Reduction of hyperlipidemia and proteinuria without growth retardation in nephritic rats by amino acids-fortified low casein diets

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Effects of low casein diets supplemented with either cystine alone, cystine plus glycine, or cystine plus threonine on hyperlipidemia and proteinuria were studied in rats with nephrotoxic serum nephritis. Rats were maintained on experimental diets for 14 days after an injection of nephrotoxic serum. An 8.5% casein diet, as compared with a basal 20% casein diet, improved both the hyperlipidemia and proteinuria incident to nephrotoxic serum nephritis but retarded the growth of rats. The supplement of 0.3% cystine to the 8.5% casein diet alleviated the growth retardation without loss of the reductive effect on hyperlipidemia and proteinuria but caused fatty liver. Glycine (2%) failed, but threonine (0.36%) succeeded in diminishing the cystine-induced fatty liver when concomitantly added with cystine (0.3%) to the 8.5% casein diet. An 8% casein diet supplemented with cystine and threonine was also found to reduce hyperlipidemia and proteinuria without growth retardation and fatty liver induction in nephritic rats. Fecal excretion of both neutral and acidic steroids in the 8% casein diet supplemented with cystine and threonine diet-fed nephritic rats was significantly higher than that in the 20% casein diet-fed nephritic rats. Hepatic cholesterol synthesis was unchanged between the two groups, whereas fatty acid synthesis of the 8% casein supplemented with cystine and threonine diet-fed rats was higher than that of 20% casein diet-fed animals. These results suggest that cystine-threonine–supplemented low casein diets have beneficial effects on hyperlipidemia and proteinuria with neither growth retardation nor fatty liver induction in nephritis. They also suggest that the hypocholesterolemic effect of the 8% casein supplemented with cystine and threonine diet in nephritic rats may be, at least in part, attributed to an increased excretion of steroids. (J. Nutr. Biochem. 5:21–27, 1994.)

Keywords: nephritis; hyperlipidemia; proteinuria; low casein diet; serum triglyceride; serum cholesterol

Introduction

Renal injuries such as puromycin aminonucleoside nephrotic syndrome[1] and nephrotoxic serum nephritis (NSN)[2] are known to be accompanied by severe hyperlipidemia. These types of hyperlipidemia should be controlled, because hyperlipidemia is believed to further deteriorate the kidney dysfunction.[3,4]

Brenner et al.[5] suggested that injury of the glomerulus is caused by increased permeability of the glomerular capillary to plasma proteins, and that high protein intake enhances the hyperfiltration of glomerulus, gradually causing progressive damage.

Many groups of investigators have reported that low protein diets ameliorate proteinuria in nephrotic patients[6] and rats.[7] Thus, we attempted to examine the effects of low protein diets on not only proteinuria but also hyperlipidemia incident to NSN.

Protein malnutrition may occur after a long period of low protein intake, even if low protein diets are effective. It is well known that low casein diets supplemented with a small amount of sulfur amino acids accelerate the growth of animals but cause lipids to accumulate in the liver.[8,9] Glycine (Gly)[9] or threonine (Thr)[8] have been reported to suppress the fatty liver caused in this manner.

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Received October 28, 1992; accepted July 28, 1993.

Here, we report that proteinuria and hyperlipidemia incident to NSN are improved by low casein diets supplemented with cystine (Cys) and Thr without growth retardation or fatty liver induction.

