Protein and calorie effects on progression of induced chronic renal failure in cats

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Objective—To determine effects of dietary protein and calories on progression of induced chronic renal failure in cats.

Animals—28 young adult female cats.

Procedure—Renal mass was reduced surgically, and glomerular filtration rate (GFR) was determined. Cats were allotted to 4 groups of 7 with similar mean GFR (1.52 to 1.55 ml/min/kg of body weight). Diets were formulated to provide: low protein and calorie (diet A), low protein and high calorie (diet B), high protein and low calorie (diet C), and high protein and calorie (diet D) intakes. Cats were fed their prescribed diet for 12 months, then blood and urine biochemical variables were measured, after which kidney specimens were examined microscopically.

Results—Protein intake by cats of groups C and D (9.0 g/d/kg) was substantially greater than that by cats of groups A and B (5.3 and 5.2 g/d/kg, respectively). Caloric intake by cats of groups B and D (73 and 71 calories/d/kg, respectively) was greater than that by cats of groups A and C (58 and 55 calories/d/kg, respectively). Renal glomerular lesions were mild and not affected by protein, calories, or their interactions. Nonglomerular lesions, though mild, were significantly influenced by calorie intake, but not by protein or calorie-protein interactions. The GFR did not decrease in any group. Urine protein-to-creatinine ratio increased significantly in all groups after reduction of renal mass, but values from all groups remained within the reference range (0 to 0.3).

Conclusions and Clinical Relevance—Diets replete in protein were not associated with increased severity of glomerular or nonglomerular renal lesions, increased proteinuria, or decreased GFR. Diets replete in calories were not associated with increased severity of glomerular lesions, but were associated with mild increase of nonglomerular lesions. Factors other than protein and calorie intake must be considered potential causes of progression of renal failure in cats. Results raise questions about the practice of restricting quantity of protein in the diet of cats with chronic renal failure, with the intention of ameliorating development of further renal damage. (*Am J Vet Res* 1998;59:575–582)

C hronic renal failure often is a progressive malady that leads to uremia. Progression is ultimately fa-

tal unless intervention with dialysis or renal transplantation is used. The remnant kidney model of renal failure has been used extensively to study progression of renal failure. In this model, renal mass is reduced surgically, usually by ligating selected branches of the renal artery in one kidney and performing contralateral nephrectomy. In certain strains of rats, residual renal tissue gradually develops lesions, leading to reduced renal function and death from uremia.[1] In those rats, dietary protein restriction ameliorates development of renal lesions and slows progression of renal failure.[1] Development of renal damage in residual tissue has been attributed to glomerular hypertension, and beneficial effects of protein restriction have been attributed to diminution of glomerular capillary blood pressure.[1] However, reduced caloric intake often accompanies use of low protein diets because of diminished food palatability. Some studies indicated that beneficial effects ascribed to protein restriction are actually attributable to decreased caloric intake.[2]

Dogs with renal failure induced by use of the remnant kidney technique gradually develop lesions in residual renal tissue,[3,4] and renal function often decreases, leading to development of uremia.[5,6] However, restricting protein intake did not ameliorate the development of renal lesions nor halt progression of renal dysfunction.[5] Those results indicate that renal responses to dietary manipulations may be species specific.

Cats with remnant kidneys have glomerular hypertension, placing them at risk for glomerular injury.[7] In a study of cats in which chronic renal failure was induced by reduction of renal mass, consumption of a low protein diet ameliorated development of glomerular lesions in the remnant kidney.[8,9] However, cats consumed less of the low protein diet, and thus, were deprived of calories and other nutrients as well. Although cats fed the low protein diet had minor renal lesion, they developed hypoalbuminemia, raising the question of whether protein malnutrition existed. The study reported here was performed to attempt to delineate the role of calories and protein in development of renal lesions in cats and to determine extrarenal effects of varied protein and caloric intake.

Materials and Methods

Cats—Thirty-five 8- to 9-month-old female cats were procured from a commercial supplier,[a] and were acclimated for 2 months while fed a commercial dry cat food. Each cat's health was assessed by physical examination, CBC, serum electrolyte and biochemical analyses[b] (anion gap, albumin, alkaline phosphatase, alanine transaminase, bicarbonate, BUN, total calcium, chloride, creatinine, glucose, potassium,

sodium, inorganic phosphorus, total protein), urinalysis, and **urine protein-to-creatinine ratio (UP:UC)** determination. Cats were housed in individual cages under conditions of controlled temperature, humidity, and light exposure.

Diets—Four experimental dry foods (A–D) were formulated to provide nutritionally adequate diets, which differed only in the quantity of protein and calories that the cats ingested. Diets A and C (low calorie) were formulated to provide 56 calories/d/kg of body weight as fed. Diets B and D were formulated to provide 75 calories/d/kg as fed. Diets A and B (low protein) were formulated to provide 5.0 g of protein/d/kg as fed, and diets C and D were formulated to provide 9.3 g of protein/d/kg. A single lot of each diet was manufactured from commonly available ingredients (Table 1). Half of each diet was stored at 20 to 22 C (room temperature) for use during the early stages of feeding trials. The remainder of each diet was stored at 4 C for use during the last 6 months of feeding trials. Each diet was analyzed to determine actual concentration of components (Table 2).

