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Long-term renal responses to high dietary protein in dogs with 75% nephrectomy

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Longterm renal responses to high dietary protein in dogs with 75% nephrectomy. It has been proposed that ingestion of large amounts of dietary protein leads to sustained renal hyperperfusion and progressive glomerulosclerosis in rats. This hypothesis was tested in dogs, with 75% reduction in renal mass, maintained for 4 years on either 56, 27, or 19% dietary protein. Twelve of 21 dogs survived 4 years, and death due to renal failure was not correlated to diet. Dogs fed 56 and 27% protein had increased GFR and C_{PAH} before and after reduction of renal mass compared to the 19% group. A pattern of deterioration of renal function, including proteinuria, was not found in any diet group. Nine of 11 dogs, fed 56, 27, or 19% protein had minimal glomerular lesions, including mesangial proliferation, GBM irregularities, adhesions, and sclerosis. Two other dogs, fed 56% protein, had more severe glomerular lesions. No significant ultrastructural differences were found in glomeruli among the three diet groups. These results do not support the hypothesis that high protein feeding had a significant adverse effect on either renal function of morphology in dogs with 75% nephrectomy.

Dietary protein has been implicated as a possible causative factor of decreased renal function. Some strains of rats have a high incidence of spontaneous glomerular and tubular lesions associated with age [1–6]. The progression and severity of these lesions can be influenced by altered dietary protein [7–9], sodium [2], and phosphate levels [10]. Surgical reduction in renal mass hastens these changes [11, 12], as does the induction of streptozotocin diabetes [13]. Likewise, the extent and severity of glomerular lesions in NZB/NZW mice with a lupus-like nephropathy can be influenced by changes in dietary protein and caloric intake [14]. While these observations are limited to the rat and mouse, they establish the possibility of dietary protein-induced nephropathy in other species. A recent hypothesis has been advanced that ingestion of excessive dietary protein leads to sustained glomerular hyperfiltration and progressive glomerulosclerosis [15].

There is a paucity of data on the effects of dietary protein on renal function and morphology in humans and non-rodent species. There is evidence that high-protein diets enhance renal function in the normal dog, although these experiments were limited to a few days or weeks [16–18]. A previous report from our laboratory describes the functional changes in dog fed varied protein diets for periods of 4 years after 75% nephrectomy [19]. Dogs fed the highest protein level in that study (56%) had higher renal function measurements compared to dogs fed reduced protein. There was no evidence of deterioration of renal function in dogs fed the high level of dietary protein. The purpose of this report is to describe the morphologic changes in those same kidneys, as seen by light and electron microscopy, and compare them to functional changes.

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Methods

Experiments were performed on 21 healthy, intact female beagle dogs purchased from a closed colony. The dogs ranged from 6 to 12 months in age at the onset. The dogs had been bred, raised, and maintained on standard and identical dietary and environmental conditions.

For the experiments, dogs were housed individually in stainless steel cages in separate air-conditioned rooms according to the diet fed. Dogs were fed exclusively a measured quantity of food sufficient to maintain body wt at the pre-experimental level. Protein intake was calculated from the amount of food actually eaten, although this seldom varied since the dogs ate to satisfy their caloric need. Dogs were fed once a day and body wts were recorded bi-monthly. Weight gain was avoided since excessive caloric intake alone has been shown to reduce renal function in rats [8].

Reduction of renal mass followed the general plan of Bricker, Klahr, and Rieselbach [20]. Control studies (Stage I) were performed 30 days after dogs were fed one of three diets to establish the level of renal function before reduction in nephron population. Renal mass was then reduced in one kidney, with the contralateral kidney unaltered. Stage II renal studies were conducted 60 days later, with simultaneous measurements made in the remnant and contralateral control kidney. Following these measurements, the control kidney was excised. Stage III functional studies were performed at various times on the remnant kidney throughout a 4-yr period. Details concerning these procedures, including surgery and anesthesia, were published previously [19].

Thirty days before Stage I measurements and throughout the experimental period, the dogs were fed exclusively one of three commercial diets which contained either 56, 27, or 19% protein (Table 1). Protein intake was 13.2, 6.9, or 3.2 g/kg/day, respec-

Table 1. Composition of dry matter of diets

Nutrient	Diet		
	A[1]	B[2]	C[3]
		%	
Protein	56	27	19
Fat	24	8	22
Carbohydrate	5	52	52
Fiber	2	4	2
Ash	13	9	5
Calcium	1.07	1.67	0.82
Phosphorus	1.03	1.03	1.01
Potassium	0.99	0.78	0.44
Sodium	0.95	0.58	0.25
Magnesium	0.04	0.18	0.11

[1] This diet consisted of canned meat and meat byproducts.
[2] This diet consisted of dry food, predominantly cereal.
[3] This diet consisted of a canned special dietary product for dogs with renal disease.

tively. While there was some variation in the electrolyte content of these diets, they were all considered complete for maintenance of adult dogs. Phosphate content of the three diets was identical.

Blood pressure was measured in each dog at Stage I, II, and III with an ultrasonic instrument (Arteriosonde, Hoffman-LaRoche, Inc., Nutley, New Jersey, USA). Blood pressure was normal throughout the 4-yr study period. Plasma volume was not measured.

Routine laboratory measurements and renal function measurements included a complete blood count, total plasma protein, albumin-globulin ratio, plasma creatinine, blood urea nitrogen (BUN), 24-hr creatinine clearance, 24-hr urinary protein excretion, routine urinalysis, and urine culture. Renal clearances of creatinine (Cr), inulin, para-aminohippuric acid (PAH), and fractional sodium reabsorption were measured at each stage (I, II, III) and at 13, 22, 30, 36, 42, 45, and 48 months after the beginning of the study. Standard clearance methods and formulae were used. Plasma amino acids were measured at 48 months. Standard profiles of plasma chemistries, electrolytes, and enzymes were performed on all dogs at intervals listed above, and were detailed in a previous report [19].

