Effects of Modification of the Arginine/Lysine Ratio of Dietary Proteins on Absorption and Turnover of Cholesterol in Rats

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Received December 5, 1988

The effects of modification of the arginine/lysine ratio of dietary protein on the cholesterol kinetics were studied in male rats. Single amino acids (lysine to soybean protein and arginine to casein) were added to approximate the arginine/lysine ratio in different proteins. After acclimation to these diets for 30 days, rats were administered intravenous \([^{14}\text{C}]\text{cholesterol}\) and oral \([^{3}\text{H}]\text{cholesterol}\). Analysis of the die-away curve of \([^{14}\text{C}]\text{cholesterol}\) showed an apparent independence of cholesterol kinetics to the dietary manipulations, but there was a moderate reduction of the size of the slowly exchangeable pool and of the biliary concentration of cholesterol when lysine was added to soybean protein. Addition of amino acids neither influenced cholesterol absorption nor the fecal excretion of the radioactivities from labeled cholesterol. The results indicate that manipulating the arginine/lysine ratio of dietary protein by adding single amino acids is not necessarily effective in ameliorating cholesterol metabolism in rats, although the arginine addition caused a significant reduction of serum cholesterol and triglyceride.

The significance of dietary protein in the development of hypercholesterolemia and atherosclerosis has frequently been demonstrated. Feeding cholesterol-free, semipurified diets containing animal proteins such as casein produces an elevation of serum cholesterol in experimental animals when compared to the corresponding diets containing vegetable proteins such as soybean protein.\(^1,2\)

It is not totally clear how soybean protein exerts its cholesterol-lowering action. The different effects between different proteins can at least in part be ascribed to the difference in their amino acid compositions. It has been indicated that one or a group of amino acids are responsible for modification of the serum cholesterol level. Kritchevsky\(^2,3\) proposed the arginine/lysine ratio as a plausible determining factor. However, previous studies in our laboratory\(^4,5\) failed to show a supplementary effect of lysine or arginine on the serum cholesterol level of rats. Huff and Carroll\(^6\) and Gibney\(^7\) also were unable to observe a crucial role of this ratio in rabbits. The significance of the arginine/lysine ratio of dietary proteins, therefore, does not necessarily lead to a conclusion. In stark contrast, when fed as proteins the serum cholesterol concentration is negatively correlated to the arginine/lysine ratio.\(^8,9\)

Recently, Vahouny et al.\(^10\) showed in lymph-cannulated rats that addition of arginine to the casein diet resulted in a slowed rate of lipid (including cholesterol) absorption, but addition of lysine to the soy protein diet increased the rate of lipid absorption. As a result of these changes, the activity of the hepatic cholesterol 7α-hydroxylase was also modified characteristically.\(^11\)

In this study, to reconfirm that the addition of single amino acids to approximate the arginine/lysine ratio may exert an effect on cholesterol metabolism, the kinetic parameters of cholesterol were measured in rats.

Materials and Methods

Animals and diets. Male Sprague-Dawley rats (Seiwa Experimental Animals, Fukuoka) initially weighing ap-