Model of renal failure—Renal mass in each cat was surgically reduced as described,[10] except that a 2-week interval was allowed between 5/6 infarction of the left kidney and surgical removal of the right kidney. Sections of right kidney were fixed, processed, stained with H&E and **periodic acid-Schiff (PAS)**, and examined by 3 viewers (CB, WC, DF) to ensure that all cats entering the study were free of renal disease. After surgery, all cats were fed diet A at a rate of 56 calories/d/kg, for a 2-month period, to allow time for renal compensatory hypertrophy to develop. One week after initiation of diet A, hypobicarbonatemia was detected in some cats. To eliminate the possibility of development of renal abnormalities secondary to acidosis,[11] potassium citrate was administered orally to cats with hypobicarbonatemia, to reestablish and maintain plasma bicarbonate concentration in the reference range (12 to 20 mmol/L). Occasionally, administration of potassium citrate was associated with vomiting; in those instances, sodium bicarbonate was administered. The dose of alkalinizing agent was adjusted to attain the desired effect on the basis of frequent measurement of plasma bicarbonate concentration.

Cat groupings and feeding trials—After the 2-month period for development of renal hypertrophy, **glomerular filtration rate (GFR)** was determined in each cat, using [¹⁴C]inulin as the marker for GFR,[10] and values (milliliters per minute per kilogram) were listed from low to high. To create 4 groups of cats with similar mean GFR values, the list of GFR values was separated into sets of 4, and 1 cat from each set was randomly assigned to each of 4 groups until there were 7 cats/group. Diets were randomly assigned to cat groups, which were identified by diet (diet A = group A, diet B = group B, diet C = group C, diet D = group D), and feeding trials were initiated. Remaining cats (with higher values for GFR) were excluded from further study.

Cats were observed daily for signs of physical abnormalities. The desired amounts of protein and calories for ingestion by each cat were computed on the basis of its presurgical body weight. The diets had been formulated so that cats of groups A and C received roughly 75% of the amount of food per kilogram as did cats of groups B and D (Table 2). The calculated weight of food was provided to each cat between 8 and 10 AM, and was available for 24 hours. The weight of uneaten food was measured at the end of each 24-hour period.

Plasma bicarbonate concentration was measured in all cats at weekly intervals during the first month of feeding trials, and bimonthly or more frequently thereafter. Potassium citrate or sodium bicarbonate was given orally to cats with hypobicarbonatemia to maintain plasma bicarbonate concentration within the reference range.

During the 12 months of the feeding trial, on the basis of body weight measurements and judgment of body con-

dition, some cats of groups A and C (low calorie) had food intake increased by 5 to 10%. At bimonthly intervals after initiating feeding trials, blood biochemical analyses (anion gap, albumin, alkaline phosphatase, alanine transaminase, bicarbonate, BUN, total calcium, chloride, creatinine, glucose, potassium, sodium, inorganic phosphorus, total protein) were repeated. At 4-month intervals, GFR, urinalysis, and UP:UC measurements were obtained.

Histologic and morphometric studies—After 12-month GFR measurements, cats were heparinized (10,000 IU of heparin, IV) and deeply anesthetized with sodium pentobarbital. The remnant kidney was quickly removed, and the renal artery was cannulated. The kidney was perfused at 120 mm of Hg pressure, using a rinsing solution[c] until effluent from the renal vein was visibly free of blood. The kidney was then perfused with fixing solution[d] at 120 mm of Hg pressure. Perfused kidney was trimmed of scar tissue (from the previous infarction), weighed, and stored in 10% formalin. Necropsy was performed on each cat, and fat depot was judged semiquantitatively on a scale of 1 (minimal amount) to 4 (liberal amount).

For histologic study of kidneys, formalin-preserved blocks of the right (nephrectomy) and left (remnant) kidneys were imbedded in plastic, processed, and stained with PAS and H&E. For PAS-stained slides, 25 outer cortical glomeruli from each cat were evaluated for mesangial matrix accumulation, using a scoring system of: 0 = normal, 1 = mild, 2 = moderate, and 3 = severe. A mean score for mesangial matrix was computed for each slide. The H&E-stained slides from remnant kidneys were used to evaluate cellular infiltration, tubular morphology, and fibrosis. Tissues were scored for severity of these lesion, using the scale: 0 = normal, 1 = mild, 2 = moderate, and 3 = severe. Slides of kidney tissue were examined by 3 investigators (CB, WC, DF) without knowledge of the group of origin of the tissue. Mean scores from the 3 viewers were used for statistical analysis.

To determine effects of diet on glomerular hypertrophy, glomerular size was measured. The area of 25 outer cortical glomeruli on each slide from remnant kidneys was measured, using a computer software device.[e]

Statistical analyses—Mean and SD values were computed. For antemortem data, ANOVA was used to determine differences between groups at each time of observation. Within groups, ANOVA was performed to determine whether differences occurred with time. A paired t-test was used when a set of paired values was compared. Simple factorial analysis (ANOVA) was used to analyze effects of protein, calories, and their interactions on mesangial matrix accumulation and on nonglomerular renal lesions. Body condition scores determined at necropsy were analyzed, using Kruskal-Wallis one-way ANOVA. For determining significant differences, $P \leq 0.05$ was chosen as indicating significant difference, and Fishers test of least significant differences was used for post-hoc evaluations with multiple comparisons.

Results

Clinical and husbandry observations—Twenty-six of 28 cats completed the study. One cat (group D) abruptly developed uremia that was nonresponsive to fluid therapy and was euthanatized during month 11 of the study. One cat (group B) was found dead during month 10 after detection of leukocytosis and treatment with antibiotics; necropsy failed to establish cause of death. One cat (group A) acutely developed severe azotemia and signs of uremia at month 3, but received fluid therapy, recovered, and completed the study. All other cats completed the study without major health abnormalities. Data collected from all 28 cats were included in the analyses.