Dogs were sacrificed by an intravenous overdose of sodium pentobarbital. Complete necropsies, with tissue collection, were performed on all dogs. Dogs that died spontaneously before the end of the study were completely necropsied and evaluated histologically. (Histologic data from dogs not completing the 4-yr study are not included in Tables 5 through 7.)

Portions of kidneys of dogs completing the 4-yr study were preserved in 10% neutral-buffered formalin for light microscopy. These tissues were dehydrated and then embedded in paraffin, sectioned, and stained with either hematoxylin-eosin, periodic acid-Schiff's, or methenamine silver stains.

Kidney samples for electron microscopy from dogs completing the 4-yr study were cut into 1 mm cubes and fixed in cold 2.3% phosphate-buffered glutaraldehyde. These samples were postfixed in 2% phosphate-buffered osmium tetraoxide, dehydrated, and then embedded in EPON 812. Thin sections were cut, stained with uranyl acetate-lead citrate stain, and examined on either a JEM 1005 (JEOL, Tokyo, Japan) or Zeiss 10A (Carl Zeiss, New York, New York, USA) electron microscope. A minimum of ten glomeruli were examined and photographed from each dog.

Four pathologists interpreted duplicate slides of kidneys without knowledge of dietary group. The following were evaluated and scored on a scale of 1 to 4 (minimal to severe):

- Presence and severity of glomerulosclerosis (% affected glomeruli, adhesions/senechiae, tuft occlusion, segmental changes, exudative lesions).
- Basement membrane alterations, including increases in thickness, membrane irregularity, or gaps in the glomerular basement membranes (GBM).
- Presence of inflammatory cells in glomeruli.
- Presence and severity of mesangial proliferation (cells and matrix).
- Presence and severity of pyelonephritis.
- Presence and severity of scarring/fibrosis, both in glomeruli and in the interstitium.
- Tubular alterations, including hyalin droplet formation, foam cells and tubular necrosis.

Two of the pathologists (JLR, GSH) also evaluated electron micrographs of kidney samples and graded glomerular lesions, including foot process effacement, mesangial proliferation, membrane irregularities, presence of inflammatory cells, and platelet aggregates, and the presence or absence of dense deposits. These gradings were also done without knowledge of diet group.

T tests were performed on scores of morphologic data by using the mean square of error from the analysis of variance. The sources of variation consisted of percentage of protein in diet, dogs in each dietary group, and the interaction between pathologists and diet group.

Statistical analysis of functional data was performed using analysis of variance, which partitioned total variance in the data into partial variances attributable to diets, time of observation, diet × time interaction, and residual variation within dogs. One dog was excluded from the analysis because of death before the 13th month. Details concerning the statistical analyses were published previously [19]. The Hotelling-Lawley trace statistic was used to test the multivariate null hypothesis of no difference among the three diets over all the dependent variables. In the case of a significant effect attributable to diet, diets were compared pairwise to identify significant differences for all variables.

Results

Functional and clinico-pathologic findings

Dogs remained healthy, ate normally, and as a group were not azotemic throughout the 4 yr. Twelve of the 21 dogs survived the 4-yr study period (Table 2). Seven of the nine deaths were unrelated to renal failure. Two dogs died in renal failure with elevated plasma creatinine values. One dog was in the 56% protein group (A), and the other in the 19% protein group (C). There was no apparent relationship between deaths, or death attributable to renal failure and diet.

Renal perfusion and filtration were effected by diet before reduction of renal mass. The baseline C_{PAH} and GFR were higher in dogs fed diets A and B compared to diet C (Figs. 1 and 2). After 75% nephrectomy, analysis of variance indicated

Table 2. Survival and cause of death[1] in 21 dogs with surgically reduced renal mass

	Number of survivors								
	0	2	13	22	30	36	42	45	48
	Months post 75% nephrectomy								
Diet A (56% protein)	10	10	9[f]	8[e]	7[g]	7	6[h]	5[a]	5[2]
Diet B (27% protein)	5	5	5	5	4[d]	4	3[c]	3	3
Diet C (19% protein)	6	6	6	5[b]	5	5	4[a]	4	4

[1] Pathologic findings in non-survivors: a, chronic renal failure; b, amyloidosis; c, cystic calculi; d, pulmonary artery thrombosis due to catheter; e, post surgical dehiscence/sepsis; f, anesthetic death; g, congestive heart failure; h, pneumonia.
[2] Kidneys examined on four of five dogs.

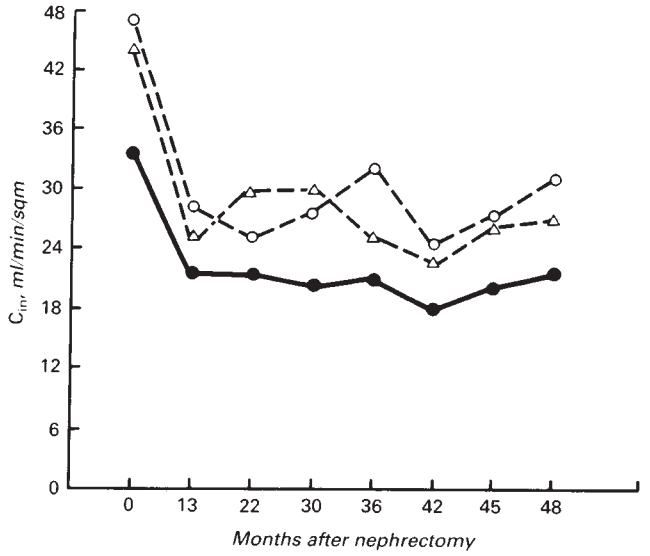

Fig. 1. *Glomerular filtration rate expressed by the clearance of inulin (C_{in}) in three groups of dogs fed different amounts of dietary protein after 75% subtotal nephrectomy.* Symbols are: △---△, diet A, 56% protein; ○---○, diet B, 27% protein; ●——●, diet C, 19% protein.

