Effect of Dietary Lysine on Endogenous Hyperlipidemia in Nephrotic Rats

Kazumi YAGASAKI, Mitsuyo MATSUMOTO, Kiyohito FUJISAWA, Akemi KUBOYA, and Ryuei FUNABIKI

Department of Applied Biological Science, Tokyo Noko University, Fuchu, Tokyo 183, Japan
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Nephrotic syndrome (NS) is well known to accompany severe hyperlipidemia as well as marked proteinuria and hypoalbuminemia. The reduction of hyperlipidemia with NS is particularly important, since the hyperlipidemic effect with NS has been suggested to deteriorate further the kidney dysfunction. Thus, pharmacological and dietary treatments of lipid abnormalities have so far been tried in nephrotic patients. The kidney plays an important role in regulating the tissue carnitine concentration. When treated with carnitine, dialyzed patients showed reduced hypertriglyceridemia and increased high density lipoprotein-cholesterol (HDL-Ch). Carnitine also reduced hypertriglyceridemia induced by feeding a high fat diet to rats. Lysine is accepted as a precursor of carnitine biosynthesis in mammals, and lysine itself has been reported to decrease the serum lipid level in normal rats. The present study was designed to know whether or not dietary-supplemented lysine would be reductive for endogenous hyperlipidemia with NS, using rats with nephrotic serum nephritis as an animal model for the disease. An excess lysine supplement to a 20% casein diet was found to reduce the hyperlipidemia, but to concurrently cause fatty liver and orotic aciduria in the nephrotic rats.

Male Wistar rats (four weeks of age) were purchased from Charles River Japan, Kanagawa. They were kept on a stock pellet diet (CE-2, CLEA Japan, Tokyo) in an air-conditioned room with a 8:00 a.m. to 8:00 p.m. light cycle. Anti-rat kidney glomerular basement membrane (GBM) rabbit serum was produced by immunizing rabbits with the supernatant of trypsin-digested rat GBM as described previously. Nephrosis was induced in the Wistar rats by a single intravenous injection of anti-GBM serum into the tail vein. In experiment 1, the rats were divided into three groups with equal body weights after feeding the stock pellet diet for 7 days (day 0), the rats of two groups receiving an antisem antibody injection (0.25 or 0.5 ml/rat). From the tail vein of the rats that had received no antisem injection (designated as normal rats), blood was collected on day 0 and left to clot to obtain serum. All the rats were fed on the stock pellet diet and water ad libitum for 21 days. On days 7 and 14, blood was collected from the tail vein of all the animals to obtain serum. Serum samples from days 0, 7, and 14 were frozen at -20°C until needed for analysis. In experiment 2, rats kept on the stock pellet diet for 5 days were then given a basal diet for another 5 days. The composition of the basal diet was 20% casein (Oriental Yeast Co., Tokyo), 5% corn oil (Hayashi Chemicals Co., Tokyo), 68.3% a-corn starch (Nihon Nosan Kogyo Co., Yokohama), 3.5% mineral mixture (AIN composition, Nihon Nosan Kogyo Co.), 1% vitamin mixture (AIN composition, Nihon Nosan Kogyo Co.), 0.2% choline bitartrate (Wako Pure Chemical Industries, Osaka), and 2% cellulose powder (Oriental Yeast Co.). All the animals were then injected intravenously with 0.25 ml/rat of anti-GBM serum (day 0), and were divided into two groups with equal body weights. The rats of one group were kept for 14 days on the basal diet, and those of the other group on the basal diet supplemented with 5% L-lysine acetic acid (Ajinomoto Co., Tokyo) at the expense of starch. Water and dietary were available at all times. In this experiment, on the day after the antisem injection (day 1), the rats were subcutaneously immunized with rabbit γ-globulin (6 mg/rat, Sigma Chemical Co., St. Louis, MO.) in 0.2 ml of Freund's complete adjuvant (Wako Pure Chemical Industries) into the hind foot pads as described previously. On days 21 and 14 in experiments 1 and 2, respectively, the rats were deprived of their diet at 9:00 a.m., but allowed free access to water until sacrifice, which was conducted 4 hr later by decapitation. Blood was collected in a glass tube and left to clot at room temperature to obtain serum. The liver and kidney were quickly removed, washed with cold 0.9% NaCl, blotted on filter paper and weighed. Urine excreted by the rats individually housed in metabolism cages was collected for the preceding 24 hr from 9:00 a.m. of each day indicated in Fig. 2. Urinary protein and orotic acid were measured by the methods of Bradford and Harris and Oberholzer, respectively. Total lipids were extracted from the liver, and total Ch (TCh), triglyceride (TG) and phospholipid (PL) were determined as described previously. The serum TG and PL levels were also determined. The serum lipoproteins were separated into HDL and very low density lipoprotein plus low density lipoprotein (VLDL+LDL) fractions by the precipitation method. The total Ch contents of unfractuated serum (TCh) and HDL (HDL-Ch) were determined by an enzymatic method with a commercial kit (Wako Pure Chemical Industries), and the difference between TCh and HDL-Ch is regarded as (VLDL+LDL)-Ch. Serum albumin was determined by a commercial kit (Wako Pure Chemical Industries). Statistical analyses were carried out as mentioned in each figure and table.

