Excess dietary lysine induces hypercholesterolemia in chickens[1]

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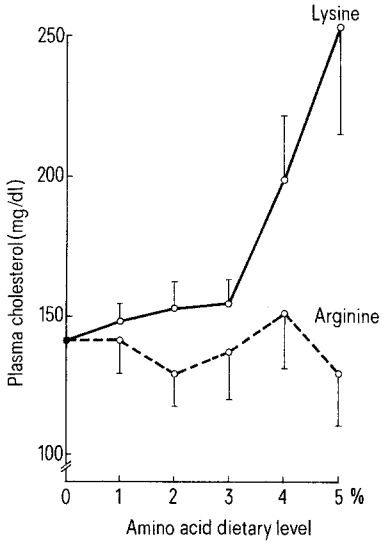
Summary. Lysine and arginine supplements were fed to 5-day-old chicks to test whether they would develop hypercholesterolemia in response to excess lysine in a fashion similar to mammals. Chicks developed hypercholesterolemia with a 4–5% lysine but not with arginine supplementation. These results indicate the lysine induced hypercholesterolemia is not due to anaplerotic interactions of arginine and lysine with the urea cycle.

Recently, the source of dietary protein has been established as a factor affecting plasma cholesterol levels. It has been observed in several animal models and also in humans that diets which contain animal protein result in higher plasma cholesterol levels than diets containing vegetable protein[3,4]. The difference between the cholesterolemic effects of animal vs vegetable dietary protein is thought to be related to their relative amino acid compositions[5]. Kritchevsky et. al.[6] hypothesized that the differential effects of dietary vegetable vs animal protein on plasma cholesterol levels were due to differences in their arginine/lysine ratios. They found that the cholesterolemic responses to dietary protein source in rabbits could be substantially modified by the addition of either supplementary lysine to vegetable protein or arginine to animal protein. In mammals, which are ureotelic, metabolic reactions involving arginine are amphibolic because arginine is not only an important substrate in the catabolic urea cycle but also serves as a substrate for the synthesis of important metabolic products (such as apolipoprotein E) which are not directly involved in the urea cycle proper[7]. Also, in mammals, lysine is inhibitory to urea cycle activity and it is possible that anaplerotic effects of excess amounts of either of these basic amino acids could be responsible for the resultant plasma cholesterolemia. Avians, however, are uricotelic and therefore do not have any measurable liver urea cycle, liver arginase or carbamyl phosphate synthetase activity. In order to test whether the effects of arginine and lysine on plasma cholesterol levels are due to urea cycle anaplerosis, we feed arginine and lysine supplements to chickens.

Materials and methods. 5-day-old New Hampshire-Columbian cross bred female chicks were divided into various groups of 6 birds each and fed diets consisting of 96% chick starter[8] 0–5% purified amino acid plus casein to 100%. Chicks were housed in heated Petersime cages and exposed to fresh water and food ad libitum. After 14 days of experimental feeding and an overnight fast, the birds were killed by decapitation, and plasma was prepared from blood stabilized with 1% tetrasodium-EDTA. Plasma total cholesterol and triglyceride levels were determined by methods previously described[8]. Plasma HDL/LDL ratios were determined by densitometric measurements of stained lipoproteins electrophoresed on polyacrylamide gels[9].

Results. The results of 2 separate 14 day long feeding trials are presented in the table. The data shows that although



Effects of different supplimentary lysine or arginine levels on chick plasma total cholesterol levels. Data is mean ± SE.

Effects of 2-week arginine or lysine supplementation on chick plasma lipids[a]

Dietary supplement	N	Mean weight gain	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL/LDL
Experiment 1					
Basal + 4% casein	6	116.5	179.4 ± 6.9	47.9 ± 3.9	
2% lysine + 2% casein	6	84.8	189.2 ± 9.7	50.2 ± 2.5	
2% Arg + 2% casein	6	110.5	154.7 ± 8.4	53.9 ± 4.9	
4% Lysine	6	89.0	219.9 ± 12.9[b]	53.0 ± 5.9	
4% Arginine	6	78.4	170.9 ± 4.9	53.8 ± 5.1	
Experiment 2					
Basal + 4% casein	6	134.4	141.1 ± 6.3	95.2 ± 6.1	10.37 ± 1.22
2% Lys + 2% casein	6	108.7	153.2 ± 9.1	111.2 ± 12.7	9.04 ± 1.80
2% Arg + 2% casein	6	111.7	128.9 ± 9.8	79.1 ± 11.8	9.45 ± 0.88
5% Lysine	6	63.3	254.5 ± 38.2[b]	129.8 ± 11.7	6.68 ± 0.97[c]
5% Arginine	6	63.9	129.4 ± 18.5	74.8 ± 14.9	12.73 ± 2.07
1% Cholesterol	6	112.6	473.6 ± 71.9	108.9 ± 15.8	0.49 ± 0.12

[a] Data presented as means ± SE. [b] Lysine fed group has cholesterol values significantly higher than basal group ($p < 0.02$) as determined by 2-tailed t-test. [c] Lysine fed group has HDL/LDL ratio significantly higher than basal group ($p < 0.05$) as determined by 2-tailed t-test.

both supplemental arginine and lysine had similar growth depressing effects, only lysine had an effect on plasma cholesterol levels which became significant at the level of 4%. In addition to hypercholesterolemia, 5% lysine supplements also altered the relative ratios of high density to low density lipoproteins; a change which was due mainly to increased levels of LDL. The results of stepwise 1% supplemental increments of both arginine and lysine up to a maximum of 5% total on chick plasma cholesterol levels are presented in the figure. It can be seen that the effects of lysine on plasma cholesterol levels are only slight from 1–3% but that 4% and 5% supplementation with lysine resulted in significant hypercholesterolemia.