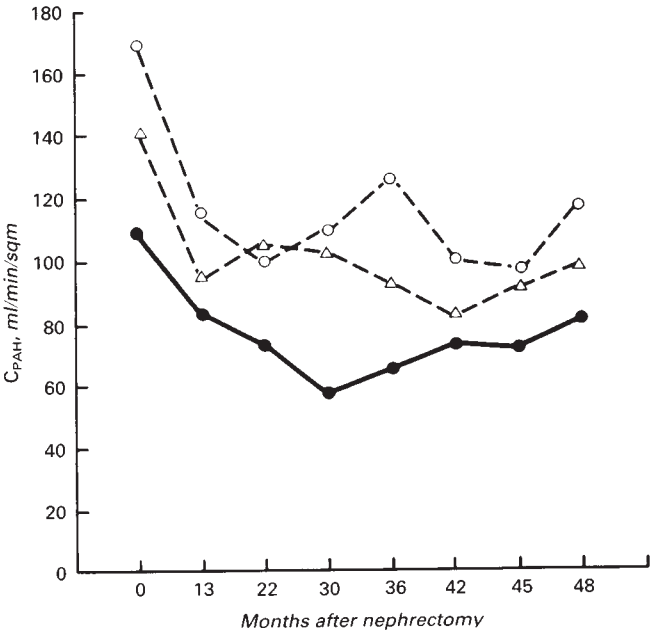

Fig. 2. *Effective renal plasma flow expressed as the clearance of para-aminohippuric acid (C_{PAH}) in three groups of dogs fed different amounts of dietary protein after 75% subtotal nephrectomy.* Symbols same as Fig. 1.

Table 3. Analysis of variance through 48 months[1,2]

Variable	Significance of effect attributable to:			
	Diet	Time	Interaction	R^2
BUN	$P < 0.001$	$P < 0.001$	$P < 0.001$	.777
Plasma creatinine	NS	$P < 0.001$	$P < 0.001$	.699
24-hr C_{cr}	$P < 0.003$	$P < 0.001$	$P < 0.05$	.820
24-hr urinary protein	NS	$P < 0.001$	NS	.560
C_{in}	$P < 0.012$	$P < 0.001$	NS	.733
C_{cr}	$P < 0.003$	$P < 0.001$	NS	.728
C_{PAH}	$P < 0.001$	$P < 0.001$	NS	.697
Fraction of Na^+ reabsorbed	$P < 0.028$	$P < 0.001$	$P < 0.027$	.810
Serum albumin	$P < 0.044$	$P < 0.001$	NS	.625
Serum globulin	NS	$P < 0.001$	NS	.696

[1] Multivariate test diet effect over all dependent variables: Hotelling-Lawley Trace = 28.307, $P < 0.002$.
[2] R^2 is the proportion of variation that is explained by the statistical model for each variable. It is a measure of the fit of the model.
Abbreviations are: C_{cr}, clearance of creatinine; C_{in}, clearance of inulin; C_{PAH}, clearance of PAH; NS, not significant.

significant differences attributable to dietary protein in BUN, 24-hr creatinine clearance, clearance of inulin and creatinine, clearance of PAH, fractional sodium reabsorption, and serum albumin concentration (Table 3). There was no significant difference in 24-hr urinary protein excretion related to diet. The clearance of inulin was significantly higher in dogs fed diet B (27%) compared to diet C (19%) ($P < 0.05$). Clearance of PAH was significantly higher in dogs fed diet B (27%) compared to diet C (19%) ($P < 0.05$). Diet A (56%) dogs had significantly higher 24-hr clearance of creatinine, serum albumin, and BUN compared to diet C (19%) dogs ($P < 0.05$). There were no significant time effects on these variables over the 4-yr study period.

The mean values of major variables measured at all study periods for the three diets are shown in Table 4. Diet B (27%) was associated with the highest clearance of PAH. Twenty-four-hr creatinine clearance was highest in dogs fed diet A (56%). Diet C (19%) was associated with the lowest clearance of PAh and lowest glomerular filtration rate (GFR) using all methods, lowest BUN, and lowest serum albumin.

When all measurements of GFR and effective renal plasma flow (ERPF) are plotted against time, it is obvious that dogs fed the lowest protein diet, diet C (19%), have the lowest values at all study periods (Figs. 1 and 2).

Data for measurement of Tm_{PAH} collected during the last study periods (42, 45, and 48 months) were pooled and averaged for each diet. The Tm_{PAH} was the same in dogs fed diet A (56%) (24.2 ± 3.8 SE mg/min/m^2 and diet B (27%) (24.6 ± 4.2), but dogs fed diet C (19%) had significantly lower values (8.1 ± 1.1) ($P < 0.005$) when evaluated with Student's t test.

No significant differences were found in complete blood

Table 4. Results of renal function tests[a]

Variable	Diet A (N = 49)	Diet B (N = 28)	Diet C (N = 34)
BUN, *mg/dl*	22.8 ± 1.3	15.6 ± 0.7	11.6 ± 0.5
Plasma creatinine, *mg/dl*	0.9 ± 0.03	0.8 ± 0.4	0.9 ± 0.03
24-hr C_{cr}, *ml/min/m²*	38.8 ± 1.8	33.1 ± 4.0	30.0 ± 0.4
24-hr urinary protein, *g*	0.25 ± 0.6	0.02 ± 0.00	0.17 ± 0.06
C_{in}, *ml/min/m²*	30.0 ± 1.5	30.8 ± 1.8	23.1 ± 1.1
C_{cr}, *ml/min/m²*	30.6 ± 1.4	31.7 ± 1.6	23.1 ± 1.2
C_{PAH}, *ml/min/m²*	104 ± 4.3	119 ± 6.8	79 ± 4.2

[a] Values are expressed as means ± standard error of the mean; N represents the number of measurements of variables over the 4-yr study period.