Table 1—Major ingredients of experimental diets

Ingredient	A	B	C	D
		Diet*		
Pregelled corn starch	33.70	50.50	1.15	26.10
Soy protein	19.50	14.50	37.60	28.10
Casein	19.50	14.50	37.60	28.10
Poultry fat	12.00	8.75	11.50	8.50
Beet pulp	4.00	3.00	4.00	3.00
Poultry digest	2.95	2.20	2.95	2.20
Potassium citrate	1.74	1.30	1.74	1.30
Sodium citrate	1.07	0.80	1.07	0.80
Taurine	0.16	0.12	0.16	0.12
Methionine	1.23	0.93	1.24	0.93

*Values are proportion (%) of total diet.

Body weight of cats decreased during the 2 months after surgery, when all received 56 calories of diet A/d/kg (Fig 1). At the time of separation into groups, body weight was 2.40 ± 0.26, 2.51 ± 0.27, 2.24 ± 0.22, and 2.52 ± 0.28 kg for groups A–D, respectively. During the 12 months of feeding trials that followed, body weight of cats of groups A and C remained stable. Body weight of cats of groups B and D increased soon after initiation of their 75 calorie/d/ kg diets, but group-B cats were unable to sustain their weight despite maintaining their food intake (Fig 1). At the conclusion of feeding trials, body weight of group-D cats was significantly greater than that of the cats of the other groups, but weight for groups A, B, and C did not differ from each other.

Food intake, and thus, protein and calorie intakes, remained stable for each group throughout the study (Fig 2). Overall, protein intake (grams per day per kilogram) was 5.3 ± 0.3, 5.2 ± 0.4, 9.0 ± 0.6, and 9.0 ± 0.9 for groups A–D, respectively. Overall, caloric intake (calories per day per kilogram) was 58 ± 4, 73 ± 10, 55 ± 4, and 71 ± 7 for groups A–D, respectively. Eating habits were evaluated subjectively throughout the study. In general, cats receiving 56 calories/d/kg (groups A and C) ate their food immediately after it was available, whereas cats receiving 75 calories/d/kg (groups B and D) ate their food throughout the 24-hour feeding period.

Three cats of each group required sporadic treatment with potassium citrate or sodium bicarbonate to maintain plasma bicarbonate concentration within the reference range. Statistically, intake of Na^+ and K^+ was not significantly influenced by administration of alkalinizing agents, and neither mean serum K^+ concentration (4.6 ± 0.3 mmol/L in untreated, 4.8 ± 0.2 mmol/

L in treated) nor serum Na^+ concentration (156.8 ± 0.7 mmol/L in untreated, 156.6 ± 0.9 mmol/L in treated) were affected by treatment.

Renal functions—The GFR was not significantly different among groups at the time that test diets were imposed. The GFR was not significantly different among groups at 4, 8, or 12 months (Table 3). Within groups, GFR increased in group-D cats after imposing diet D; the increase was significant at 4 months, but not at 8 and 12 months (Table 3). Plasma creatinine concentration was not significantly different among groups at any of the times tested, but a trend for higher values in cats of groups A and B existed (Table 4).

The UP:UC values were not significantly different among groups for any of the times tested, except at the time of separation of cats into groups (month 0) when group-A cats had significantly greater UP:UC than did cats of the other groups. In each group, a significant increase in UP:UC between presurgical values and values after 4, 8, and 12 months of feeding was apparent. However, mean values for each group at all times were within the reference range for clinically normal cats (0 to 0.3).

Urine specific gravity was not different among groups for any of the periods studied, but presurgical values for specific gravity were significantly greater than all subsequent measurements (Table 3).

Blood biochemical variables—Cats had normal blood biochemical values prior to surgical reduction of renal mass, and values for the various groups did not differ once cats were allotted to groups on the basis of GFR (time 0, Table 4). Anion gap, alkaline phosphatase, alanine transaminase, chloride, and glucose values did not differ among groups at any of the time intervals after imposing of diets. Serum K^+ concentration was normal, and not significantly different among groups for all times tested. Plasma bicarbonate concentration was not different among groups, except at months 2 and 8, and was within the reference range (12 to 20 mmol/L) for all groups at all times.

Plasma albumin concentration in the groups varied at specific times; values were significantly higher in groups C and D when differences existed. However, values for all groups were within the reference range (2.6 to 3.7 g/dl) at all times. Serum total protein and phosphorus concentrations were usually significantly higher for groups C and D as well (Table 4).

Table 2—Analysis of feed and amount of nutrients delivered to cats*

Component	Diet A Actual†	Diet A Delivered†	Diet B	Diet C Actual	Diet C Delivered	Diet D
Protein	37.94	28.46	29.08	69.31	51.98	51.91
Fat	15.46	11.60	11.25	14.54	10.91	11.56
K	1.08	0.81	0.81	1.07	0.80	0.86
Ash	8.30	6.23	6.44	8.25	6.19	6.53
Fiber	1.39	1.04	0.98	1.32	0.99	1.68
Ca	1.18	0.89	0.93	1.24	0.93	0.98
P	1.16	0.87	0.92	1.20	0.90	0.96
Na	0.66	0.50	0.60	0.70	0.53	0.65
Mg	0.09	0.07	0.08	0.10	0.08	0.07
Cu	0.02	0.02	0.02	0.02	0.02	0.02
Fe	0.03	0.02	0.03	0.04	0.03	0.03
Mn	0.07	0.05	0.06	0.07	0.05	0.05
Moisture	6.24	6.24	7.81	9.43	9.43	0.05
Metabolizable energy	4.24	4.24	4.10	4.17	4.17	4.10