The ability of anti-GBM rabbit serum to induce nephrosis and hence hyperlipidemia is known to fluctuate depending on rabbits immunized with orotic acid. The results of the present study showed that dietary-supplemented lysine was effective in reducing hyperlipidemia in nephrotic rats. These findings suggest that dietary lysine may be an effective treatment for nephrotic syndrome.

Fig. 1. Changes in Serum Cholesterol Level with Time after Injecting Anti-GBM Serum to Rats (Exp. 1)

The rats (average weight of 143 g) received no intravenous injection (C), or 0.25 (a) or 0.5 (b) mL/rat of anti-GBM serum on day 0. All the rats were maintained on a stock pellet diet (CE-2) throughout the experimental period. Serum total cholestrol (TCh) concentration was determined as described in the text. The inset panel shows the Ch distribution among serum lipoproteins on day 21. Each value represents the mean of 4 rats, and vertical bars indicate standard errors. Values not sharing a common letter are significantly different at p<0.05 within each indicated day (by Duncan's multiple-range test).

Abbreviations: NS, nephrotic syndrome; GBM, glomerular basement membrane; Ch, cholesterol; TCh, total cholesterol; TG, triglyceride; PL, phospholipid; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.
with GBM. Accordingly, changes in the serum TCh level were monitored after injecting different doses of anti-GBM serum to rats kept on the stock pellet diet throughout the experimental period (Fig. 1). The serum TCh level in the normal rats was almost constant over the experimental period of 21 days. The injection of 0.5 ml/rat of antisemur to the rats caused a significant elevation in the serum TCh level on days 7, 14, and 21, when compared with the level in the normal rats on each indicated day. The lower dose (0.25 ml/rat) of antisemur injection also significantly elevated the serum TCh level on days 7 and 14, but no significant difference from the normal group was observed on day 21. The severity of hypercholesterolemia was gradually reduced in both cases as time went on. However, no significant difference was observed on days 7 and 14 between the two groups treated with 0.25 and 0.5 ml/rat of antisemur.

Cholesterol distribution among the serum lipoproteins on day 21 is also shown in Fig. 1. The serum (VLDL + LDL)−Ch level was elevated depending on the injected doses of antisemur, although the elevation was significant only in the higher dose (0.5 ml/rat) group. The serum HDL-Ch level was increased by the antisemur injection, but the rise was neither significant nor dose-dependent. The injection of 0.25 or 0.5 ml/rat of antisemur caused proteinuria (data not shown). These results indicate that the injection of either 0.25 or 0.5 ml/rat of anti-GBM serum was capable of inducing hypercholesterolemia and proteinuria for at least 14 days. In a separate experiment, the injection of 0.5 ml/rat of antisemur to the rats fed on a 20% casein diet was found to cause hypoalbuminemia and hypertriglyceridemia in addition to hypercholesterolemia, which was characterized by an increase in (VLDL + LDL)−Ch with no significant increase in HDL-Ch; 43% of the rats died within 7 days (unpublished observation). We assume that the dose (0.5 ml/rat) might have been too much to induce moderate nephrosis in those rats fed on the 20% casein diet in place of the stock pellets. The injection of 0.25 ml/rat of antisemur could also preserve the hypercholesterolemic state for at least 14 days (Fig. 1). In the subsequent experiment, we therefore decided to employ a dose of 0.25 ml/rat of antisemur, to immunize the rats against rabbit γ-globulin in order to attain stably lasting nephrosis,190 and to give the experimental diets to the rats for 14 days. The effect of excess lysine on the abnormal lipid and protein metabolism in nephrotic rats was studied under the above-mentioned conditions (experiment 2). The time-course of urinary protein excretion is illustrated in Fig. 2. Urinary protein excretion from the nephrotic control rats increased rapidly and linearly during the initial 2 days, and thereafter, the high excretion rate was maintained for up to 14 days. Excess lysine exerted no influence on proteinuria at any time when compared with the control. As shown in Table I, no significant changes were observed in body weight gain, food intake and relative kidney weight in the lysine-supplemented group. The liver was significantly enlarged by the lysine supplement. Urinary excretion of orotic acid was greatly increased by the lysine supplement, as was expected from previous reports describing orotic aciduria caused by feeding excess lysine to normal rats.111,186 The serum albumin level was not influenced by the lysine supplement, while the serum TCh, TG and PL levels were significantly lowered. Assessment of the reduction of TG and PL is based on the relation of (VLDL + LDL)−Ch. The lysine supplement caused severe fatty liver due mainly to TG accumulation and partially to TCh accumulation, while the PL content in the liver was slightly (12%) but significantly reduced by the lysine supplement. The liver enlargement caused by the lysine supplement might have been, at least in part, due to the lipid accumulation in the organ.