Table 5. Incidence and severity of light microscopic glomerular pathology in 11 dogs with reduced renal mass fed diets with either 56, 27, or 19% protein content[a]

Animal	Diet used *(% protein)*	Pathologist 1	Pathologist 2	Pathologist 3	Pathologist 4	Group mean
1	A (56)	4	3.5	3.5	3.5	3.625
2	(56)	3.5	3.5	2	2.5	2.875
3	(56)	2.5	NE	1	1	1.500
4	(56)	2	2	1	1	1.500
5	B (27)	2	1	1.5	1	1.375
6	(27)	2	2	1	0	1.250
7	(27)	1	2	1	1.5	1.375
8	C (19)	0	1.5	1	0	0.650
9	(19)	1	2	1	0	1.000
10	(19)	2	2	0	0	1.000
11	(19)	2	2	2	1.5	1.875

[a] Grades of severity of pathology are: 0, no changes; 1, minimal; 2, moderate; 3, marked; 4, severe; NE, not examined.

Note: One dog in the 56% protein group (diet A) died of post-surgical sepsis in the 46th month of the study. Microscopy of kidneys from this dog are not included in this analysis.

counts, plasma chemistries, electrolytes, or enzymes, except for higher serum cholesterol in dogs fed diet A (56%). Also, serum iron concentrations were slightly lower in dogs fed diet A (56%). No significant differences in plasma amino acid concentration were found between diet groups.

Routine urinalyses were not remarkable and no changes attributable to diet were found. Six dogs developed cystic calculi during this study. Retrospective review of GFR, ERPF, and other function studies did not indicate an effect of the calculi on renal function.

Body wts of dogs were maintained constant by intakes of energy and protein. Expressed in terms of body wt 0.75, that is, metabolic body size, energy intakes were 24 and 10% greater in dogs fed diets A (56%) and B (27%), respectively, than in those fed diet C (19%). Protein intakes were 295 and 120% greater in dogs fed diet A (56%) and B (27%) than in those fed diet C (19%).

Light microscopic findings

Dogs fed 27% protein (diet B; dogs 5 through 7, Table 5) or 19% protein (diet C; dogs 8 through 11, Table 5) had a number of minimal glomerular and tubulointerstitial lesions. Focal minimal thickening of glomerular basement membranes and a minimal amount of mesangial proliferation (Fig. 3) were seen in a few glomeruli from most dogs. Some dogs had focal segmental sclerosis in a few glomeruli. There were focal tuft adhesions to Bowman's capsule in some glomeruli, while others were more severely affected. Fetal glomeruli were seen in the kidneys of most dogs. The overall incidence of glomerular lesions was graded minimal (Tables 5 and 6). Glomerular lesions, in dogs 5 through 11, occurred in 10 to 20% of glomeruli. Other lesions were also seen in kidneys of these dogs. Small focal infiltrates of lymphocytes and plasma cells were present in the interstitium. There were scattered and focal areas of minimal interstitial fibrosis. Some tubules were dilated and filled with amorphous eosinophilic debris. Five of seven dogs had minimal to moderate pyelitis with epithelial cell proliferation and neutrophil infiltration.

Two of four dogs (dogs 3 and 4, Table 5) fed 56% protein (diet A) had lesions similar in incidence and severity to those just described for dogs fed diet B (27% protein) or diet C (19% protein). The glomerular lesions consisted of minimal focal segmental glomerulosclerosis, focal proliferation of mesangial matrix and cells, fetal glomeruli, and focal thickening or splitting of GBM. Similar tubulointerstitial lesions were also seen, including mild pyelitis, slight focal tubulointerstitial nephritis, tubular dilatation and deposition of intraluminal eosinophilic debris, and minimal focal interstitial fibrosis.

Two other dogs (dogs 1 and 2, Table 5) fed 56% protein (diet A) had moderate sclerotic lesions in approximately 40% of the glomeruli. Most of the affected glomeruli had minimal lesions which consisted of focal segmental sclerosis (Fig. 4) and tuft adhesions. About 25% of the sclerotic glomeruli (roughly 6 to 10% of all glomeruli) had more severe lesions, with sclerosis and obliteration of the entire tuft. In addition, other glomeruli without frank segmental sclerosis had either focal or diffuse thickening and splitting of glomerular basement membranes. This was not accompanied by glomerular cellular proliferation. Focal adhesions of capillary loops to Bowman's capsule were present in some nonsclerotic glomeruli, as were a few cellular crescents. There were a number of atrophic glomeruli present, with shrunken tufts and amorphous eosinophilic debris filling the uriniferous space. Tubulointerstitial lesions in these two dogs (1 and 2) were similar in incidence and severity to other dogs in their diet group and to other diet groups. These lesions included mild pyelitis, slight focal tubulointerstitial nephritis, tubular dilatation and deposition of intraluminal eosinophilic debris, and minimal focal interstitial fibrosis. There was no correlation of the incidence or severity of tubulointerstitial lesions with a dietary effect in any groups.

The incidence and severity of glomerular lesions, evaluated by light microscopy, increased as the level of dietary protein increased (Tables 5 and 6). This was most pronounced in the high-dose group. As can be seen from Tables 5 and 6, the incidence and severity of glomerular lesions in most dogs was graded minimal. Glomerular pathology was graded high moderate in one animal (dog 2) and marked in the most severely affected animal (dog 1).

