Utility of Fasting Essential Amino Acid Plasma Levels in Formulation of Nutritionally Adequate Diets III: Lowering of Rat Serum Cholesterol Levels by Lysine Supplementation

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Abstract ☐ A lysine-supplemented ration lowered the serum cholesterol levels of rats. The level of lysine supplementation was derived from the fasting plasma essential amino acid profile.

Keyphrases ☐ Lysine supplementation—effect on rat serum cholesterol levels ☐ Amino acid supplementation—effect of added lysine on rat serum cholesterol levels ☐ Cholesterol levels—effect of lysine supplementation, rats ☐ Nutrition—effect of lysine supplementation on rat serum cholesterol levels

Earlier studies demonstrated that the molar proportions of the essential amino acids in the fasting plasma of Sprague–Dawley rats (1) and Wistar rats (2) were useful in: (*a*) determining the order of limiting essential amino acids in rations, (*b*) preparing chemically defined synthetic diets, and (*c*) ranking diets according to their biological value and net protein utilization. The average values of fasting plasma essential amino acids of human adults were also used to rank a series of protein diets fed to premature infants (2).

This paper presents an additional application of the fasting plasma profile in a nutritional study conducted with Sprague–Dawley rats. An increase in the dietary protein level resulted in a decrease in the total plasma cholesterol in chicks (3) and mice (4). The cholesterol blood levels of calves were reduced by a higher level of dietary protein (5).

It was of interest to determine how the serum cholesterol level would be influenced by feeding a lysine-supplemented ration. The level of lysine supplementation was derived from the fasting essential amino acid profile of Sprague–Dawley rats.

Table I—Calculation of the Order of Limiting Essential Amino Acids Based on the Fasting Plasma Profile of Rats[a]

Essential Amino Acid	*A*, mmoles/liter Plasma	*B*, mmoles/100 g Ration	*B/A*
L-Lysine	0.389	10.80	27
L-Tryptophan	0.054	1.56	29
L-Threonine	0.199	8.22	41
L-Histidine	0.062	3.86	62
L-Arginine	0.146	9.24	63
L-Methionine + 1/2 cystine	0.052	3.86	74
L-Phenylalanine + tyrosine	0.115	14.86	129
L-Leucine	0.100	15.16	151
L-Valine	0.101	15.95	157
L-Isoleucine	0.060	10.51	175

[a] L-Lysine is limiting since the *B/A* value is the smallest:

L-tryptophan *B/A* value = 29
L-lysine *B/A* value = 27
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Therefore, 2 × 0.389 = 0.778 mmole = 0.114 g of L-lysine to be added to 100 g of ration to make L-tryptophan and L-lysine equally limiting.

Table II—Serum Cholesterol Levels of Rats with and without Supplementary L-Lysine

Rat	Serum Cholesterol Levels, mg %: Ration	Ration plus Lysine (0.11%)	Ration plus Lysine for 30 Days, then Ration for 11 Days
1	128	82	113
2	120	76	117
3	134	99	131
4	130	100	119
5	122	84	109
6	126	100	124
Average	127	90	119
Average ± *SD*	127 ± 5.2	90 ± 12.4	119 ± 7.9

EXPERIMENTAL

Two groups of 20 female Sprague–Dawley rats, 216 ± 8.8 g, were fed a complete diet[1] and a lysine-supplemented ration for 30 days (0.114 g/100 g of ration). The method for calculating the L-lysine level is shown in Table I. A third group of 20 rats was fed the lysine-supplemented ration for 30 days and was then switched to the unsupplemented ration for 11 days.

After an 18-hr fast, groups of six rats were sacrificed and the collected blood was allowed to clot. Serum cholesterol was determined by the method of Levine and Zak (6) (Table II).

DISCUSSION

The data in Table II were subjected to a statistical evaluation. The serum cholesterol levels for the rats fed the lysine-supplemented ration were significantly lower than those for the control group. They also differed significantly from the group fed the control ration for 11 days. The value for the control *versus* the lysine-supplemented ration was $p < 0.001$, and the value for the rats fed the control ration for 11 days *versus* the lysine-supplemented ration was $p < 0.01$. The value for the control *versus* the group fed the control ration for 11 days was $p < 0.1$.

