

Effect of dietary protein on functional, morphologic, and histologic changes of the kidney during compensatory renal growth in dogs

J. V. White, DVM; D. R. Finco, DVM, PhD; W. A. Crowell, DVM; S. A. Brown, DVM, PhD; D. A. Hirakawa, PhD

SUMMARY

Two diets similar in caloric density and mineral content, but markedly different in protein content, were used to study the effects of dietary protein on renal function and morphologic and histopathologic changes in dogs that had functional renal tissue reduced by seven-eighths nephrectomy. The effects of moderate protein intake (MPrI = 15% protein; dry-matter basis) and high-protein intake (HPrI = 31% protein; dry-matter basis) were studied for the initial 7 months (period 1 [P1]) after renal mass reduction. Diets were then switched between groups during the following 7 months (period 2 [P2]) to evaluate the effects of increased or decreased protein intake.

The HPrI caused significantly ($P < 0.05$) greater glomerular filtration rate (GFR) and renal growth than did MPrI during P1. Dogs that maintained HPrI during P1 and MPrI during P2 (group 1) had significant ($P < 0.05$) reduction in GFR during P2. Dogs that maintained MPrI during P1 and HPrI during P2 (group 2) had significant ($P < 0.05$) improvement in GFR and renal growth during P2. At the end of the study, renal reserve was evaluated in both groups of dogs before and after group 1 was returned to HPrI for 2 weeks. During this 2-week feeding trial, group-1 dogs had marked improvement in renal reserve, relative to group 2, and GFR increased to the terminal P1 values. Results indicate a possible residual benefit from HPrI during the early phase of compensatory renal growth in the form of functional compensatory memory to HPrI.

The severity of renal lesions was indistinguishable between dogs of dietary groups during both study phases. Plasma electrolyte concentrations rapidly returned to normal range after renal ablation, but mild azotemia and proteinuria persisted throughout most of the study. High protein intake was not associated with increased degree or progression of proteinuria.

Naturally acquired renal disease frequently is characterized by reduction in the number of viable nephrons and decrease in glomerular filtration rate (GFR). Severely damaged nephrons atrophy and cease to function, while remaining viable nephrons enlarge by cellular hypertro-

phy and, to a lesser extent, cellular hyperplasia. This compensatory renal growth (CRG) permits partial recovery of lost renal mass and GFR without generation of new nephrons. As a result, GFR and tubular solute transport capacity improve during CRG.¹ Usually, the greater the growth response, the greater the improvement in renal function.²

In mammalian species that have been studied, high-protein intake increases the magnitude of CRG and GFR, whereas protein restriction has the opposite effect.¹⁻⁴ It would seem advantageous, therefore, to increase dietary protein intake during convalescence from acute renal disease. However, this therapeutic approach has not gained acceptance in veterinary medicine, because reduced protein intake decreases accumulation of nitrogenous waste products in uremic dogs and may have protective effect against progression of renal failure in rats and human beings.⁵ However, to the authors knowledge, association between high-protein intake and progression of renal failure has not been clearly documented in dogs. Four studies, using high-protein diets in dogs with reduced renal mass, have failed to detect significant correlation between high-protein intake and decrease in GFR with time.^{6,7-9} In 3 of these studies,^{6,7,9} protein intake was restricted in all dogs until after the initial phase of compensatory renal growth had been completed and renal function had stabilized. Therefore, the experimental model used was more analogous to chronic, rather than to acute renal failure in dogs.

Considering the negative findings of those studies and the known stimulatory effect of protein on renal function and compensatory growth, we hypothesized that recovery of renal function might be less than maximal when protein intake is limited in dogs recuperating from acute renal insult. If so, high-protein intake might be beneficial for dogs with acute renal failure, provided the increased protein intake does not appreciably increase the risk of severe uremia or development of renal lesions. To test our hypothesis, we evaluated biochemical, morphologic, histologic, and functional changes in remnant kidneys of dogs consuming either high- or moderate-protein diet. Considering the possibility of a temporal effect of dietary protein, we used a dietary cross-over experimental design and recorded response to 2 levels of protein intake immediately after renal mass reduction (first 7 months—period 1 [P1]), and response to altered (increased or decreased) protein intake during the second 7 months of study (period 2 [P2]).