In none of the dogs was there a correlation of the degree of glomerular pathology to renal functional impairment as assessed by GFR, C_{PAH}, proteinuria, and clinical pathology

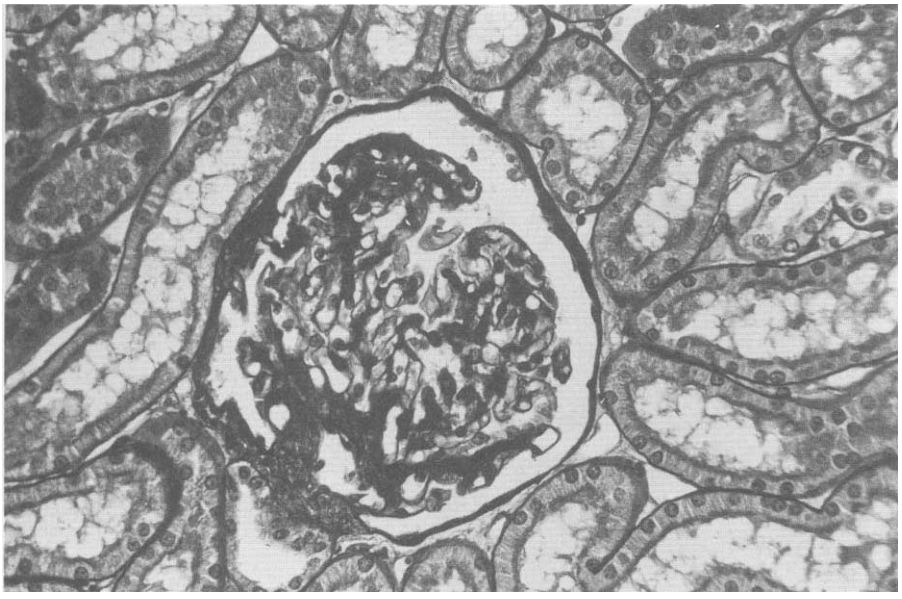

Fig. 3. *Minimal mesangial proliferation is seen extending from the glomerular hilus.* Glomerular basement membranes are uniform and thin and there are no sclerotic capillary loops. (Periodic acid-Schiff's stain, ×80)

Table 6. Statistical analysis of glomerular pathology gradings by light microscopy

Diet used *(% protein)*	Pathologist 1	Pathologist 2	Pathologist 3	Pathologist 4	Average
A (56)	3.00 ± 0.91	3.00 ± 0.87	1.88 ± 1.18	2.00 ± 1.22	2.43 ± 1.10[b]
B (27)	1.67 ± 0.58	1.67 ± 0.58	1.17 ± 0.29	0.83 ± 0.76	1.33 ± 0.62[b,c]
C (19)	1.25 ± 0.96	1.88 ± 0.25	1.00 ± 0.81	0.38 ± 0.75	1.13 ± 0.87[d]

Grading system same as in Table 5.
Significance of effects of dietary protein: [a] 56 vs. 19%, $P < 0.01$; [b] 56 vs. 27%, $P < 0.05$; [c] 27 vs. 19%, not significant; [d] These is a significant ($P < 0.01$) linear dose response trend for increased incidence and severity of glomerular pathology with increasing levels of dietary protein.

measurements. This lack of correlation emphasizes the minimal effect of these lesions on renal function.

Electron microscopic findings

In contrast to the light microscopic findings, no statistically significant difference in glomerular pathology could be found between diet groups with electron microscopy (Table 7). A minimal amount of foot process effacement (Fusion) occurred in all dogs in all dietary groups. This lesion was present in a scattered focal distribution and appeared as areas of fusion of three to five foot processes (Fig. 5). Dense arrays of microfilaments frequently were seen near these fused areas. Proliferation of mesangial cells and matrix was seen in all dietary groups. Matrix proliferation frequently accompanied cell proliferation and appeared as an increase in dense amorphous material in mesangial stalks. In some areas, the mesangium had a frothy or lytic appearance (Fig. 6). Mesangial changes were seen in all diet groups and were not correlated with dietary protein levels.

Most dogs had focal areas of irregularity in GBM. These irregularities included splitting and folding of GBM and humps and lytic areas within GBM. The incidence and severity of these lesions was not correlated with dietary protein. Lesions of this type are common in dogs of this age and breed.

Other glomerular lesions, such as the presence of dense deposits, tuft adhesions, and the presence of inflammatory cells occurred in all dietary groups and was not correlated with dietary protein. Likewise, there was no correlation of the occurrence of ultrastructural glomerular lesions with any impairment of renal function. There appeared to be little effect of dietary protein on glomeruli where evaluated with the electron microscope.

A number of dogs died before the end of the study (Table 2). Most of the deaths were related to anesthesia, repeated surgical procedures, and catheterizations. The kidneys of four dogs that died after completing at least 30 months on the study were examined histologically. Two dogs fed diet C had a minimal amount of diffuse mesangial proliferation. One of these dogs died at 42 months and also had a minimal amount of focal glomerulosclerosis and moderate diffuse subacute pyelitis. Two other dogs fed diet A also had renal lesions. The dog that died of congestive heart failure at 30 months had a minimal amount of diffuse mesangial proliferation. The other dog died at 42 months of a ruptured pulmonary abscess, but had marked diffuse glomerulosclerosis and interstitial fibrosis. Other kidneys were not available for examination.

Discussion

These data in dogs question whether observations in rats about ingestion of dietary protein can be transferred to other species. Dogs with reduced renal mass, which were fed 56% or 27% protein, had increased renal hemodynamics (measured by elevated GFR and (C_{PAH}) compared to dogs fed 19% protein.