Methods and materials

Animals and diets

Male Wistar rats (4 weeks of age) were obtained from Charles River Japan, Kanagawa, Japan. They were kept on a stock pellet diet (CE-2, CLEA Japan, Tokyo, Japan) for 5 days and fed on a basal diet for another 5 days in an air-conditioned room with a temperature of 22 ± 2° C, a relative humidity of 60 ± 5% and an 8:00 a.m. to 8:00 p.m. light cycle. All the animals were moved on day 7 of the preliminary feeding from individual cages with wire bottoms into metabolism cages to collect urine. The composition of the basal diet (20C) was 20% casein (Oriental Yeast Co., Tokyo, Japan), 5% corn oil (Hayashi Chemicals Co., Tokyo, Japan), 68.3% α-corn starch (Nihon Nosan Kogyo Co., Yokohama, Japan), 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, Japan), and 2% cellulose powder (Oriental Yeast Co.). On day 10 of the preliminary feeding (day 0), rats weighing 140 to 165 g were divided into groups with equal body weights and received a single intravenous injection in the tail vein of anti-rat kidney glomerular basement membrane (GBM) rabbit antiserum, which was produced by immunizing rabbits with the supernatant of trypsin-digested rat GBM[10,11]; a constant dose was used in each experiment (0.25 to 0.70 mL/rat). The following day (day 1), the animals were subcutaneously immunized with rabbit γ-globulin (6 mg/rat, Sigma Chemical Co., St. Louis, MO USA) in 0.2 mL of Freund's complete adjuvant (Wako Pure Chemical Industries) into the hind foot pads as described previously.[12,13] This nephritic model is documented to reveal that the glomerulus is swollen and has a crescent in Bowman's space, adhesion of capillary wall and Bowman's capsule, proliferated mesangium, thickness of capillary wall, and hypercellularity after the antiserum injection.[10-12] Immediately after the injection of antiserum (day 0), rats of each group were kept on a particular experimental diet for 14 days. The composition of each experimental diet (see below) was adjusted to 100% by changing the amounts of α-corn starch to keep all the diets isocaloric. Water and each diet were available at all times.

In experiment 1, rats were divided into two groups, injected with antiserum, and each group was fed on either the 20C or an 8.5% casein diet (8.5C). In experiment 2, rats were divided into four groups, injected with antiserum, and each group was given either 20C, 8.5C, 8.5C supplemented with 0.3% L-Cys (Nippon Rikagakuyakuhin Co., Tokyo, Japan, designated as 8.5CC), or 8.5C supplemented with 0.3% L-Cys and 2% Gly (Wako Pure Chemical Industries, designated as 8.5CCG). In experiment 3, rats were divided into three groups, injected with antiserum, and each group was kept on either 20C, 8.5C, or 8.5C supplemented with 0.3% L-Cys and 0.36% L-Thr (Anjinomoto Co., Inc., Tokyo, Japan, designated as 8.5CCT). In experiment 4, rats were divided into three groups. Animals of two groups received antiserum injection and were fed either the 20C or an 8% casein diet supplemented with 0.3% L-Cys and 0.36% L-Thr (designated as 8CCT). Rats of the third group (normal group) were given no antiserum injection and maintained on the basal diet (20C).

In all experiments, urine excreted by the rats that were individually housed in metabolism cages was collected from 9:00 a.m. of each day for the preceding 24 hours as indicated in *Figures 1 and 2*. In all experiments, the animals were deprived of their diet at 9:00 a.m. on day 14, but allowed free access to water until sacrifice, which was performed 4 hours later by decapitation. Blood was collected and left to clot at room temperature. The liver and kidney were quickly removed, washed with cold 0.9% NaCl, blotted on filter paper, and weighed.

Lipid analyses

From the liver, total lipids were extracted according to the procedure of Folch et al.[14] After aliquots of the chloroform phase had been dried, cholesterol (Ch),[15] triglyceride (TG),[16] and phospholipid (PL)[17] levels were determined as described previously.[13] The serum TG and PL levels were also determined as described above. The serum Ch level was enzymatically determined with a commercial kit (Wako Pure Chemical Industries).

Serum albumin and urinary protein determination

Serum albumin level was determined with a commercial kit (Wako Pure Chemical Industries). Urinary protein was measured by the Bradford method (Bio-Rad Protein Assay, Bio-Rad Laboratories, Richmond, CA).[18]

Cholesterol and fatty acid syntheses from [^{14}C]acetate in liver slices

In experiment 4, liver slices weighing 100 to 120 mg were placed in 1 mL of Krebs-Ringer phosphate buffer (pH 7.4)