*All values are percentage, except metabolizable energy which is calories per gram of food as fed. †Actual is the composition of food as analyzed; delivered (diets A and C) reflects the reduction in amount provided to cats to achieve the desired intake of protein and calories.
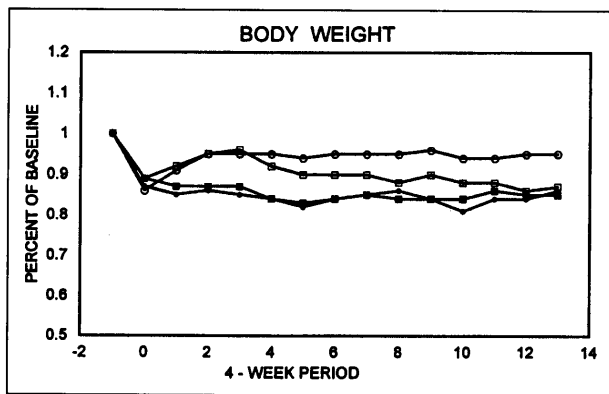
Figure 1—Body weight measurements in cats before and during the 1-year feeding trial. Body weight prior to surgery was designated as 100%, and subsequent weights were expressed as a percentage of that value. Group A is solid squares, group B is hollow squares, group C is solid circles, and group D is hollow circles.
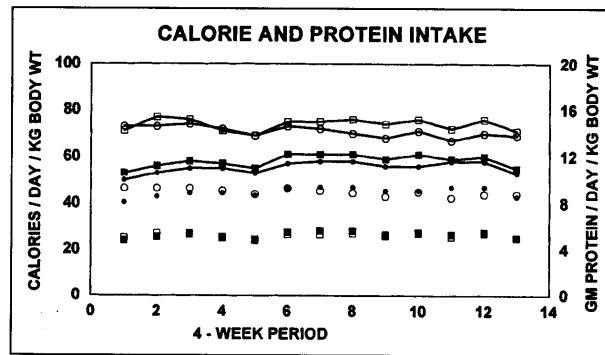

Figure 2—Calorie and protein intake by cats during each 4-week period of the 1-year study. Connected points depict caloric intake; nonconnected points depict protein intake. *See* Figure 1 for key.

Overall values for BUN concentration during the 12 months of dietary testing were 51.5 ± 16.1, 48.6 ± 12.1, 75.4 ± 17.3, and 67.1 ± 27.7 mg/dl for groups A–D, respectively. Overall mean values for serum creatinine concentration were 2.0 ± 0.7, 2.1 ± 0.5, 1.7 ± 0.4, and 1.9 ± 0.6 mg/dl for groups A–D, respectively. The BUN-to-serum creatinine ratio was 25.2, 23.5, 45.1, and 35.3 for groups A–D, respectively.

The PCV values were not significantly different among groups prior to surgical reduction of renal mass (overall mean = 31.3 ± 2.8%) or when diets were begun (overall mean = 23.3 ± 3.3%). For groups A, B, and D, PCV did not change significantly with time during the 12-month feeding trial, whereas in group-C cats, PCV increased to presurgical values at 4, 8, and 12 months.

Necropsy and tissue studies—Scores for body fat deposits were not significantly different among groups, although values for group-D cats approached significance ($P = 0.076$). Right kidneys removed during nephrectomy weighed 11.3 ± 1.1, 11.8 ± 2.0, 10.9 ± 1.6, and 11.5 ± 1.6 g for groups A–D, respectively. At necropsy 14 months later, gross lesions were not relevant in any cat, except for those in remnant kid-

neys and lesions consistent with uremia in the euthanatized cat of group D. Remnant kidneys from groups A–D weighed 7.8 ± 1.6, 9.7 ± 1.9, 9.2 ± 2.5, and 12.5 ± 1.7 g, respectively, and group-D kidneys were significantly larger than those from the other groups.

Scoring of remnant kidney glomeruli for mesangial matrix accumulation indicated a significant increase in mesangial matrix in remnant kidneys, compared with surgically removed kidneys, but differences in matrix scores among cat groups were not significant for surgically removed or remnant kidneys (Table 5). A trend toward higher scores for groups C and D was subjected to power analysis. Assuming that the difference in a protein effect was real, for a 90% chance of detecting a significant effect of protein on masangial matrix accumulation, 196 cats would have been required for the experiment. Glomerular area was 18,185 ± 6,644 μm²; 18,113 ± 5,803 μm²; 21,033 ± 7,690 μm²; and 20,058 ± 10,922 μm² for groups A–D, respectively; values for groups C and D were significantly greater than those for groups A and B.