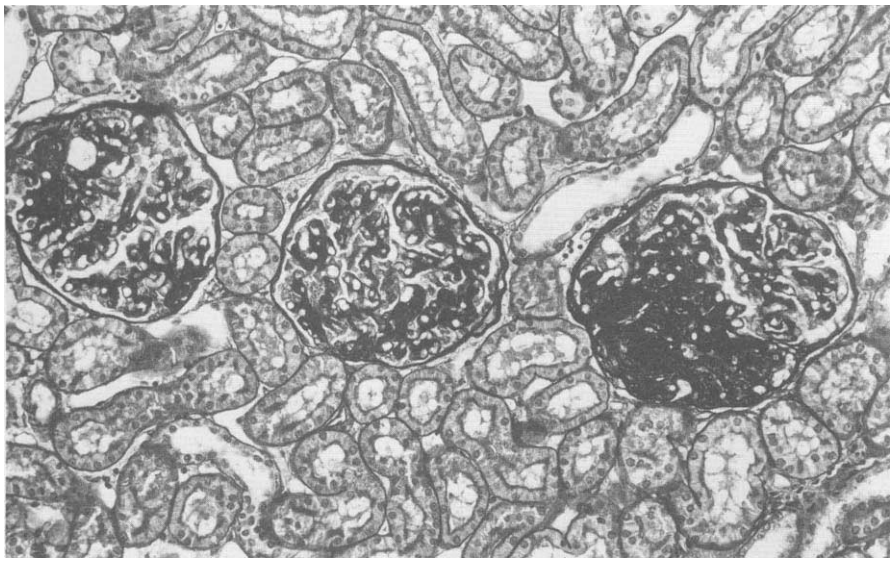

Fig. 4. *Minimal focal glomerulosclerosis is seen in two glomeruli from a dog fed 56% protein; a third glomerulus has more extensive sclerosis with tuft occlusion and the formation of adherent senechia.* (Period acid-Schiff's stain, ×50)

Table 7. Incidence and severity of ultrastructural glomerular pathology

Animal	Percentage of protein in diet	Foot process fusion		Mesangial proliferation		Basement membrane irregularities		Mesangial froth		Dense deposits		Tuft adhesions	
		A	B	A	B	A	B	A	B	A	B	A	B
1	56	2.0	3.0	3.0	3.0	2.5	2.0	3.0	3.0	2.0	2.0	2.5	2.0
2	56	3.0	3.0	3.0	4.0	3.5	2.5	3.0	2.5	3.5	4.0	1.0	1.0
3	56	1.0	1.0	1.5	1.5	3.0	1.0	3.0	1.0	2.0	1.0	0	0
4	56	1.0	1.5	2.5	2.5	3.0	1.5	1.0	2.0	1.0	1.5	0	0.5
5	27	1.0	1.5	2.5	1.0	3.5	2.5	2.0	1.0	2.0	1.0	0	0
6	27	2.5	1.5	1.0	2.0	3.5	2.5	3.0	1.0	2.0	1.0	0	0
7	27	2.0	2.0	2.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	0	0
8	19	1.0	1.5	1.0	2.0	2.5	2.5	2.0	3.0	1.0	2.0	0	1.5
9	19	1.5	1.1	2.0	2.5	1.5	1.0	2.0	3.0	1.5	2.0	0	1.0
10	19	2.5	2.0	3.0	3.0	1.5	2.0	2.5	4.0	1.0	2.0	2.5	3.0
11	19	1.5	1.0	2.5	0.5	3.5	2.5	2.5	3.0	0	1.0	1.5	3.0

Grading system same as Table 5.
Abbreviations are: A and B, scores of two pathologists.

The higher GFR and C_{PAH} were present in dogs fed higher protein before reduction of renal mass and persisted throughout 4 yr without a pattern of deterioration.

While there was considerable variation in the amount of urinary protein excreted by these dogs and there was increased protein excretion per surviving nephron, there was no significant dietary effect on protein excretion in the urine. While dogs fed 56% protein excreted an average of 0.25 ± 0.6 g per 24 hr, dogs fed 19% protein excreted 0.17 ± 0.0 g per 24 hr, and those fed 27% protein 0.02 ± 0.0 g per 24 hr. These values are within the normal range of values of 0.3 to 0.4 g per 24 hr for dogs of a variety of breeds housed under various conditions [21]. As was stated previously, there was no correlation of light and electron microscopic lesions with urinary protein values or other functional tests.

Much of the previous work that supports a deleterious role of dietary protein in progressive glomerular disease was done in albino rats. Several investigators established that high protein feeding accelerated the development of chronic nephrosis and proteinuria. Saxton and Kimball [7] found that rats fed 30 to 40% protein had over three times the incidence of chronic nephrosis as did rats fed 7 to 10% protein. They also found a higher incidence of chronic nephrosis in rats fed a 33% casein-lactalbumin protein diet than in rats fed a 41% dry liver protein diet, suggesting the type of protein may also play a role.

In other studies, renal mass was reduced or experimental disease was induced to compromise renal function, and then dietary manipulations were performed. Hostetter et al [22] studied renal function and the appearance of glomerular lesions in Munich-Wistar rats 7 days after 90% renal ablation. They found higher single nephron GFR in rats fed a normal protein diet (24% protein) compared to those fed a low-protein diet (6% protein) or to sham-operated controls. Minimal glomerular lesions were seen in those rats fed normal dietary protein. Glomerular lesions of these types in rats fed low dietary protein or sham-operated controls were much less severe. No sclerotic lesions were seen in any rats, possibly due to the short duration of the study. Glomerular lesions were reported by Shimamura and Morrison [11] in Wistar rats fed normal protein for 10–50 weeks after