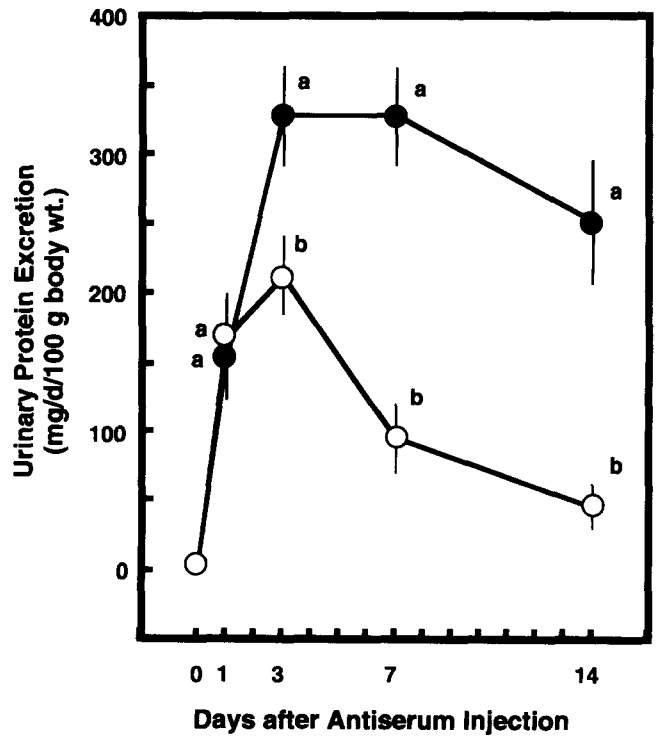

Figure 1 Effects of dietary casein levels on urinary protein excretion in rats with nephrotoxic serum nephritis. Each value and vertical bar represents the mean and standard error, respectively. Values not sharing a common letter are significantly different at $P < 0.05$ within each indicated day by Student's *t* test.

Table 1 Effects of dietary casein levels on the growth, food intake, liver and kidney weights, serum albumin and lipid concentrations, and liver lipid contents in rats with nephrotoxic serum nephritis

Measurement	20 C	8.5 C
Body weight gain (g/14 days)	87 ± 4[a]	33 ± 9[b]
Food intake (g/14 days)	254 ± 6[a]	215 ± 14[b]
Liver weight (g/100 g body weight)	5.7 ± 0.2[a]	4.9 ± 0.1[b]
Kidney weight (g/100 g body weight)	1.3 ± 0.1[a]	0.9 ± 0.1[b]
Serum albumin (g/L)	30 ± 1[a]	32 ± 1[a]
Serum lipids (mmol/L)		
Ch	7.2 ± 0.9[a]	4.0 ± 0.6[b]
TG	7.6 ± 1.5[a]	2.2 ± 0.4[b]
PL	6.7 ± 0.8[a]	4.1 ± 0.4[b]
Liver lipids (μmol/g liver)		
Ch	4.4 ± 0.1[a]	4.0 ± 0.2[a]
TG	8.5 ± 0.8[a]	12.2 ± 4.4[a]
PL	33.9 ± 1.2[a]	27.7 ± 0.8[b]

Values are means ± SEM for seven rats. Rats were treated as described in Materials and Methods section. Values not sharing a common letter are significantly different at $P < 0.05$ (by Student's t test).

containing 37 kBq of [1-^{14}C]acetate (37 MBq/mmole, Amersham International plc, Buckinghamshire, UK). Total fatty acid (FA) and Ch syntheses were estimated as described previously.[19,20]

Fecal steroid excretion

In experiment 4, feces were collected individually for 2 days before sacrifice. Neutral sterols (NS) and bile acids (BA) were extracted according to the method of Yamanaka et al.[21] NS and BA were enzymatically determined with commercial kits (Wako Pure Chemical Industries) as described previously.[22]

Statistical methods

Statistical analysis was carried out using Student's t test (experiment 1) or Duncan's multiple-range test (experiments 2 through 4), and a P value of <0.05 was considered significant.

