Methods. Mature, male Mongolian gerbils, 40–80 g weight, were obtained from Beaumanor Farms, Cleveland, Ohio. Adult male rats, 200–300 g weight, were obtained from the St. Louis University Colony (Doisy-Wistar Strain). Adult rabbits, 1.5–2.0 kg weight, were obtained from Eldridge Rabbitry, St. Louis, Missouri, while 3-week-old chickens were obtained from Kenroy Hatchery, Berger, Missouri.

All dietary amino acids were purchased from General Biochemicals, Chagrin Falls, Ohio. Vitamin supplements were obtained through the courtesy of Hoffmann-La Roche Inc., Nutley, New Jersey. Cholesterol, chramotographically pure, was purchased from Merck & Co., Inc., Rahway, New Jersey. Rations appropriate for each species tested were obtained from Ralston Purina, St. Louis, Missouri. Serum cholesterol was determined by the method of Abell *et al.* (5).

In early experiments the animals were divided into three groups. Group 1 was maintained on the commercial ration to which lard had been added to bring the total fat content of the diet up to 10% by weight (FCD). Group 2 was fed the control amino acid formula (AAF) while group 3 was maintained on the amino acid–glutamate formula (AA FG). The composition of these diets is reported in Table I.

All animals were fed *ad libitum* for 2 weeks and weights and blood samples obtained at the beginning and at the end of the periods. In later work the experimental design included sequential dietary experiences in various combinations using each animal as its own control.

Results. All animals tolerated the formula diets fairly well after a few days of adjustment. In general they maintained their weight throughout the experimental period. During the formula feeding the gerbils' fur assumed a more glossy appearance; no changes were observed in the rabbits, rats, or chicks. In all animals tested, maintenance on the AAF regimen produced serum cholesterol levels which were not significantly different from those observed on the control ration (FCD).

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TABLE I. Ingredients of the Amino Acid Formula Diet.[a]

Food	g/kg
Lard	100.00
Cornstarch	163.50
Sucrose	420.00
Mineral mix	40.00
Nonessential nitrogen	
Ammonium citrate[b]	110.00
Glycine[b]	110.00
Essential amino acids	56.00
Arginine	2.00
Histidine	4.00
Lysine	6.30
Phenylalanine	8.66
Tryptophan	1.97
Methionine	8.66
Threonine	3.94
Leucine	8.66
Isoleucine	5.51
Valine	6.30
Cholesterol	0.50

[a] Vitamin supplements, much in excess of requirement (6), were as follows (unit/100 g): Vitamin A, 3500 IU; vitamin C, 396 mg; vitamin D, 360 IU; vitamin B_{12}, 5.0 μg; folic acid, 360 μg; thiamine, 12.4 mg; riboflavin, 12.4 mg; nicotinic acid, 84 mg; pantothenate, 17.6 mg; pyridoxine, 3.96 mg; inositol, 30 mg; choline, 100 mg; biotin, 50 μg; vitamin E, 3 mg.

[b] In the AAFG diet ammonium citrate and glycine are replaced by glutamic acid (g/g).

The chicks and gerbils responded to the glutamate formula, however, by significant decreases in the serum cholesterol levels (Table II).

The rabbit did not respond and, the rat paradoxically, showed higher serum cholesterol levels when fed the high glutamate regimen.

In previous clinical experiments (2) human subjects were maintained for successive 4-week periods on the dietary sequence FCD, AAF, AAFG, AAF. To determine if the gerbil would respond in a manner similar to that observed in humans, the experiment shown in Table III was performed. In this experiment each animal was used as its own control. The effect of the glutamate formula on serum cholesterol was even more impressive here: a 53% decrease in serum cholesterol levels was observed upon the first dietary change from AAF to AAFG. During the second AAF feeding, on the fourth week, the serum cholesterol increased significantly even though it did not reach the levels observed during the previous AAF regimen (second week). During the fifth week (second AAFG) the characteristic response of serum cholesterol to glutamate feeding was again shown with an additional 45% drop from the previous AAF (fourth week). These results clearly demonstrate a consistent repetitive hypocholesterolemic effect of glutamic acid in the gerbil when compared to levels observed when glycine and ammonium acetate are fed as a source of nonessential nitrogen in the formula diet.

Discussion. Of all animals tested in this study, the gerbil and chick appear to be the only two in which the hypocholesteremic effect of glutamate formula diets was observed. Because of the greater magnitude of the changes, the gerbil appears to be the experimental animal of choice.

The curious hypercholesteremic effect of the AAFG feeding in the rat was not studied further, hence it remains unexplained. No effect whatsoever was demonstrated in the adult male rabbit.

In humans the hypocholesteremic effect of glutamate is not accompanied by overt changes in plasma free amino acid concentration, nor in nitrogen balance; there is, however, an increase in the ratio of nonessential to essential amino acids in the plasma. It appears that the fall in plasma cholesterol which occurs in human subjects fed the glutamate formula is accompanied by a reduction in cholesterol biosynthesis by the liver (8). It is hoped the gerbil will aid in the elucidation of the mechanism of this interesting phenomenon. This hope is supported by the finding that the gerbil responds to changes in dietary regimen in a like manner to the human being.

Summary. Several animals have been tested in search of a model to study the mechanism of the hypocholesteremic effect of glutamic acid formula diets, previously demonstrated

TABLE II. Serum Cholesterol Values ± SEM in Animals Maintained for 2 Weeks on the Designated Diet.

Animal	Dietary regimen		
	FCD[a]	AAF	AAFG
Chick	145 ± 6.7 (21)	167 ± 10.7 (12)	124 ± 6.9 (12)[b]
Rabbit	59 ± 10.5 (5)	—	79 ± 10.1 (5)
Rat	77 ± 7.2 (4)	84 ± 3.9 (12)	102 ± 3.7 (10)[c]
Gerbil	128 ± 11.2 (8)	138 ± 14.6 (12)	80 ± 4.2 (12)[d]

[a] Commercial rations (FCD), after addition of lard to bring the crude fat content up to 10%, contained: (a) for gerbils and rats: crude protein > 23.0%, crude fiber > 6.0%; (b) for rabbits and chicks: crude protein > 15%, crude fiber >18%.

[b] AAFG serum cholesterol significantly ($p < .02$) lower than AAF.

[c] AAFG serum cholesterol significantly ($p = .005$) higher than AAF.

[d] AAFG serum cholesterol significantly ($p = .002$) lower than AAF; all other differences were insignificant at $p = .05$.

Number of animals in each experiment given in parentheses.

TABLE III. Serum Cholesterol Values ± SEM in Gerbils Fed the Designated Diets, for 7-Day Periods.

	Dietary regimen[a]					
	FCD	AAF	AAFG	AAF	AAFG	FCD
Period no.	1	2	3	4	5	6
Mean serum cholesterol (mg/100 ml)	155 ±12.9 (14)	169 ±11.1 (14)	80 ±8.4 (14)	115 ±11.2 (14)	63 ±6.2 (10)	101 ±9.6 (4)
Significance of change		$p = .35$	$p < .0005$	$p < .01$	$p < .005$	$p < .01$

[a] Number of animals in each experiment given in parentheses.

in human beings. Upon feeding the glutamate formula diet to the Mongolian gerbil, the characteristic decline in serum cholesterol has been demonstrated. These data support the view that the Mongolian gerbil is an excellent animal model for the study of the mechanism by which dietary glutamic acid, under our experimental conditions, lowers serum cholesterol in human beings.

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