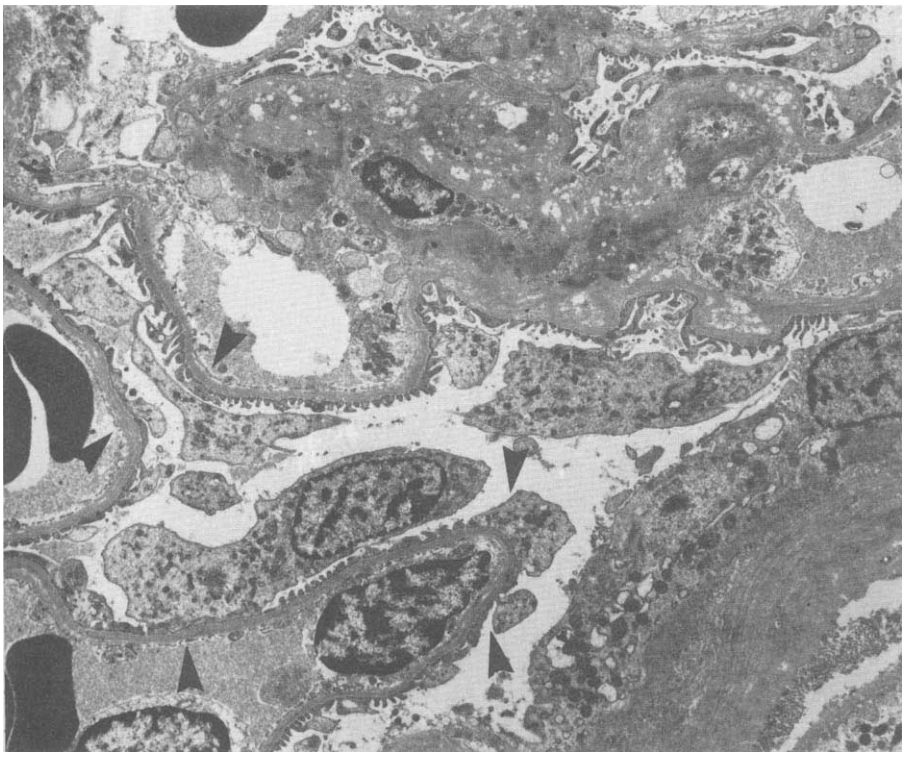

Fig. 5. *Focal foot process effacement, with dense microfilament arrays* (arrows), *was seen in all dogs on the study.* The mesangium of one capillary loop has a lytic and moth-eaten appearance. (Uranyl acetate-lead citrate stain, ×2000)

85% renal ablation. Glomerular hyalinization began by 25 weeks and was extensive by 50 weeks in remnant kidneys. Foot process effacement, mesangial proliferation, and progressive capillary occlusion were seen by electron microscopy.

Neugarten et al [23] were able to ameliorate glomerular damage during the course of nephrotoxic serum nephritis (NSN) in Sprague-Dawley rats by restriction of dietary protein. Mesangial widening, segmental proliferation, and glomerulosclerosis, accompanied by proteinuria and azotemia, were significantly more severe in rats with NSN fed 50% protein than in those fed either 20 or 4.0% protein.

There were significant differences in response to protein between the dogs in the present study and rats reported by previous investigators. We were not able to establish significant effect of high protein feeding on the incidence of glomerular lesions. We also were unable to establish a protective effect of reduced protein feeding on the incidence of glomerular pathology. Glomerular lesions such as protein resorption droplets, attenuation of visceral epithelial cell cytoplasm, and mesangial proliferation, shown in rats to be associated with ablation-induced hyperfiltration, were not seen in the present study. Such lesions were not significant in dogs fed 27 or 56% protein, even though elevated GFR, and presumably hyperfiltration, were present. Rats with reduced renal mass fed high dietary protein became proteinuric and azotemic [12], while dogs in the present study did not.

Both light and electron microscopic evaluation of glomerular pathology were used in the present study and there were significant differences between the results obtained with each technique. There was a trend toward increasing incidence and severity of glomerular lesions with increasing levels of dietary protein, as assessed by light microscopy. However, this trend was not seen with electron microscopy. Electron microscopic samples, taken at random, allow evaluation of fewer glomeruli but with greater precision for diagnosis of subtle lesions, which may be indicative of hyperfiltration/hyperfunction lesions. We believe the electron microscope sampling procedure may be less biased than light microscopy where the observation of prominently stained, sclerotic glomeruli may influence grading of lesions in all glomeruli.

These data using dogs with 75% nephrectomy fed varied protein diets indicate that dogs do not respond to high dietary protein the same as rats. There may be several reasons for these differences. Dogs and rats have a different spectrum of age-related glomerular diseases. Middle-aged and old albino rats of several different strains (Wistar, Sprague-Dawley) have a high incidence of spontaneous primary glomerulosclerosis [1–6], but dogs do not [24–28]. Dogs are much more commonly affected by membranous and membranoproliferative glomerulonephritis, amyloidosis, and tubulointerstitial disease when older [24–28]. Thus, the feeding of high levels of dietary proteins to rats may exacerbate the development of spontaneous glomerulosclerosis, a process which does not seem to occur frequently in dogs.