Results

In our preliminary experiment, a 10% casein diet, as compared with the basal diet (20C), was found to suppress proteinuria but failed to improve hyperlipidemia incident to NSN (data not shown). Thus, in experiment 1, the dietary casein level was reduced from 10% to 8.5%, and the effect of an 8.5% casein diet (8.5C) was examined in comparison with that of the 20C in nephritic rats. As illustrated in *Figure 1*, urinary protein excretion from the nephritic control (20C) rats increased rapidly and linearly during the initial 3 days, and this high excretion rate was maintained for up to 14 days. The low casein diet (8.5C) commenced to significantly suppress proteinuria 3 days after the feeding, and a gradual decrease in urinary protein excretion was successively observed during the rest of the experimental period. As shown in *Table 1*, body weight gain, food intake, and liver and kidney weights were significantly lower in the 8.5C group than in the 20C group. No significant difference was observed in the serum albumin level between the two groups. In contrast, the 8.5C group showed a hypolipidemic action, that is, the serum Ch, TG, and PL levels were significantly lower in the 8.5C group than in the 20C group. The liver Ch and TG contents were unchanged between the two groups, while the PL content was slightly (18%) but significantly reduced in the 8.5C group *(Table 1)*. The 8.5C feeding was found to suppress the induction of both proteinuria and hyperlipidemia in nephritic rats, but retarded growth of these animals.

In experiment 2 we studied the effects of 8.5C, 8.5C supplemented with either Cys (8.5CC) or Cys plus Gly in combination (8.5CCG), using 20C as the control, in nephritic rats. The time course of urinary protein excretion is illustrated in *Figure 2*. Urinary protein excretion of the 20C (control) group rapidly increased until day 3, and this high excretion rate was retained up to day 14. The excretion rate of the 8.5C group was the lowest throughout the experimental period, and it decreased significantly 3 days after the feeding and thereafter, confirming the finding in experiment 1 *(Figure 1)*. The excretion rates of the 8.5CC and 8.5CCG groups were significantly lower than those of the control group on days 7 and 14. On day 14, the rates of both

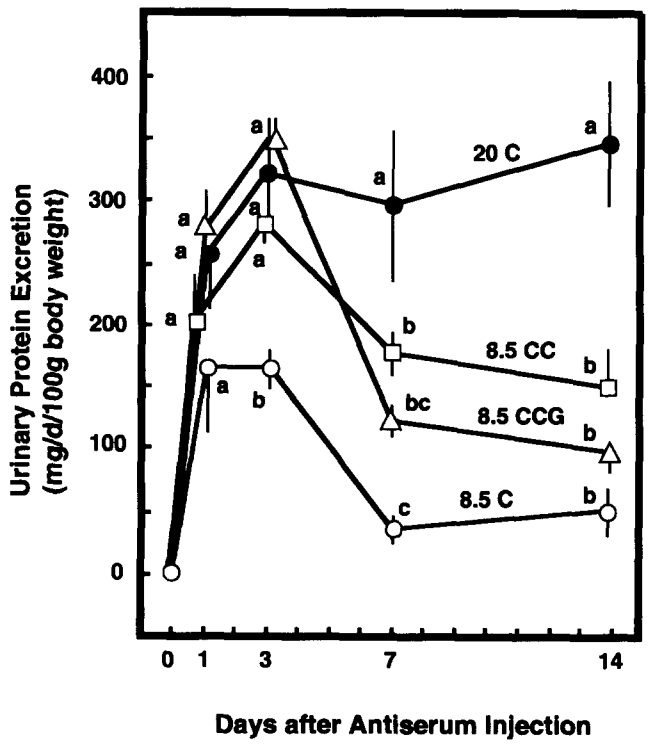

Figure 2 Effects of 8.5% casein diets supplemented with either cystine or cystine and glycine in combination on urinary protein excretion in rats with nephrotoxic serum nephritis. Each value and vertical bar represents the mean and standard error, respectively. Values not sharing a common letter are significantly different at $P < 0.05$ within each indicated day by Duncan's multiple-range test.