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approximately 110 g were used throughout. Animals were housed individually in metabolic cages and were kept in an air-conditioned room and a 12 hr light-dark cycle (illuminated 0800 to 2000 hr). They were fed purified diets ad libitum. The composition of the basal diet was, in weight percent: protein 20, corn oil 5, mineral mixture (Harper) 4, vitamin mixture (Harper) 1, choline chloride 0.2, cellulose 2, and sucrose to 100.4% Isolated soybean protein (Fujipro R, Fuji Oil Co., Osaka) and casein (vitamin-free, ICN Pharmaceuticals, Inc., Cleveland, OH) were used as dietary protein sources. Two sets of experiments were done in succession. In the first experiment L-lysine-HCl (1.72%) was added at the expense of sucrose to the soybean protein diet to give an arginine/lysine ratio comparable to that in the casein diet. In the second experiment L-arginine (0.86%) was added to the casein diet to give a ratio comparable to that in the soybean protein diet. After 30 days on these experimental diets, rats were starved for 18 hr and received intravenous [4-14C]cholesterol (Amersham International plc, Buckinghamshire, 1.95 μCi or 2.48 μCi per 0.2 ml in the first and second experiments, respectively) into the tail vein followed by intragastric [1,2(n)-3H]cholesterol (Amersham International, 1 7.2 μCi or 24.6 μCi per 0.5 ml in the first and second experiments, respectively) under light ether anesthesia. The labeled cholesterol was purified by thin-layer chromatography, dispersed in physiological saline containing 5% ethanol and a small amount of lecithin by sonication, and given within 2 hr of preparation.12) Starvation was continued for an additional 6 hr. Rats were then allowed free access to their own diets, and were bled from the tail vein periodically up to 30 days for measurement of the radioactivity and analysis of cholesterol. Feces were collected daily for the first 7 days and lyophilized. On days 31 to 33, the bile duct was cannulated,13) the bile collected for 2 hr, and the animals were decapitated.

Cholesterol absorption. Cholesterol absorption was measured by the dual isotope ratio method of Zilversmit and Hughes.12,14) For 2 to 7 days after the dose of the isotopes, the cholesterol absorption rate was calculated, and the data were pooled and averaged.

Cholesterol kinetics. The decay curve of serum cholesterol specific activity after intravenous [14C]cholesterol was analyzed by the two-pool model system, and the parameters of cholesterol kinetics were calculated.15)

Lipid analyses. The concentrations of serum cholesterol from periodical blood samples were measured enzymatically (Cholesterol C-Test, Wako Pure Chemicals, Inc., Osaka). Biliary cholesterol and bile acid were measured enzymatically.13) The radioactivity of serum and feces was measured as described elsewhere.12,16)

Results

In each experiment, addition of a single amino acid did not influence food intake or weight gain of rats. The liver weight after bile drainage also was the same between the corresponding two groups.

Concentration of serum cholesterol

Addition of arginine to the casein diet induced a significant reduction (mean values 103 and 88.0 mg/dl) of serum cholesterol levels throughout the post-injection periods. A marginal and insignificant reduction (mean values 86.9 and 75.6 mg/dl) was observed when lysine was added to the soybean protein diet.

Absorption and excretion of cholesterol

As shown in Table I, the cholesterol absorption rate was comparable between the corresponding paired groups. Also, only a small difference was observed between soybean protein and casein, although direct comparison may not be adequate since the experiments were done separately. As shown in Fig. 1, fecal excretion of the radioactivity from the labeled cholesterol administered intravenously or orally was virtually uninfluenced by the addition of amino acid in both trials. The magnitude of excretion appeared to be somewhat greater in rats fed soybean protein diets than in those fed casein diets. The same excretion pattern was also observed between the corresponding two groups when the steroids were separated into neutral and acidic constituents (data not shown).

Table I. Effects of Addition of Lysine to Soybean Protein or Arginine to Casein on Cholesterol Absorption

<table>
<thead>
<tr>
<th>Groups</th>
<th>Absorption (%)</th>
</tr>
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<tbody>
<tr>
<td>Soybean protein</td>
<td>50.7 ± 3.2</td>
</tr>
<tr>
<td>Soybean protein + lysine</td>
<td>55.7 ± 3.2</td>
</tr>
<tr>
<td>Casein</td>
<td>55.8 ± 2.3</td>
</tr>
<tr>
<td>Casein + arginine</td>
<td>52.8 ± 1.7</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of 8 rats.
Arginine/Lysine Ratio and Cholesterol Metabolism

Fig. 1. Effects of Addition of Lysine to Soybean Protein or Arginine to Casein on Fecal Excretion of Radioactivity Following Administration of Intragastric [3H]Cholesterol and Intravenous [14C]Cholesterol.