A suggested mechanism for these observations is that the addition of 0.114 g of lysine to 100 g of ration improves the biological value and net protein utilization. Evidence for this finding is the improved growth consistently obtained with the lysine-supplemented ration (7). Therefore, it follows that by increasing net protein utilization, less essential and nonessential amino acids will remain for intermediary metabolic conversion to cholesterol intermediates (acetate, acetoacetic acid, acetone, pyruvic acid, *etc.*).

Thus, it appears that it is possible to lower cholesterol serum levels by strategic supplementation with limiting essential amino

[1] Rockland Farms Mouse/Rat Complete Diet.

acids. The reported reversible atherosclerosis in Rhesus monkeys adds further potential significance to these observations in rats (8). This reversible atherosclerosis was achieved through dietary control. The ingestion of lysine-supplemented rations, wherein the protein component has been improved biologically by the addition of an essential amino acid, may prove to be practical in human nutrition.

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Utility of Fasting Essential Amino Acid Plasma Levels in Formulation of Nutritionally Adequate Diets IV: Lowering of Human Plasma Cholesterol and Triglyceride Levels by Lysine and Tryptophan Supplementation

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Abstract □ The administration of capsules containing L-lysine monohydrochloride (0.205 g) and L-tryptophan (0.069 g) three times daily after meals resulted in a significant drop in plasma cholesterol and triglyceride levels. The proportion of amino acids in the blend was derived from the average fasting plasma essential amino acid profile.

Keyphrases □ Lysine and tryptophan supplementation—effect on human plasma cholesterol and triglyceride levels □ Tryptophan and lysine supplementation—effect on human plasma cholesterol and triglyceride levels □ Amino acid supplementation—effect of added lysine and tryptophan on human plasma cholesterol and triglyceride levels □ Cholesterol and triglyceride levels—effect of lysine and tryptophan supplementation, humans □ Nutrition—effect of lysine and tryptophan supplementation on human plasma cholesterol and triglyceride levels

Earlier studies demonstrated that the molar proportions of the essential amino acids in the fasting plasma of Sprague–Dawley rats (1–3), Wistar rats, and humans (4) were useful in *(a)* determining the order of limiting essential amino acids in rations, *(b)* preparing chemically defined synthetic diets, *(c)* ranking diets according to their biological value and net protein utilization, and *(d)* lowering serum cholesterol levels in Sprague–Dawley rats by L-lysine supplementation.

This paper presents another application of the fasting plasma profile concept in a nutritional study conducted with adult humans. Supplementation of the normally varied human dietary regimen with L-lysine and L-tryptophan was investigated since these essential amino acids are most frequently limiting. It was reasoned that provision of two essential amino acids usually found to be limiting would improve the biological value and net protein utilization of each meal. The great variety of protein combinations consumed by humans on *ad libitum* dietary regimens would be a complicating feature in a supplementation study. Lowering of serum cholesterol levels in Sprague–Dawley rats had been achieved by lysine supplementation of a standardized ration (2). Nevertheless, it was decided to determine the effect of L-lysine and L-tryptophan supplementation on the plasma levels of cholesterol and triglycerides in humans.

EXPERIMENTAL

Derivation of Capsule Formula—The average fasting human plasma concentrations of lysine and tryptophan are 0.170 and 0.052 mmole/liter, respectively (4). Thus, the ratio of lysine to tryptophan on a molar basis is 3.27 to 1. A blend of 595 g of L-lysine monohydrochloride (3.27 moles) and 204 g of L-tryptophan (1 mole) was prepared. Hard gelatin capsules (No. 1) were hand filled with 0.274 g of the blend. Each capsule contained 0.069 g of L-tryptophan and 0.205 g of L-lysine monohydrochloride. This level of supplementation was judged to be satisfactory. The minimum daily requirement of L-tryptophan and L-lysine for adult males had been established (5, 6) to be 0.25 and 0.80 g, respectively.

Human Volunteers Studied—Six persons participated in the study[1]. Phenotyping of the hyperlipidemia or hyperlipoproteinemia was established by zone electrophoresis, using agarose as the

[1] M.S.C., 48 years old, and S.C., 22 years old, mother and daughter, respectively, were members of a family with a history of hyperlipidemia. M.B. and D.G. were 56-year-old females. A.Z. was a 40-year-old male who was included in the study in spite of his being a moderate alcoholic. G.C. was a 42-year-old male who had been on clofibrate for several months.