In surgically removed and remnant kidneys, cellular infiltrate, tubular lesions, and fibrosis were mild and uncommon. In remnant kidneys, scores for groups A–D, respectively, were: tubular lesions = 0.05 ± 0.15, 0.21 ± 0.30, 0.10 ± 0.20, and 0.33 ± 0.43; cellular infiltrate = 0.17 ± 0.24, 0.33 ± 0.33, 0.10

Table 3—Renal functions and urine composition

Variable/group		Before	Month			
			0	4	8	12
GFR (ml/min/kg)	A	ND	1.52 ± 0.48	1.59 ± 0.65[a]	1.52 ± 0.62	1.57 ± 0.57
	B	ND	1.53 ± 0.40	1.31 ± 0.15[a]	1.60 ± 0.29	1.57 ± 0.25
	C	ND	1.55 ± 0.52	1.53 ± 0.39[a]	1.67 ± 0.49	1.42 ± 0.32
	D	ND	1.51 ± 0.36	1.76 ± 0.47[b]	1.74 ± 0.55	1.70 ± 0.57
Urine P/C	A	0.07 ± 0.03[1]	0.17 ± 0.08[a,1,2]	0.24 ± 0.12[2,3]	0.24 ± 0.09[2,3]	0.27 ± 0.12[3]
	B	0.06 ± 0.03[1]	0.10 ± 0.03[b,1,2]	0.18 ± 0.08[2,3]	0.27 ± 0.15[4]	0.25 ± 0.06[3]
	C	0.08 ± 0.04[1]	0.11 ± 0.03[b,1]	0.24 ± 0.12[2]	0.26 ± 0.11[2]	0.27 ± 0.13[2]
	D	0.07 ± 0.01[1]	0.14 ± 0.06[b,2]	0.24 ± 0.05[3]	0.25 ± 0.08[3]	0.24 ± 0.07[3]
Urine pH	A	5.86 ± 0.38[1]	ND	6.14 ± 0.63[1,3]	6.57 ± 0.53[a,2,3]	6.79 ± 0.57[a,2]
	B	6.36 ± 0.85	ND	6.21 ± 0.70	5.93 ± 0.45[b]	6.58 ± 0.58[a,b]
	C	6.21 ± 1.11	ND	6.00 ± 0.50	5.86 ± 0.48[b]	5.86 ± 0.63[b]
	D	5.71 ± 0.70[1]	ND	6.14 ± 0.8[1]	6.43 ± 0.73[a,b,1]	6.67 ± 0.75[a,2]
Urine specific gravity	A	1.047 ± 0.010[1]	ND	1.032 ± 0.12[2]	1.033 ± 0.012[2]	1.032 ± 0.011[2]
	B	1.052 ± 0.011[1]	ND	1.033 ± 0.16[2]	1.034 ± 0.007[2]	1.033 ± 0.009[2]
	C	1.054 ± 0.010[1]	ND	1.031 ± 0.01[2]	1.030 ± 0.007[2]	1.030 ± 0.008[2]
	D	1.051 ± 0.005[1]	ND	1.035 ± 0.014[2]	1.035 ± 0.015[2]	1.035 ± 0.016[2]

ND = no data; P/C = urine protein-to-creatinine ratio

For each measurement, lack of common superscript letters or lack of letters in each column signify statistically significant differences between groups; common superscript letters or no letters signify lack of significance. For each measurement, lack of common superscript numbers or lack of numbers in rows signify statistically significant differences within a group at different times; common superscript numbers or no numbers signify lack of significance.