Fig. 2. Effects of Addition of Lysine to Soybean Protein or Arginine to Casein on Turnover of Intravenously Administered [14C]Cholesterol in Serum.

Cholesterol kinetics
The specific activity of serum cholesterol as a function of time is shown in Fig. 2. Modification of the arginine/lysine ratio by adding amino acids did not exert a noticeable effect on the contour of die-away curves. These curves were found to fit with a two-pool model, from which the kinetic parameters of
Table II. Effects of Addition of Lysine to Soybean Protein or Arginine to Casein on Cholesterol Kinetics

<table>
<thead>
<tr>
<th>Groups</th>
<th>Kinetic parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( M_A (\text{mg}) )</td>
</tr>
<tr>
<td>Soybean protein</td>
<td>85.2±8.9</td>
</tr>
<tr>
<td>Soybean protein + lysine</td>
<td>76.0±4.6</td>
</tr>
<tr>
<td>Casein</td>
<td>121±5</td>
</tr>
<tr>
<td>Casein + arginine</td>
<td>108±7</td>
</tr>
</tbody>
</table>

Values are mean±S.E. of 8 rats. \( M_A \), mass of cholesterol in Pool A; \( PR_A \), the metabolic turnover rate (production rate) of cholesterol; \( K_{AB} \), the rate constant for the rate of transfer of cholesterol from Pool A to Pool B; \( K_A \), the rate constant for the rate of removal of cholesterol from Pool A; \( M_B \), the minimum mass of cholesterol in Pool B.

* Significantly different from the corresponding amino acid supplemented group at \( p < 0.05 \).

Table III. Effects of Addition of Lysine to Soybean Protein or Arginine to Casein on Bile Flow and Concentration of Biliary Cholesterol and Bile Acids

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bile flow (ml/hr)</th>
<th>Cholesterol (μg/ml)</th>
<th>Bile acids (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean protein</td>
<td>0.70±0.07</td>
<td>99.1±4.4*</td>
<td>14.8±1.4</td>
</tr>
<tr>
<td>Soybean protein + lysine</td>
<td>0.73±0.08</td>
<td>84.6±3.0</td>
<td>12.2±1.1</td>
</tr>
<tr>
<td>Casein</td>
<td>0.98±0.05</td>
<td>63.8±3.3</td>
<td>8.97±0.25</td>
</tr>
<tr>
<td>Casein + arginine</td>
<td>1.01±0.06</td>
<td>68.0±4.4</td>
<td>9.41±0.35</td>
</tr>
</tbody>
</table>

Values are mean±S.E. of 8 rats.

* Significantly different from the corresponding amino acid supplemented group at \( p < 0.05 \).

Cholesterol turnover were calculated, and the results are described in Table II. The size of Pool A (rapidly exchangeable cholesterol pool) and Pool B (slowly exchangeable cholesterol pool) and the production rate of cholesterol in Pool A tended to be decreased by the addition of lysine to soybean protein but the difference was significant only in Pool B. Addition of arginine to casein caused a slight and insignificant reduction of the size of Pool A and an increase in the rate constant \( K_{AB} \). Casein as compared to soybean protein appeared to considerably increase the size and production rate of Pool A.

Biliary excretion of cholesterol and bile acid

The bile flow and the concentration of biliary cholesterol and bile acid are shown in Table III. In the experiment with soybean protein, the bile flow and biliary bile acid level were not influenced by supplementary lysine but the manipulation caused a slight but significant reduction of the biliary cholesterol level. In the experiment with casein no arginine effect was found on these parameters.