Table 2 Effects of 8.5% casein diets supplemented with either cystine or cystine and glycine in combination on the growth, food intake, liver and kidney weights, serum albumin and lipid concentrations, and liver lipid contents in rats with nephrotoxic serum nephritis

Measurement	20 C	8.5 C	8.5 CC	8.5 CCG
Body weight gain (g/14 days)	81 ± 4[a]	28 ± 7[b]	65 ± 5[a]	64 ± 6[a]
Food intake (g/14 days)	256 ± 7[a]	196 ± 11[b]	249 ± 15[a]	268 ± 9[a]
Liver weight (g/100 g body weight)	6.1 ± 0.3[a]	5.2 ± 0.2[b]	6.2 ± 0.3[a]	6.4 ± 0.2[a]
Kidney weight (g/100 g body weight)	1.4 ± 0.1[a]	0.9 ± 0.1[b]	1.1 ± 0.1[c]	1.1 ± 0.1[c]
Serum albumin (g/L)	27 ± 2[a]	29 ± 1[a]	25 ± 1[a]	27 ± 1[a]
Serum lipids (mmol/L)				
Ch	8.9 ± 1.7[a]	3.9 ± 0.7[b]	4.8 ± 0.8[b]	4.9 ± 0.4[b]
TG	8.7 ± 2.2[a]	3.3 ± 0.4[b]	5.3 ± 1.5[ab]	6.0 ± 0.7[ab]
PL	7.5 ± 1.4[a]	3.9 ± 0.3[b]	4.9 ± 0.6[b]	4.9 ± 0.3[b]
Liver lipids (μmol/g liver)				
Ch	6.2 ± 0.1[ac]	5.1 ± 0.2[b]	6.3 ± 0.3[a]	5.5 ± 0.4[bc]
TG	12.0 ± 1.9[a]	16.0 ± 3.7[a]	39.2 ± 6.7[b]	33.1 ± 5.8[b]
PL	39.0 ± 0.9[a]	31.9 ± 1.4[b]	31.0 ± 0.5[b]	32.8 ± 1.2[b]

Values are means ± SEM for five (8.5 CC) or seven (20 C, 8.5 C and 8.5 CCG) rats. Rats were treated as described in Methods and materials section. Values not sharing a common letter are significantly different at $P < 0.05$ (by Duncan's multiple-range test).

Table 3 Effect of an 8.5% casein diet supplemented with cystine and threonine in combination on the growth, food intake, liver and kidney weights, urinary protein excretion, serum albumin and lipid concentrations, and liver lipid contents in rats with nephrotoxic serum nephritis

Measurement	20 C	8.5 C	8.5 CCT
Body weight gain (g/14 days)	88 ± 4[a]	15 ± 7[b]	81 ± 6[a]
Food intake (g/14 days)	245 ± 5[a]	170 ± 10[b]	263 ± 10[a]
Liver weight (g/100 g body weight)	5.5 ± 0.1[a]	5.1 ± 0.3[a]	5.2 ± 0.1[a]
Kidney weight (g/100 g body weight)	1.0 ± 0.1[a]	0.8 ± 0.1[b]	0.9 ± 0.1[ab]
Urinary protein excretion (days 6–7) (mg/d/100 g body weight)	334 ± 37[a]	163 ± 53[b]	168 ± 24[b]
Serum albumin (g/L)	31 ± 1[a]	29 ± 2[a]	32 ± 1[a]
Serum lipids (mmol/L)			
Ch	5.4 ± 0.8[a]	3.6 ± 0.6[b]	3.5 ± 0.3[b]
TG	3.8 ± 0.8[a]	1.5 ± 0.4[b]	1.8 ± 0.2[b]
PL	4.5 ± 0.4[a]	3.2 ± 0.4[b]	3.1 ± 0.2[b]
Liver lipids (μmol/g liver)			
Ch	4.3 ± 0.3[a]	4.1 ± 0.3[a]	3.3 ± 0.1[b]
TG	8.2 ± 2.5[a]	16.7 ± 2.0[b]	5.2 ± 0.9[a]
PL	32.4 ± 1.8[a]	25.3 ± 1.6[b]	28.3 ± 1.0[ab]

Values are means ± SEM for six rats. Rats were treated as described in Methods and materials section. Values not sharing a common letter are significantly different at $P < 0.05$ (by Duncan's multiple-range test).