Table 4—Blood biochemical determinations for cats of Groups A, B, C, and D

Variable		Month						
		0	2	4	6	8	10	12
Albumin (g/dl)	A	2.97 ± 0.08	3.21 ± 0.16[a]	2.88 ± 0.34[a]	3.29 ± 0.18[a]	2.89 ± 0.29[a]	3.29 ± 0.19	2.84 ± 0.13[a]
	B	2.97 ± 0.15	3.36 ± 0.26[a]	3.14 ± 0.17[b]	3.44 ± 0.27[a]	2.99 ± 0.21[a,c]	3.47 ± 0.24	2.95 ± 0.14[a,b]
	C	3.07 ± 0.13	3.53 ± 0.19[b]	3.27 ± 0.20[b]	3.62 ± 0.15[b]	3.23 ± 0.11[b]	3.50 ± 0.20	3.06 ± 0.15[b]
	D	2.96 ± 0.05	3.33 ± 0.10[a]	3.14 ± 0.10[b]	3.47 ± 0.13[a]	3.16 ± 0.05[b,c]	3.33 ± 0.16	3.03 ± 0.12[b]
Bicarbonate (mmol/L)	A	14.6 ± 2.6	16.3 ± 2.4[a]	16.4 ± 2.0	15.3 ± 1.0	14.0 ± 1.2[a,b]	14.6 ± 1.1	14.0 ± 1.0
	B	13.6 ± 1.0	15.1 ± 1.7[a]	15.4 ± 1.5	13.4 ± 2.4	13.7 ± 2.2[a,b]	14.7 ± 1.5	14.3 ± 2.5
	C	14.7 ± 2.0	13.0 ± 1.4[b]	15.3 ± 1.6	13.2 ± 1.1	12.4 ± 1.0[a]	12.7 ± 1.8	12.9 ± 1.3
	D	13.3 ± 2.4	16.6 ± 2.0[a]	15.9 ± 1.9	14.7 ± 2.4	14.9 ± 1.3[b]	15.7 ± 1.6	14.7 ± 2.3
BUN (mg/dl)	A	50.1 ± 16.9	53.1 ± 15.8[a,b]	49.4 ± 13.9[a]	49.7 ± 12.3[a]	51.7 ± 15.3[a]	56.3 ± 25.8[a]	48.7 ± 15.4
	B	44.1 ± 14.3	49.9 ± 12.8[a]	49.3 ± 10.5[a]	50.6 ± 15.1[b]	48.6 ± 13.0[a]	46.9 ± 12.3[a]	46.0 ± 13.0
	C	50.6 ± 12.0	76.9 ± 18.3[c]	74.2 ± 15.4[b]	78.7 ± 18.1[c]	78.4 ± 18.8[b]	76.1 ± 18.5[b]	68.0 ± 19.1
	D	46.0 ± 14.6	75.1 ± 30.5[b,c]	67.6 ± 26.5[a]	66.0 ± 27.2[a,b,c]	65.9 ± 28.1[a]	65.5 ± 34.1[a]	61.5 ± 30.1
Creatinine (mg/dl)	A	2.6 ± 0.7	2.1 ± 0.6	2.0 ± 0.7	2.0 ± 0.7	1.9 ± 0.7	2.1 ± 0.8	2.0 ± 0.6
	B	2.5 ± 0.6	2.2 ± 0.5	2.2 ± 0.4	2.1 ± 0.6	1.9 ± 0.4	2.0 ± 0.6	1.9 ± 0.6
	C	2.5 ± 0.6	1.8 ± 0.4	1.8 ± 0.5	1.7 ± 0.4	1.6 ± 0.3	1.5 ± 0.3	1.7 ± 0.3
	D	2.3 ± 0.5	1.9 ± 0.5	1.9 ± 0.5	2.0 ± 0.7	1.8 ± 0.6	1.9 ± 1.0	1.9 ± 0.8
Potassium (mmol/L)	A	4.3 ± 0.4	5.2 ± 0.7	4.6 ± 0.6	5.1 ± 0.4	4.0 ± 0.3	5.0 ± 0.4	4.2 ± 0.2
	B	4.4 ± 0.3	5.2 ± 0.6	4.2 ± 0.3	5.1 ± 0.4	4.1 ± 0.4	5.2 ± 0.5	4.2 ± 0.4
	C	4.5 ± 0.4	5.1 ± 0.5	4.3 ± 0.2	5.1 ± 0.4	4.2 ± 0.2	5.0 ± 0.4	4.4 ± 0.4
	D	4.4 ± 0.5	5.2 ± 0.4	4.3 ± 0.2	5.1 ± 0.4	4.2 ± 0.4	5.1 ± 0.4	4.3 ± 0.4
Total Ca (mg/dl)	A	9.3 ± 0.5	10.2 ± 0.7[a]	9.1 ± 0.7	9.9 ± 0.5	9.3 ± 0.6	10.4 ± 0.6	9.2 ± 0.5
	B	9.4 ± 0.7	11.1 ± 0.7[b]	9.9 ± 1.1	10.4 ± 0.9	10.4 ± 1.8	11.5 ± 1.6	10.0 ± 1.3
	C	9.8 ± 0.5	10.9 ± 0.5[b]	9.7 ± 0.8	10.5 ± 0.5	9.8 ± 0.5	10.6 ± 0.5	9.9 ± 0.6
	D	9.7 ± 0.5	11.0 ± 0.6[b]	9.4 ± 0.4	10.4 ± 0.5	10.0 ± 0.6	10.7 ± 0.8	9.9 ± 1.0
P (mg/dl)	A	4.8 ± 0.4	4.3 ± 0.5[a]	4.1 ± 0.3[a]	4.2 ± 0.2[a]	4.0 ± 0.4[a]	4.0 ± 0.5	3.6 ± 0.4[a]
	B	4.9 ± 0.6	4.6 ± 0.3[a]	4.0 ± 0.2[a]	4.4 ± 0.4[a]	4.3 ± 0.7[a]	3.9 ± 0.6	4.0 ± 0.5[a,c]
	C	4.6 ± 0.4	4.7 ± 0.3[a]	4.1 ± 0.3[a]	5.0 ± 0.6[b]	4.5 ± 0.6[a,b]	4.3 ± 0.3	4.2 ± 0.6[b,c]
	D	4.8 ± 0.3	5.3 ± 0.6[b]	4.8 ± 0.4[b]	5.0 ± 0.5[b]	5.0 ± 0.9[b]	4.5 ± 0.8	4.9 ± 0.6[d]
Total protein (g/dl)	A	6.6 ± 0.3	6.9 ± 0.5[a]	6.3 ± 0.8[a]	7.3 ± 0.3[a]	6.5 ± 0.3[a]	7.3 ± 0.4[a]	6.5 ± 0.4[a]
	B	6.6 ± 0.4	7.5 ± 0.4[b]	6.9 ± 0.4[a,b]	7.8 ± 0.5[a,c]	6.7 ± 0.2[a]	7.6 ± 0.2[a]	6.8 ± 0.3[a,b]
	C	7.1 ± 0.4	7.8 ± 0.4[b]	7.2 ± 0.4[b]	8.2 ± 0.5[b,c]	7.4 ± 0.6[b]	8.0 ± 0.4[b]	7.3 ± 0.6[b]
	D	6.8 ± 0.3	7.6 ± 0.5[b]	7.1 ± 0.3[b]	7.9 ± 0.4[b,c]	7.3 ± 0.5[b]	7.6 ± 0.8[a]	7.3 ± 0.6[b]

ND = no data.
See Table 3 for key.

± 0.20, and 0.45 ± 0.47; and fibrosis = 0.10 ± 0.20, 0.31 ± 0.40, 0.07 ± 0.18, and 0.36 ± 0.55. Calories had a significant effect on cellular infiltrate and tubular lesions, and effects of calories on fibrosis approached significance ($P = 0.058$). Protein percentage did not affect cellular infiltrate, tubular lesions, or fibrosis.