The present data show the number of excess (5%) lysine to the 20% casein diet caused a clear reductive action on endogenous hyperlipidemia in nephrotic rats. This hypolipidemic effect of dietary lysine is comparable to that of drugs with nephrotic patients44 and rats.175 However, severe fatty liver was concurrently brought about by the excess lysine. These results are similar to those from the study by Hevia et al.,16 who reported the hypolipidemic action and induction of fatty liver by feeding 5% lysine hydrochloride with 15% casein and glucose as the sole carbohydrate source to normal rats for 14 days. Aoyama et al.161 have suggested that an increase in the urinary excretion of orotic acid might be associated with the stimulation of orotic acid biosynthesis and with lipid accumulation in the liver. In the present study, the lysine-fed, nephrotic rats excreted 28-fold as much orotic acid into the urine as did nephrotic control rats. Ammonia is a precursor of carbamoylphosphate in mammalian liver, and carbamoylphosphate is metabolized to either urea through the urea cycle or to pyrimidines including orotic acid.193 Excess lysine stimulates orotic acid biosynthesis by inhibiting the urea cycle operation via arginase inhibition or by liberating excess ammonia from the lysine molecule.193 Orotic acid feeding is well known to induce fatty liver through the inhibition of hepatic lipoprotein secretion.200 Thus, an overproduction of orotic acid is one possible explanation for the fatty liver observed in the lysine-fed, nephrotic rats. Since 5% lysine acetic acid was added to the basal diet in the present study, the lysine-enriched diet was supplied with about 1.5% acetic acid that was absent.

### Table I. Effect of Dietary Supplemented Lysine on the Growth, Food Intake, Liver and Kidney Weights, Urinary Excretion of Orotic Acid, Serum Albumin and Lipid Concentrations, and Liver Lipid Contents in Nephrotic Rats (Exp. 2)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>+ Lysine</th>
</tr>
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<tbody>
<tr>
<td>Body weight gain (g/14 days)</td>
<td>85 ± 3</td>
<td>71 ± 9</td>
</tr>
<tr>
<td>Food intake (g/14 days)</td>
<td>265 ± 6</td>
<td>233 ± 14</td>
</tr>
<tr>
<td>Liver weight (g/100 g of body weight)</td>
<td>5.5 ± 0.2</td>
<td>8.0 ± 0.7**</td>
</tr>
<tr>
<td>Kidney weight (g/100 g of body weight)</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Orotic acid excretion (days 13−14) (nmol/day/100 g of body weight)</td>
<td>38 ± 7</td>
<td>1089 ± 485*</td>
</tr>
<tr>
<td>Serum albumin (g/100 ml)</td>
<td>3.2 ± 0.1</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>Serum lipids (mg/100 ml)</td>
<td>207 ± 12</td>
<td>153 ± 15*</td>
</tr>
<tr>
<td>TCh</td>
<td>80 ± 6</td>
<td>70 ± 7</td>
</tr>
<tr>
<td>HDL-Ch</td>
<td>127 ± 9</td>
<td>84 ± 18*</td>
</tr>
<tr>
<td>(VLDL + LDL)−Ch</td>
<td>412 ± 57</td>
<td>158 ± 35**</td>
</tr>
<tr>
<td>TG</td>
<td>372 ± 27</td>
<td>214 ± 19**</td>
</tr>
<tr>
<td>PL</td>
<td>2.1 ± 0.1</td>
<td>5.7 ± 0.8**</td>
</tr>
<tr>
<td>Liver lipids (mg/g of liver)</td>
<td>11.9 ± 2.0</td>
<td>135.9 ± 29.0**</td>
</tr>
<tr>
<td>TCH</td>
<td>30.5 ± 0.5</td>
<td>26.7 ± 0.9**</td>
</tr>
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The rats were treated as described in the legend to Fig. 2, before being sacrificed 14 days after the injection of anti-GBM serum. Urinary orotic acid, serum albumin, and serum and liver lipids were measured as described in the text. Each value represents the mean ± standard error for 7 rats. **Significantly different from the control group at *p < 0.05 and **p < 0.01, respectively (by Student's t-test).
from the basal diet. Acetic acid was available for fatty acid\textsuperscript{21} and Ch\textsuperscript{22} biosyntheses via acetyl-CoA in the liver. An unusual acetic acid supply to the diet is, therefore, another possible explanation for the severe fatty liver resulting in the lysine-fed, nephrotic rats. The hyperlipidemia in NS appears to be the consequence of an increased formation of hepatic lipoproteins\textsuperscript{3} accompanied by increased lipogenesis in the liver\textsuperscript{7} and/or a decreased lipoprotein catabolism in plasma.\textsuperscript{5,23,24} The hypolipidemic effect of excess lysine in nephrotic rats may be attributed to a suppression of the increased formation of hepatic lipoproteins via the lysine-induced stimulation of orotic acid biosynthesis, since orotic acid inhibits hepatic lipoprotein secretion.\textsuperscript{25} Carnitine, a metabolite of lysine, reduced not only the hypertriglyceridemia but also the fatty liver induced in rats by feeding a high fat diet.\textsuperscript{7} In the present study, however, excess lysine caused fatty liver, although dietary acetic acid might partially have supported its induction. Thus, the role of carnitine may be little, if any, in the action of excess lysine in nephrotic rats.

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References