groups showed no significant differences from those of the 8.5C group. *Table 2* shows overall data on experiment 2. Body weight gain and food intake of the 8.5C group were significantly lower than those of the control group. These reductions returned to the control level when the 8.5C was supplemented with Cys or Cys plus Gly. The same tendency was seen in liver and kidney weights, although the reduction in the kidney weight of the 8.5C group was not completely reversed by amino acid supplements. No dietary effect was observed on the serum albumin level. All serum lipid levels determined were significantly lower in the 8.5C group than in the control, confirming the above-mentioned results *(Table 1)*. Likewise, the serum lipid levels were also lower in the 8.5CC and 8.5CCG groups than in the control, although the reduction in the serum TG level was not significant. Liver Ch content was significantly lower in the 8.5C group than in the control. This decrease was reversed to the control level by the Cys supplement (8.5C versus 8.5CC) but Gly interfered with the Cys action (8.5CC versus 8.5CCG). TG in the liver of the 8.5CC group accumulated significantly when compared with the control and 8.5C groups, as was expected from the early observations in normal rats.[8] Glycine, which was reported to prevent a methionine-induced TG accumulation in the liver,[9] failed to ameliorate the Cys-induced fatty liver. Liver PL content was significantly lower in the 8.5C, 8.5CC, and 8.5CCG groups than in the control group.

In experiment 3, nephritic rats were fed the 20C, 8.5C, and 8.5C supplemented concomitantly with Cys and Thr (8.5CCT) instead of Cys and Gly. As seen in *Table 3*, the growth retardation and reduced food intake induced by feeding the 8.5C (20C versus 8.5C) were completely restored to the control level by the concomitant addition of Cys and Thr to the 8.5C (8.5C versus 8.5CCT, 20C versus 8.5CCT). Reduced liver and kidney weights in the 8.5C group were unaffected by further

amino acid supplements, nor was any dietary effect noted on the serum albumin level. The time course of urinary protein excretion revealed identical effects of 8.5C and 8.5CCT on proteinuria (data not shown), the suppressive effects of both diets becoming significantly different from the control diet 7 days after their feeding *(Table 3)*. The hypolipidemic effect of the 8.5C (20C versus 8.5C) was maintained even when the 8.5C was supplemented with Cys and Thr (8.5C versus 8.5CCT, 20C versus 8.5CCT). Liver Ch content was significantly lower in the 8.5CCT group than in the control and 8.5C groups. Liver TG content of the 8.5C group increased to twice that of the control, but the rise was reversed and dropped to the control level in the 8.5CCT group, suggesting the ability of Thr to prevent the Cys-induced fatty liver observed in experiment 2 *(Table 2)*. A decreased content of liver PL in the 8.5C group (20C versus 8.5C) was unaffected by the supplementation of Cys and Thr (8.5C versus 8.5CCT).

In experiment 4 we studied the mechanisms for the induction, as well as the improvement, of hyperlipidemia incident to NSN, employing normal rats fed the 20C in addition to nephritic rats fed the 20C or an 8% casein diet supplemented with 0.3% Cys and 0.36% Thr (8CCT). In this experiment, the dietary casein level of the above-mentioned hypolipidemic diet (8.5CCT) was reduced by 0.5% to attain almost the same nitrogen intake as that found with the 8.5C. *Table 4* summarizes the results. Body weight gain and food intake were reduced and liver and kidney weights were enlarged (Nor-20C versus Nep-20C) by nephritis induction; feeding 8CCT to nephritic rats had no significant influence on this reduction or enlargement (Nep-20C versus Nep-8CCT). While urinary protein excretion remained constant at low levels (3 to 9 mg/day/100 g body weight) over the experimental period in normal rats, that of nephritic control rats rapidly increased and was maintained at a high rate until day 14. As indicated in *Table 4*, the excretion rate of nephritic rats fed the 8CCT diet began to significantly decrease on day 7 (Nep-20C versus Nep-8CCT). Hypoalbuminemia was induced in nephritic rats (Nor-20C versus Nep-20C). Feeding the 8CCT diet to nephritic rats did not influence the serum albumin level (Nep-20C versus Nep-8CCT). Hyperlipidemia characterized by elevations in Ch, TG, and PL was confirmed in nephritic control rats as compared with normal rats (Nor-20C versus Nep-20C), and hyperlipidemia in nephritic rats was significantly reduced by feeding the 8CCT diet (Nep-20C versus Nep-8CCT). As high density lipoprotein (HDL)-Ch measured by the precipitation method[23] was unchanged among the three groups (data not shown), hypercholesterolemia in nephritic rats was due not to the elevation in HDL-Ch but to that in very low density lipoprotein + low density lipoprotein (VLDL + LDL)-Ch. This method, however, does not precipitate all non-HDL lipoproteins when serum has high concentrations of TG-rich lipoproteins, so that serum lipoproteins should be separated by the ultracentrifugal method. Liver Ch content increased slightly but significantly with nephritis induction, this increase tending to be suppressed by 8CCT feeding. No significant changes were observed in liver TG content among the three groups, indicating that the 8CCT diet did not induce fatty liver. No significant change was seen in liver PL content between the Nor-20C and Nep-20C groups. Feeding the 8CCT diet to nephritic rats

