Hypocholesterolemic Effect of α-Ketoglutarate in the Mongolian Gerbil[1] (36390)

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We have recently reported that the Mongolian gerbil responds with a fall in serum cholesterol when fed a chemically defined formula diet containing glutamic acid (AA FG) as the sole source of nonessential nitrogen (1). Comparable results had been obtained in humans fed similar glutamate diets (2). When the nonessential nitrogen in the diet was supplied as ammonium acetate or citrate and glycine (AAF), no hypocholesterolemic effect was observed in humans or in gerbils. The fall in the plasma cholesterol and $S_{f0\text{-}12}$ β-lipoprotein appears to be accompanied by a reduction in sterol biosynthesis.

In efforts to elucidate the mechanism of this hypocholesterolemic effect of glutamic acid, and in order to determine whether glutamic acid *per se* or a metabolite of glutamic acid is responsible for this effect, two other amino acids added to the basic formula diet have been studied. These amino acids could be metabolized to intermediates common to glutamic acid or to glutamic acid itself. We have also included in this study α-ketoglutarate, since it appears to be the primary glutamic acid metabolite.

Methods and Materials. Mature, male Mongolian gerbils (50–70 g wt) were obtained from Tumblebrook Farms, Brant Lake, NY.

All dietary amino acids were purchased from General Biochemicals, Chagrin Falls, OH. Vitamin supplements were obtained through the courtesy of Hoffman-LaRoche, Inc., Nutley, NJ. Cholesterol, chromatographically pure, was purchased from Merck & Company, Rahway, NJ.

The animals were divided into seven groups of 10 gerbils each. 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). Cholesterol was also supplemented to bring the total to 500 mg/kg of diet.

Group 2 was maintained on FCD plus glutamate (FCDG) (100 g of glutamate/kg FCD). Groups 3–7 were fed one of the following amino acid formula diets for 1 to 2 week periods: amino acid formula with glycine (AAF), amino acid formula with lysine (AAL), amino acid formula with proline (AAP), amino acid formula with α-ketoglutarate (AAK), or the amino acid formula with glutamic acid (AAFG). The composition of these diets is reported in Table I. All animals were fed *ad libitum* and were weighed twice a week. Blood samples were obtained at the beginning, at 1 week, and at the end of the experimental dietary periods by cardiac puncture.

Liver lipid extraction was done by the method of Bligh and Dyer (5). The lipid extract was taken up in 1 ml of chloroform and spotted on ITLC-SG silica gel impregnated fiber-glass paper from Gelman Instrument Co., Ann Arbor, MI. The solvent systems routinely used for the separation of the lipids were isooctane:isopropyl acetate:acetic acid (100:3:0.1) and isooctane:benzene (100:20).

Serum and hepatic cholesterol and squalene were determined by the method of Abell *et al.* (3) and/or by the densitometric tech-

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TABLE I. Contents of the Amino-Acid Formula Diets.[a]

Food	(g/kg)
Lard	100.00
Cornstarch	163.50
Sucrose	420.00
Mineral mix	40.00
Major nitrogen source[b]	
Ammonium citrate	110.00
Glycine	110.00
Essential amino acids	56.00
Cholesterol	0.50

[a] Essential amino acid composition and vitamin supplements as in Ref. (1).

[b] In the AAFG diet, ammonium citrate and glycine are replaced by glutamic acid (g/g); in the AAL diet, they are replaced by lysine (g/g) and in the AAP diet, by proline (g/g). In the AAK diet, α-ketoglutarate (g/g) plus ammonium chloride (60.5 g/kg) are used.

nique of Privett *et al.* (4) using a Photovolt densitometer.

Results. All animals tolerated the formula diets fairly well after a few days of adjustment, although some weight loss was observed in all groups during the experimental period. The weight losses, as shown in Table II, were maximal (23%) in the gerbils on the AAL regimen and minimal (13%) in the gerbils on the AAP regimen. Although these weight losses are considered significant, no specific correlation could be made between weight and cholesterol changes. In fact, the AAF and AAL gerbils which showed the most severe weight losses (22 and 23%, respectively) did not show any significant decrease in serum cholesterol; on the contrary, increases were noted (17 and 13%, respectively).

Because of the observed variability inherent in our animal population, the serum cholesterol levels are reported before and after 1 week on each dietary regimen. It was felt that in this way, each animal would serve as its own control and this would increase the confidence limits in the reliability of the results.

Maintenance on the AAF regimen produced serum cholesterol levels which were not significantly different from those observed on the FCD regimen. The addition of glutamate to the FCD was also without any effect on serum cholesterol, a finding previously observed in humans (6).

The AAF regimen appeared to have a slight, although statistically not significant, hypercholesterolemic effect. A similar effect was observed in the gerbils on the AAL diet, while the AAP regimen caused a 41% decrease in serum cholesterol. The most striking changes were observed in the animals on the AAK regimen. In this group, a decrease of 66% in serum cholesterol level was observed. This decrease is even greater than that found with the AAFG dietary regimen (48.9%).

In Table III, the results of the liver squalene and cholesterol determinations are presented. All livers contained approximately the same amount of squalene per gram of liver, with the exception of FCDG and AAP.