Discussion

A previous study[8,9] indicated that cats with reduced renal mass which consumed 75 calories and 6.8 g of protein/d/kg developed severe renal lesions, compared with cats with reduced renal mass which consumed 56 calories and 2.7 g of protein/d/kg. Cats of the 75 calorie, 6.8 g of protein group had > 21% of glomeruli with adhesions, and glomerulosclerosis score of 1.37, compared with 0.57% glomerular adhesions and glomerulosclerosis score of 0.51 in cats of the 56 calorie, 2.7 g of protein group. The study reported here was designed to distinguish the effects of protein from calories in the genesis of renal lesions. In contrast to those of the previous study, cats of this study had mild to minimal glomerular lesions, regardless of diet, and no significant difference existed among groups in the magnitude of these mild lesions. Cats of this study also developed nonglomerular renal lesions that were mild to minimal. Calories, but not protein, were significantly related to magnitude of nonglomerular renal scores.

Several similarities existed between the 2 studies that should have resulted in detection of a protein, calorie, or combined effect on glomeruli. Young adult female cats and the remnant kidney model were used in both studies, and an identical schedule was used for reduction of renal mass, time allocated for renal compensatory hypertrophy, and time of dietary study. Both studies involved diets that had the same protein concentration to represent low and high protein intakes; in the study reported here, good compliance by cats in consuming the food resulted in good duplication of caloric intake between the 2 studies. The degree of renal dysfunction was similar, as judged by plasma creatinine concentration and percentage reduction of GFR from values for normal cats in each laboratory.

Because strikingly different results were obtained from the 2 studies, the differences between the studies should be analyzed to assess their role in the results. Diets differed considerably in fat content (previous study = about 37%, this study = 12%), and in K+ content (about 0.4% vs 0.8 % in this study). Although both studies involved diets with the same protein concentration to represent low (about 28%) and high (about 52%) protein diets, actual intake of protein was markedly different because of the effect of the fat on caloric density. Thus, cats of groups A and B of this study consumed 5.2 and 5.3 g of protein/d/kg, respectively, compared with 2.7 g/d/kg consumed by cats of the previous study, and cats of groups C and D consumed 9 g of protein/d/kg, compared with 6.8 g/d/kg consumed by cats of the previous study. This higher protein intake by cats of this study was reflected in the BUN-to-serum creatinine ratio, which was about 25 and 23 in cats of groups A and B, respectively, compared with 15 in cats of the previous study, and 45 and 35 in cats of groups C and D, respectively, compared with 27 in cats of the previous study. Another difference between this and the previous study was body weight change during the 12 months of feeding trials. In both studies, cats lost or only maintained body weight during the 2 months after surgical reduction of renal mass. After imposing the test diets, the

Table 5—Mesangial matrix scores from kidney obtained before (nephrectomy) and after (remnant kidney) the 12-month feeding period

Variable	Group A	B	C	D
Nephrectomy				
Mean ± SD	0.12 ± 0.17	0.10 ± 0.08	0.16 ± 0.25	0.06 ± 0.05
Median	0.08	0.08	0.08	0.08
Minimum	0.0	0.03	0.0	0.0
Maximum	0.51	0.25	0.72	0.13
Remnant kidney				
Mean ± SD	0.77 ± 0.33	0.66 ± 0.31	0.88 ± 0.31	0.88 ± 0.33
Median	0.93	0.63	1.04	0.85
Minimum	0.16	0.35	0.48	0.47
Maximum	1.15	1.09	1.17	1.35
Difference	(remnant minus nephrectomy)			
Mean ± SD	0.65 ± 0.26	0.56 ± 0.25	0.72 ± 0.50	0.82 ± 0.33
Median	0.67	0.56	0.96	0.77
Minimum	0.13	0.27	−0.17	0.40
Maximum	0.89	0.93	1.17	1.27

Accumulation of mesangial matrix in each glomerulus examined was scored as 0 = normal, 1 = mild accumulation, 2 = moderate accumulation, 3 = severe accumulation. Each viewer scored 25 outer cortical glomeruli from each kidney, and the viewer's mean score was tabulated. The mean scores from the 3 viewers was used for computations.

high protein group of the previous study had > 40% increase in body weight that was maintained for at least 6 months, whereas in this study, no group exceeded its presurgical weight during subsequent study.

Identification of differences between the 2 studies in intake of protein, K^{+}, and fat warrant consideration of each as a potential cause of the difference in development of renal lesions. In addition, interactions between the factors should be considered.

Protein intake—Results of this study do not support the hypothesis that quantity of protein ingested is a relevant factor in development of renal lesions in cats with reduced renal mass. Cats of groups C and D ingested considerably more protein than did cats of the high protein group in the previous study, without development of lesions of the magnitude as reported in that study. However, another factor to be considered is source of protein. In this study, casein and soy protein were the major protein sources, whereas in the previous study, pork liver and casein were protein sources. Results of studies in human beings indicate that renal hemodynamic effects of protein differ depending, in general, on whether the protein is of animal or vegetable origin.[12-18] Because renal hemodynamic effects of protein are believed to be a mechanism for renal damage, it has been proposed that a vegan diet may be beneficial in the prevention of glomerular sclerotic changes in health and disease of human beings.[19,20] Studies in rats with remnant kidneys documented improved survival, less proteinuria, and milder renal lesions when rats were fed soy protein, compared with casein.[21,22] Other studies in rats have suggested that protein source may affect serum lipid concentrations and lipid metabolism,[23,24] which also could affect the kidneys. We are not aware of studies in cats or dogs that address the issue of protein source as a relevant factor in the genesis of renal damage.