Discussion

In this study various parameters of cholesterol kinetics including the absorption rate were, like those in rabbits,\(^6,7\) essentially unaltered to a great extent by changing the arginine/lysine ratio. Although there was a slight reduction of the serum cholesterol levels after addition of arginine to casein, this was in conflict with that reported previously.\(^5\) The reason for the discrepancy is not apparent at the moment, but the hypocholesterolemic effect of supplementary arginine may in part be ascribed to an increasing trend of the cholesterol removal from Pool A. Kritchevsky\(^2,3\) observed that the addition of lysine to soybean protein resulted in an elevation of plasma cholesterol and the degree of atherosclerosis, while the effect of arginine addition to casein was not unequivocal.

There appears to be an increasing trend of biliary steroid excretion in rats fed soybean protein as compared to those fed casein,\(^13\) but the effect of amino acid addition was not evident except for a slight reduction of the concentration of biliary cholesterol when the soybean protein diet was supplemented with
lysine. Since the rate of fecal excretion of acidic as well as neutral steroids was uninfluenced by the addition of single amino acids, the significance of the observed difference is obscure. The cholesterol absorption rate not being very different between rats fed soybean protein and casein disagreed with our previous observation, where it was significantly lower in the soybean protein group compared to the casein group. The discrepancy may be due to the difference in the level of dietary fat; 5% in the present and 1% in the previous experiments. The dietary protein-dependent effect on intestinal cholesterol absorption appears to be weakened when the dietary fat level is increased. In rats fed a 5% corn oil diet for a short time, the serum cholesterol-lowering action of soybean protein becomes less significant than in those fed a 1% corn oil diet, although, even at the higher fat level, soybean protein still exerted a hypocholesterolemic effect. The different response of serum cholesterol to amino acid supplementation may also be relevant to the difference in the dietary fat level. The different effects of added amino acids on the rate of lymphatic absorption of cholesterol as observed by Vahouny et al. could not be confirmed currently because of the difference in the methodology.

The arginine/lysine ratio seemed to influence the serum level of triglyceride rather than cholesterol. Addition of arginine resulted in a marked reduction of triglyceride in serum harvested after bile drainage (748 ± 108 vs. 344 ± 43 mg/dl, \( p < 0.05 \)). The decrease in serum triglyceride accompanies a concomitant increase in plasma glucagon. Glucagon reduces hepatic synthesis and secretion of triglyceride-rich lipoproteins. The lysine supplementation to soybean protein had no effect on the serum triglyceride level (327 ± 38 and 308 ± 16 mg/dl for the groups with or without lysine supplementation, respectively).

Although studies on the effects of excess dietary lysine suggest a role of this amino acid in the metabolism of cholesterol, the lysine content is comparable in casein and soybean protein. Available information does not necessarily support the view that lysine is the principle determinant for the plasma cholesterol level. Since the arginine content in soybean protein is about twice that in casein, this amino acid may be responsible for the hypocholesterolemic action of soybean protein. Eklund and Sjöblom showed in rats, a significant negative correlation between the arginine content in various proteins and the serum VLDL+ LDL level. However, although Katan et al. and Sugiyama et al. reported that supplementation of an amino acid modifies the serum cholesterol concentration, it is doubtful whether a single amino acid is a principle determinant for serum cholesterol levels.

Kritchevsky et al. and Sugano et al. observed that there is a negative relationship between the arginine/lysine ratio of dietary protein and serum cholesterol concentration; this is quite in contrast to the observation reported for amino acid supplementation studies. It is thus presumable that added amino acids behave differently in the digestive tracts from those in peptides, since the mode and rate of intestinal absorption of two types of amino acids, free or peptide-bound, differ. Thus, the structure of proteins could possibly influence their cholesterolemic effect.

In conclusion, modification of the ratio of arginine/lysine alone did not greatly affect the cholesterol metabolism in rats. The results of this study support the view that the amino acid sequence in addition to the amino acid composition of dietary protein is significant in the regulation of the serum cholesterol level.

Acknowledgment. We thank Dr. R. Chanderbhan (George Washington University, Washington, D. C.) for his important suggestions during the preparation of this manuscript.

References