Table 4 Effect of an 8% casein diet supplemented with cystine and threonine in combination on the growth, food intake, liver and kidney weights, urinary protein excretion, serum albumin and lipid concentrations, liver lipid contents, hepatic lipid syntheses, and fecal steroid excretion in rats with nephrotoxic serum nephritis

Measurement	Nor-20C	Nep-20C	Nep-8CCT
Body weight gain (g/14 days)	120 ± 3[a]	60 ± 4[b]	50 ± 4[b]
Food intake (g/14 days)	298 ± 12[a]	248 ± 6[b]	257 ± 8[b]
Liver weight (g/100 g body weight	4.2 ± 0.1[a]	6.5 ± 0.2[b]	6.6 ± 0.3[b]
Kidney weight (g/100 g body weight)	0.7 ± 0.0[a]	1.4 ± 0.1[b]	1.3 ± 0.1[b]
Urinary protein excretion (days 6–7) (mg/d/100 g body weight)	5 ± 1[a]	485 ± 19[b]	323 ± 18[c]
Serum albumin (g/L)	36 ± 2[a]	24 ± 1[b]	22 ± 1[b]
Serum lipids (mmol/L)			
Ch	2.2 ± 0.2[a]	13.2 ± 1.0[b]	9.4 ± 0.9[c]
TG	0.8 ± 0.1[a]	19.8 ± 5.7[b]	4.8 ± 1.3[a]
PL	2.8 ± 0.2[a]	10.4 ± 0.7[b]	7.6 ± 0.6[c]
Liver lipids (μmol/g liver)			
Ch	3.8 ± 0.1[a]	4.6 ± 0.1[b]	4.2 ± 0.2[b]
TG	11.2 ± 1.6[a]	10.8 ± 2.2[a]	17.0 ± 2.5[a]
PL	39.3 ± 1.9[a]	35.1 ± 1.7[a]	25.3 ± 1.9[b]
Hepatic lipid syntheses (dpm × 10^{-4}/2 hr/g liver)			
Ch	1.9 ± 0.4[a]	6.9 ± 1.6[b]	5.7 ± 0.6[b]
FA	6.2 ± 0.7[a]	20.5 ± 6.4[a]	46.5 ± 8.6[b]
Fecal steroid excretion (μmol/2 d/100 g body weight)			
NS	8.7 ± 1.2[a]	10.3 ± 0.4[a]	17.6 ± 1.8[b]
BA	9.6 ± 1.4[ab]	6.4 ± 0.8[a]	11.5 ± 1.1[b]

Values are means ± SEM for five (Nor-20C) or seven (Nep-20C, Nep-8CCT) rats. Rats were treated as described in Methods and materials section. Values not sharing a common letter are significantly different at $P < 0.05$ (by Duncan's multiple-range test).

significantly decreased liver PL content (Nep-20C versus Nep-8CCT). Liver Ch synthesis in nephritic control rats increased 3.6 fold over that in normal rats (Nor-20C versus Nep-20C), but the treatment of nephritic rats with the 8CCT did not alter this (Nep-20C versus Nep-8CCT). Liver FA synthesis in nephritic control rats also increased 3.3 fold to that in normal rats, though not significantly, and the 8CCT treatment of nephritic rats further increased the FA synthesis. No significant differences were observed in the fecal excretion of NS and BA between normal and nephritic control rats, although BA excretion showed a tendency to decrease with nephritis. Fecal excretion of both NS and BA was significantly stimulated in nephritic rats fed the 8CCT diet as compared with those fed the 20C diet.

