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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ACKNOWLEDGMENTS AND ADDRESSES

Received July 3, 1974, from the *Department of Allied Health and Industrial Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY 11439*

Accepted for publication September 26, 1974.

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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.

Table I—Effect of Lysine and Tryptophan Supplementation on Plasma Levels of Triglycerides and Cholesterol in Humans

Subject		Plasma Concentration (mg %) of Triglycerides and Cholesterol				
M.S.C.	Triglycerides	1725, 0 days	1200, 10 days	1365, 20 days	1565, 24 days	—
	Cholesterol	380, 0 days	302, 10 days	380, 20 days[a]	335, 24 days	—
S.C.	Triglycerides	215, 0 days	180, 6 days	162, 15 days	150, 30 days	102, 60 days
	Cholesterol	216, 0 days	203, 6 days	203, 15 days	210, 30 days	180, 60 days
M.B.	Triglycerides	250, 0 days	230, 7 days	200, 15 days	140, 30 days	148, 60 days
	Cholesterol	350, 0 days	310, 7 days	310, 15 days	302, 30 days	288, 60 days
A.Z.	Triglycerides	235, 0 days	195, 7 days	257, 17 days	195, 24 days	—
	Cholesterol	200, 0 days	185, 7 days	210, 17 days[b]	188, 24 days	—
D.G.	Triglycerides	288, 0 days	172, 7 days	—	—	—
	Cholesterol	240, 0 days	200, 7 days	—	—	—
G.C.	Triglycerides	255, 0 days	205, 7 days	—	—	—
	Cholesterol	252, 0 days	265, 7 days	—	—	—

[a] Subject consumed 1 dozen clams as an appetizer the night before. [b] Subject attended a cocktail party the night before.

support medium (7–9). This correlated well with the fasting plasma lipid assays as well as the appearance of the plasma samples after storage at 2–6° for 24 hr. All hyperlipoproteinemias were classified as Type IV, with the exception of Subject M.S.C. whose hyperlipoproteinemia was classified as Type V.

Protocol—All volunteers were instructed to continue their *ad libitum* dietary regimens but to take one capsule of the amino acid blend after each meal. In the case of Subject G.C., clofibrate was discontinued.

Analytical Procedure—After a 12–14-hr fast, approximately 20 ml of blood was drawn and placed in a tube containing edetic acid. After centrifuging to remove the cellular elements, the supernate was analyzed by established colorimetric procedures for cholesterol (10) and triglycerides (11). Averages for the duplicate assays performed are summarized in Table I.

DISCUSSION

The data in Table I show a downward trend in triglyceride levels in all six patients. Significant decreases were observed for S.C. (53%), M.B. (41%), and D.G. (41%). Subject G.C., formerly on clofibrate, showed a 20% decrease in plasma triglycerides after taking the amino acid pair for 7 days. The decrease in plasma cholesterol in five subjects ranged from 6 to 18%, but G.C. showed a 5% increase.

Hyperlipidemia or hyperlipoproteinemia can occur because of an increased exogenous load of cholesterol or triglyceride entering from the intestine as chylomicrons. It can also occur because of an increased release of endogenous glyceride and cholesterol entering from the liver and intestine as pre-β-lipoproteins. This increased release of lipid may be secondary to a greater flux of lipid precursors like fatty acids or carbohydrates or because of *de novo* synthesis. Hyperlipoproteinemia can occur because of a failure of the end organs, adipose tissue, and muscle sometimes to assimilate lipid due to defects in the clearing enzymes. Hyperlipoproteinemia can also occur because of abnormal solubility properties of the plasma or abnormalities in the lipoproteins themselves.

All of these factors are further modified by the general state of protein and carbohydrate metabolism and the action of a number of hormones acting predominantly by promoting lipogenesis or lipolysis in the adipose tissue, muscle, or liver. Hyperlipidemia or hyperlipoproteinemia is thus a symptom of a heterogeneous group of disorders that differ in clinical manifestations, prognosis, and responsiveness to therapy (12).

It has not been established whether lowering of serum cholesterol and triglyceride levels has a detrimental, a beneficial, or no effect on the morbidity or mortality due to atherosclerosis or coronary heart disease. Albrink (13), however, reported that the average incidence rate of coronary heart disease is doubled for individuals having cholesterol serum levels in the range of 239–261 mg % as compared with those individuals having levels in the 96–195-mg % range. In addition, the incidence of coronary heart disease was greater than fivefold for individuals having a serum triglyceride concentration in the 176–490-mg % range as compared with those with a range in concentration of 20–75 mg %. Thus, the downward trend in plasma levels of cholesterol and triglyceride achieved in this investigation by the administration of two essential amino acids given after meals is most encouraging. No toxic effects were expected and none has been encountered in any of the six subjects to date. The data obtained have led to the broadening of the scope of the investigations to attempt to establish the optimal dosage regimen, the effect of other amino acids and combinations, and the mechanism for the observed lipid lowering.

Levy and Langer (14) differentiated the hyperlipidemic drugs as follows: those that act by decreasing lipoprotein synthesis (nicotinic acid) and those that promote lipoprotein catabolism (cholestyramine). In the present investigation, the intermediary metabolic conversion of L-tryptophan to nicotinic acid was considered to be a possible mechanism. However, hypolipidemic doses of nicotinic acid range from 4.5 to 6 g/day. A mechanism proposed at this time is that, with the greater net protein utilization achieved with strategic supplementation, less essential and nonessential amino acids will be available for intermediary metabolic conversion to cholesterol and triglyceride intermediates (acetate, acetoacetic acid, acetone, pyruvic acid, *etc.*).

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