Dietary potassium—In the previous study,[8,9] hypokalemia and muscle weakness developed in 4 of 7 cats with remnant kidneys fed the high protein diet, which resolved after addition of K^{+} to the diet. In contrast, hypokalemia was never detected in the cats of this study. In another report,[25] an association between hypokalemia and renal dysfunction in cats was iden-

tified. Restriction of dietary K^{+} caused hypokalemia and transient reduction in GFR in clinically normal cats, but renal tissues were not examined to determine whether renal lesions developed.[26] At present, it is well established that hypokalemia and renal dysfunction may be associated in cats, but the cause-effect relation remains conjectural.[27] Epidemiologic studies in human beings have documented an inverse relation between K^{+} intake and hypertension. Potassium depletion induced by dietary K^{+} restriction increases blood pressure in normal and hypertensive people.[28] The hypothesis that hypokalemia and K^{+} depletion could cause renal damage in cats via hypertension apparently has not been tested.

Dietary lipids—In the previous studies[8,9] and this study, poultry fat was used in the diets. Because there was a threefold difference in fat content of the diets used in this study and those of the previous studies, cats developing severe renal lesions had a substantially higher intake of fat. Alterations in serum lipid profiles are associated with renal disease in human beings[29] and dogs,[30] including increases in total cholesterol, low-density lipoprotein, and triglyceride concentrations. Dietary lipids have been incriminated as a cause of renal damage in other species because of differences in unsaturated fatty acid composition of lipid sources and the influence of these unsaturated fatty acids on generation of eicosanoids. Vasomotor, inflammatory, and platelet-aggregating properties of the eicosanoids may affect several organs, including the kidneys. Studies of the relevance of dietary lipids in progression of renal failure in cats have not been reported, to our knowledge.

Body weight and utilization of calories—The difference between studies of weight gain by cats receiving 75 calories/d/kg was remarkable. In this study, cats of groups B and D failed to exceed presurgical weight, whereas weight gain > 40% was achieved by cats of the previous studies. A discrepancy in use of calories has been encountered in rodents pair-fed for caloric intake, and has been attributed to continuous versus sporadic food intake.[31] The same phenomenon of weight gain associated with sporadic versus continuous feeding probably occurs in cats as well, because

body weight is maintained with less food intake by sporadic, compared with continuous, feeding.[32] The metabolic differences that are associated with sporadic, compared with continuous, feeding do not seem well defined. However, such metabolic differences could have a role in development of renal damage. It has been hypothesized that sporadic versus continuous eating may affect renal hemodynamics.[1] Ironically, in considering the renal damage associated with protein intake, sporadic feeding has been hypothesized to be protective rather than harmful.[1] In this study, group-C cats consumed their food abruptly, but group-D cats consumed their food over a longer period. Because both groups consumed 9 g of protein/d/kg, the effect of feeding pattern on renal damage does not seem too important.

In the study reported here, nonglomerular renal lesions were minimal, but a significant effect of calories was observed. Recent studies have indicated that a tubulointerstitial lesion may be relevant in development and progression of primary glomerular diseases.[33,34] Results of this study suggest that calorie intake should receive further attention as a factor in progression of renal failure in cats.

Interaction of factors—In the previous study,[8,9] intake of pork liver and poultry fat was shared by cats developing severe lesions (high protein, high calorie intake) and those with minimal lesions (low protein, low calorie intake), although the latter group consumed less of each than did the former. Unless quantity consumed was a factor, consumption of neither pork liver or poultry fat would explain renal damage in the high protein, high calorie group. It is possible that, although neither pork liver nor poultry fat alone was responsible for the renal damage, interaction of these factors with each other, or with hypokalemia or other factors, could explain the severity of the renal lesions in cats of the previous study.

Other nutritional considerations—In the previous study,[8,9] cats ingesting 2.7 g of protein/d/kg had significantly lower PCV and serum albumin concentration than did cats ingesting 6.8 g/d/kg. In the present study, albumin concentration was maintained in the reference range, although at some times, values were significantly lower in cats of groups A and B. The PCV values in these cats are difficult to interpret, because a small but significantly higher hematocrit existed in group-C cats, compared with cats of the other 3 groups. It is unclear whether the decreased serum albumin concentration found in cats of the previous study was harmful, but it might represent generalized body protein depletion. Because results of this study indicate that protein intake per se is not a risk factor for progression of renal lesions, the clinical practice of such severe protein restriction is questionable.

Cats of group B initially gained weight after initiation of the feeding trial, but later lost weight and completed the study at the same weight as cats consuming the lower calorie diets (groups A and C). Group-B cats had the same caloric intake as group-D cats, but the restricted protein intake apparently was a limiting factor in maintaining body weight.

Group-C cats had adequate protein for anabolism, but apparently the need for calories precluded optimal protein anabolism. The higher BUN-to-serum creatinine ratio in these group-C, compared with group-D, cats supports the theory that dietary protein catabolism was greater when calories were restricted. The glomerular area in cats of groups C and D was greater than in that in cats of groups A and B, suggesting a protein effect on glomerular hypertrophy. However, the greater kidney weight in group-D cats than in cats of groups A, B, and C suggests that renal tubular elements did not have the same degree of hypertrophy in group-C as in group-D cats, possibly because of limited protein anabolism.

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