Discussion. Arginine-lysine antagonism in the chick is a well known phenomenon[10]. This antagonism affects the rate of growth, and the metabolic site of the antagonism is the kidney where a high concentration of lysine is believed to induce kidney arginase activity. However, since in both mammals and birds the liver is the major site of lipoprotein metabolism and since compared to mammals only trace amounts of arginase are present in the chicken liver, it is not clear whether lipoprotein metabolism would be affected by arginine-lysine antagonism in the chick. Our results from chicks fed supplemental crystalline lysine and arginine show that, as in ureotelic mammals, excess dietary lysine does stimulate hypercholesterolemia. It can be concluded, therefore, that the cholesterolemic effects of arginine-lysine imbalance are not mediated by urea cycle anaplerosis.

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Somatic cell hybrids producing inhibitors of melanotic melanoma tyrosine hydroxylase

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Summary. Splenic lymphocytes from BALB/c mice pre-immunized with purified tyrosine hydroxylase (TH) were fused with murine myeloma N/1 cells. Supernatants of only 2 from large number of cloned cell hybrids contained an inhibitor of TH.

Human malignant melanoma cells have 2 characteristics that vary in magnitude from a cell line to another: Melanin synthesis is a specialized function of pigment cells and differentiates melanotic melanoma from other tumor cells. It arises from the oxidation of tyrosine by tyrosine hydroxylase. In human melanomas, tyrosine hydroxylase activity varies with the degree of melanization. The magnitude of response and binding of estrogens also vary from one melanoma cell line to another. Depending on the culture medium, an estradiol-non-responsive melanotic cell line (E^-Mel^+) could be derived from an estradiol-responsive melanotic line (E^+Mel^+) by cultivation of the latter cells in the absence of estradiol[5,6], and melanotic cells (Mel^+) could be converted into amelanotic by deprivation from tyrosine. In general, melanotic melanoma cells bind and respond to estradiol, whereas amelanotic cells do not[7].

In melanocytes tyrosine hydroxylase exists in multimolecular forms, a soluble cytoplasmic and an insoluble membrane form[8]. In mouse and human tumors the T_1 and T_2 tyrosine hydroxylases are soluble, while T_3 is a particulate membrane associated enzyme[9–12].

The present studies examined the characteristics of a tyrosine hydroxylase inhibitor (M-THI) found in spent culture media of cloned cell hybrids obtained from fusion of murine myeloma cells with splenic lymphocytes of BALB/c mice pre-immunized with highly purified tyrosine hydroxylase.

Materials and methods. Purified tyrosine hydroxylase (polyphenol oxidase) was obtained from Sigma Company, and was purified by gel filtration to yield 20,000 U/mg[13].

Human malignant melanotic melanoma HMMC-ShA, and HMMC-WJP were established using tumor biopsies as explants from 2 male adults, whereas HMMC-ZBJ was from a female adult. The amelanotic melanoma cell lines HMMC-Sr and HMMC-KM were also established from tumor biopsies from 2 male adults[14].

A group of 10 BALB/c (10–20 g) female mice were immunized with purified tyrosine hydroxylase (TH). Spleenocyte suspensions were prepared and splenic lymphocytes were separated on Ficol Hypaque columns.

An equal number of 10^6 of spleen lymphocytes and murine myelome ($NS_1/1$) cells were mixed in DMEM and centrifuged at $600 \times g$ for 10 min. The supernatant was removed, and the cell pellets were resuspended in 1.5 ml of 50% polyethylene glycol 1500 in DMEM (pH 7.5–7.8). The cells were washed, and suspended in DMEM containing 20% fetal calf serum, hypoxanthine (0.1 mM), amethopterin (0.4 µM) and thymidine (3 µM) (DMEM-HAT). Aliquots of each suspension were dispensed into 96 well Coster plates with 50 µl of DMEM per well and incubated in a humidified 5% CO_2 air atmosphere. The wells were fed every 72 h with 100 µl of HAT-free DMEM supplemented with 20% FCS. Wells that became acidic 7–14 days after fusion and containing cell clusters were assayed for anti-tyrosine hydroxylase (M-THI). 48 out of the 96 wells showed active cellular proliferation. Supernatant of only 1 (Hy-7) out of the 48 wells contained the TH-inhibitor. The cells of this well were once more cloned and 3 times subcultured in vitro, then injected s.c. into athymic Nu/Nu mice. 4 out of 5 inoculated animals developed tumors.