The influence of systemic hypertension on arterial sclerosis or glomerulosclerotic changes in dogs is unknown. The lack of hypertension in the present dog study appears to be a species difference compared to rats. Hypertension is not found in dogs with 75% nephrectomy unless salt loading is used [29]. One would not expect hypertension in the present study since all diets contained less than 1% sodium. It is interesting to note diet A contained the highest protein and highest sodium content, yet hypertension or deterioration of renal hemodynamics was not found. In the hypertensive rat or those with reduced renal mass, it has been shown that intrarenal hypertension is

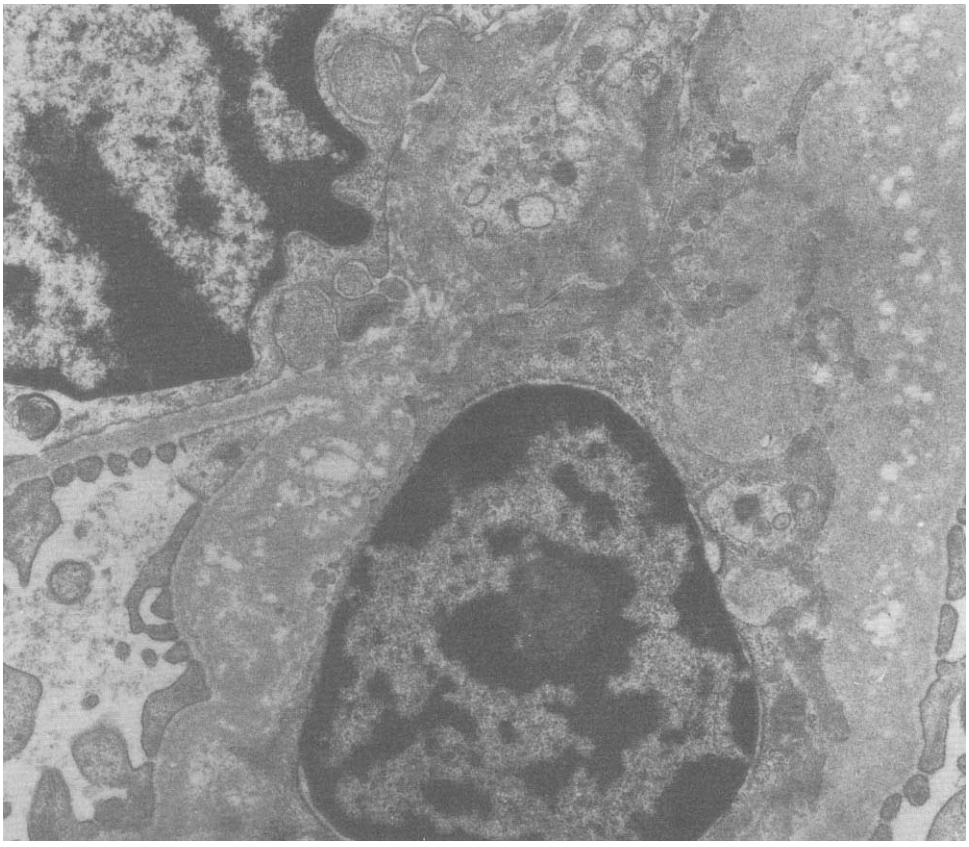

Fig. 6. *Lytic and frothy areas of mesangium were seen in glomeruli from most dogs.* Lesions of this type are relatively common in dogs of this age and apparently unrelated to dietary protein intake. (Uranyl acetate-lead citrate stain, ×910)

more critical than systemic blood pressure to cause glomerular morphologic changes [22, 30].

The dietary content of linoleic acid has been shown to influence the progression of glomerular sclerosis in rats with 75% nephrectomy [31]. The high dietary intake of linoleic acid, 27 vs. 2% of calories, in a normal diet prevented glomerular sclerosis, presumably by increased PGE_2 generation in the renal cortex which led to vasodilation or altered coagulation status. In the present study, the diets contained varied fat content, but, in all cases, a low linoleic acid content. Diet B, with the lowest fat content, had the highest linoleic acid, 0.58%. Therefore, this low linoleic acid content would be unlikely to have an influence on the progression of renal disease in these dogs.

It is interesting to note that while the two diets with higher protein content had significantly increased GRF and C_{PAH} compared to the reduced protein diet, there was no significant difference in renal perfusion between the two higher protein diets. In another study from our laboratory, it was reported that dogs with chronic renal failure fed diets containing 54 and 26% protein had higher GFR compared to those fed 18 and 8% protein [32]. Again, there was no significant difference between the two high-protein diets or between the two lower protein diets. This finding was valid even when diets were changed using a Latin Square design between dogs. Therefore, it appears that GRF and C_{PAH} are significantly increased when dietary protein exceeds 26%, but further increases in protein intake do not change these measurements.

While these results indicate that the dog is different from the rat in response to dietary protein, several important questions are raised that cannot be answered with this study. Progressive deterioration of function was not reached in these dogs. What portion of renal mass removal is critical in dogs? It may be that a greater portion of the renal mass must be removed in dogs, compared to rats, to trigger progressive deterioration, or a longer time may be required to trigger deterioration. Whole kidney functional loss may not have been apparent with the present measurements until more advanced damage has occurred. What is the nature of hyperfiltration in the remnant dog kidney? The present study could not assess the influence of dietary protein on the single nephron hyperfiltration response. It is possible that single nephron hyperperfusion resulting from high protein intake could mask the effects of glomerular damage. To investigate these possibilities, further studies to measure intrarenal hypertension and glomerular hyperfiltration are needed in the dog model.

Thus, dogs maintained for 4 yr on a high-protein diet after 75% nephrectomy show no deterioration of GFR or C_{PAH}. It was assumed that glomerular hyperfiltration and hyperperfusion occurred in these dogs when renal mass was reduced. These results of increased GFR agree with previous reports from dog studies [20]. These results suggest that the deleterious effects of high protein feeding seen in rats do not occur in dogs with comparable reduction in renal mass. In the present studies, GFR was reduced to approximately 50% of normal after 75% nephrectomy. Rats subjected to uninephrectomy have marked

renal lesions after eating a high-protein diet [12]. Therefore, the dog appears to react differently than rats in regard to dietary protein when the GFR of dogs is even lower than that of rats. The response to dietary protein in dogs with further reduction in GFR remains uncertain. The present study emphasizes the species difference in regard to protein effect on renal function and morphology. Comparative usefulness of the rat or dog as a model for dietary protein effects in humans is open to question.

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