Discussion

Rats with nephrotoxic serum nephritis, as compared with normal controls, exhibited hyperlipidemia, proteinuria, and hypoalbuminemia *(Table 4)*, the latter two symptoms essentially characterizing the nephrotic syndrome. Low casein diets were demonstrated to improve both the proteinuria and hyperlipidemia in these nephritic animals. The critical level for dietary casein to reduce hyperlipidemia appeared to be lower than that required to suppress proteinuria, because a 10% casein diet failed, but an 8.5% casein diet succeeded in reducing hyperlipidemia in these rats *(Table 1)*.

Low protein diets cause protein malnutrition and growth retardation, which should be avoided, particularly in infants with renal failure. To solve this problem, the effects of low casein diets fortified with amino acids were examined in nephritic rats. Cystine-threonine–supplemented low casein diets (8.5CCT, 8CCT) alleviated growth retardation without inducing fatty liver, the dietary manipulation preserving the suppressive effects on hyperlipidemia and proteinuria in nephritic animals.

Hyperlipidemia incident to nephrosis is generally thought to occur as a result of an increased synthesis of lipids and apoproteins in the liver[24] and/or a decreased clearance of serum lipoproteins.[25–27] In the present study, hepatic Ch and FA syntheses were stimulated in nephritic rats *(Table 4)*. Treatment of these rats with 8CCT exerted no influence on hepatic Ch synthesis, but stimulated fecal excretion of NS and BA. Hence, the hypocholesterolemic action of 8CCT may be, at least in part, due to a stimulation of steroid excretion into feces, although the possibility that Ch absorption from the intestine may be suppressed by 8CCT treatment cannot be excluded. In contrast, the 8CCT treatment stimulated further hepatic FA synthesis in nephritic rats. Nonetheless, this dietary manipulation showed a hypotriglyceridemic effect and no TG accumulation in the liver of nephritic rats *(Table 4)*. The hypotriglyceridemic effect of the 8CCT treatment might therefore be due to factors other than modulating hepatic FA synthesis. Such factors could be (a) a suppression of FA mobilization from peripheral adipose tissue; (b) an increase in FA uptake and FA oxidation by the liver; and (c) an increase in clearance of TG-rich lipoproteins, VLDL, and chylomicron. Factors a and b would curtail a supply of fatty acyl-coenzyme A to be esterified to PL as well as TG, and hence formation and secretion of VLDL from the liver. As for c, three kinds of factors should be taken into consideration: TG lipolytic enzymes, substrate compositions, and regulators of enzyme activity. Extensive studies are needed, with careful consideration given to the above-mentioned points, to determine the exact mechanisms for the hypotriglyceridemic effect of the 8CCT treatment in nephritic rats. Inclusion of normal rats fed on the 8CCT would also help to clarify those nutritional effects.

In this study, the severity of nephritis was not always to the same extent, particularly in the nephritic control (Nep-20C) group. Administration of antisera obtained from different rabbits in each experiment may be one cause for the phenomenon. Despite this, the results obtained here indicate that low casein diets supplemented with limiting amino acids (Cys and Thr) alleviate protein malnutrition that appears in the treatment of nephritic rats fed a low casein diet alone. In our separate experiments, similar effects of limiting amino acid supplements on low protein diets containing proteins other than casein have been found in nephritic rats (unpublished observations). Thus, the results on this rat model point to a possibility that low protein diets, provided specific limiting amino acids are supplemented to meet amino acid requirements, would ameliorate protein malnutrition due to treatments of nephritis with low protein diets. Because of the different requirements of amino acids between humans and rats, quantity of supplemental amino acids should be modulated in accordance with human requirements when this kind of dietary manipulation is applied to subjects with nephritis.

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