TABLE II. Serum Cholesterol Values (mg/100 ml ± SEM) and Changes in Body Weights in Gerbils Before and After 1 Week on the Designated Diet.

Dietary regimen[a]	Cholesterol Before		Cholesterol After	Body wt changes (%)	Significance of change[b]
FCD (54)		109 ± 8.1		+9	
FCDG (10)	120 ± 5.3	$p = .10$	110 ± 3.3	+4	NS
AAF (10)	90 ± 13.4	NS	106 ± 21.6	—22	NS
AAL (10)	72 ± 7.5	NS	81 ± 9.2	—23	NS
AAP (10)	106 ± 12.6	$p < .01$	63 ± 4.4	—13	$p < .02$
AAFG (8)	138 ± 12.8	$p = .005$	71 ± 5.8	—14	$p < .05$
AAK (10)	110 ± 4.3	$p < .001$	37 ± 8.9	—15	$p < .001$

[a] Number in parentheses indicates the number of gerbils on each regimen.

[b] Relative to the comparison of "after" values to FCD; NS = not significant.

TABLE III. Liver Squalene and Cholesterol Concentrations in Gerbils on Various Diets After 1 Week (± SEM).

Dietary regimen[a]	Squalene (μg/g)	Significance of change[b]	Cholesterol (mg/g)	Significance of change
FCD (10)	107 ± 7.0		2.7 ± .14	
FCDG (10)	160 ± 5.7	$p < .001$	2.6 ± .45	NS
AAF (10)	125 ± 7.7	NS	1.9 ± .15	$p < .001$
AAL (10)	127 ± 13.1	NS	3.0 ± .75	NS
AAP (10)	159 ± 13.1	$p < .005$	1.6 ± .17	$p < .001$
AAFG (8)	109 ± 14.0	NS	1.8 ± .20	$p < .001$
AAK (10)	132 ± 12.7	NS	1.6 ± .18	$p < .001$

[a] Figure in parentheses indicates the number of gerbils on each regimen.
[b] Relative to the comparison of each value to the FCD value; NS = not significant.

The liver from these two sets of gerbils contained significantly higher squalene than any other. Interestingly enough, this did not correlate with a higher liver cholesterol; the liver cholesterol content of the AAP animals was the lowest of all groups. No specific correlation could also be made between liver and serum cholesterol. However, the AAK group had the lowest serum cholesterol with a liver cholesterol similar to the AAP group.

Discussion. The results cited above suggest that the degree of hypocholesterolemia produced by the formula diets may be related to the extent of conversion of the major amino acid furnished by the formula to α-ketoglutarate or one of its metabolites.

Since the only source of nitrogen apart from the essential amino acid in these diets is supplied in the form of the specific compound (proline, lysine, glutamate, etc.), the animal is forced to obtain the nitrogen needed for amino acid and protein synthesis by transamination or deamination of the primary amino acid provided. Using well-recognized biochemical pathways (7), the following sequence can be considered:

(1) Glycine → Glyoxylate
↓
Formate

(2) Lysine → Pipecolate
↓
α-Aminoadipate
↓
Glutaryl-CoA

(3) Proline → 4-Hydroxyproline
↓
γ-Hydroxyglutamate
↓
γ-Hydroxy-α-ketoglutarate

(4) Glutamic acid → α-Ketoglutarate

(5) α-Ketoglutarate

Dietary regimens furnishing (1) and (2) are not hypocholesterolemic, (3) and (4) are hypocholesterolemic (40.5% and 48.9% decreases, respectively), while (5) is the most hypocholesterolemic (66.5% decrease).

Since α-ketoglutarate is the main metabolite found in gerbil liver after intraperitoneal injection of glutamic acid-U-^{14}C (to be published elsewhere), this further suggests that α-ketoglutarate or one of its metabolites — and not glutamic acid itself — may be the compound responsible for the hypocholesterolemia found in gerbils fed a formula diet containing glutamate. In support of this hypothesis is also the observation previously reported (8) that, in humans, plasma aminograms of fasting subjects fed the AAFG diet (137 g of L-glutamic acid/day) did not show any increase in the plasma glutamate levels.

The lack of hypocholesterolemic effect of glutamate added to a normal diet (FCDG), which has also been previously observed in humans (6), may be explained by the fact that glutamate in this case is not the only source of nitrogen. The animal, therefore, may not accumulate α-ketoglutarate or its metabolites in such large amounts, and this may not trigger an α-ketoglutarate-related pathway responsible for the hypocholesterolemia.

Summary. In an effort to elucidate the mechanism of the hypocholesterolemic effect of glutamic acid previously demonstrated in the Mongolian gerbil, several amino acid formula diets have been studied. The diets containing amino acids which could be readily converted to α-ketoglutarate were hypocholesterolemic, while the diets containing amino acids *not* readily converted to α-ketoglutarate were not. The diet containing α-ketoglutarate was the most effective.

No significant correlation could be made between the serum cholesterol and the hepatic squalene or cholesterol content. However, the animals with the lowest serum cholesterol levels had correspondingly low hepatic cholesterol levels.

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