Mechanisms responsible for maintaining, decreasing, or increasing renal mass and function are poorly understood. Some factors, such as reduction of renal mass or feeding of a high-protein diet, are known to stimulate CRG

Received for publication Jan 22, 1990.
From the Departments of Physiology and Pharmacology (White, Finco, Brown), Pathology (Crowell), College of Veterinary Medicine, University of Georgia, Athens, GA 30602, and The Research and Development Division (Hirakawa), The IAMS Company, Lewisburg, OH 45338.

Supported by The IAMS Co, Lewisburg, Ohio, and the Veterinary Medical Experimental Station, University of Georgia, Athens.

Address reprint requests to D. Finco.

and increase single nephron GFR. Such CRG in rats may rapidly reverse once the stimulus is removed.¹⁰⁻¹² On the other hand, rats with 2 kidneys, then given an additional kidney by transplantation, apparently do not have reduction of their original kidney mass,¹¹ even as GFR returns to the 2-kidney level.¹³ Because these observations indicate that controls on normal growth and CRG may differ, adjustment of renal function associated with the 2 forms of growth may also differ. To our knowledge, the matter of regression of CRG and functional change has not been studied well in any species. In rats, improvement of GFR gained during CRG is lost with removal of the stimulus, yet it is not known whether a residual functional benefit is retained by the nephrons conditioned by the stimulus. We considered the possibility that maximal stimulation of CRG with dietary protein during the acute growth response to mass reduction (first 7 months) could result in morphologic or functional benefit later, regardless of dietary protein intake at that later time. To investigate this possibility, renal reserve was evaluated terminally in dogs that had initially maintained high-protein intake (HPrI) and in dogs that initially had maintained moderate-protein intake (MPrI).

Materials and Methods

Dogs—Young adult mixed-breed dogs were obtained from the University of Georgia, College of Veterinary Medicine Laboratory Animal Resource Service and were housed individually indoors. All dogs had been vaccinated, treated for ectoparasites and endoparasites, and were free of blood microfilariae. During a 30-day quarantine, dogs were fed a commercial dry dog food^a (22% protein on a dry-matter basis), given water ad libitum, and were conditioned to frequent handling and periodic restraint in a Pavlov sling. At the end of quarantine, 24 dogs were selected for study on the basis of normal physical findings, normal plasma electrolyte, urea nitrogen, protein, and glucose values, normal CBC and urinalysis results, and culture-negative urine status.

Diets—Two diets^b with markedly different protein content were prepared for use in this study (Table 1). Nutrients, other than protein, were closely balanced between

^a Eucanuba, The IAMS CO, Lewisberg, Ohio.

^b Research diets, The IAMS Co, Lewisberg, Ohio.

Table 1—Composition of moderate- and high-protein diets fed during study periods 1 and 2

Component	Moderate-protein	High-protein
Moisture	7.6	7.6
Protein	15.2	31.7
Fat	16.1	17.3
Crude fiber	4.4	3.9
Ash	7.9	8.1
Sodium	0.51	0.45
Potassium	0.82	0.99
Chloride	1.05	0.93
Calcium	1.36	1.40
Phosphorus	0.91	1.01
Magnesium	0.09	0.09
Caloric density	4.63	4.78

Moisture is expressed as percentage of diet as fed. Caloric density is expressed as kcal/g of diet. All other dietary components are expressed as percentage of dry matter.

diets because nonprotein components of diets may influence renal responses.^{4,14-18} Each diet was formulated with the same animal and cereal proteins, but additional casein was added to the high-protein diet to attain a higher final protein content. Each dog was fed once daily to provide 132 kcal/(kg of body weight)^{0.75/d}.¹⁹ Water was given ad libitum. Individual food intake and body weight were measured daily and monthly, respectively.

Renal mass reduction—Renal failure was induced by use of a 1-stage nephrectomy-infarction procedure. Via right flank incision, the right kidney was removed from dogs under halothane anesthesia. The kidney was weighed and sagittally sectioned to obtain control tissue from each dog studied. One section was quick-frozen in liquid nitrogen and stored at -70°C for subsequent chemical analyses. The other section was immediately placed in buffered 10% formalin for subsequent processing and evaluation by light microscopy. The left kidney was exteriorized via a left flank incision, and approximately 75% of the organ mass was infarcted by ligation of selected interlobar renal arteries.

Experimental design—Glomerular filtration rate was determined for each dog on the third and seventh days after surgery, as subsequently described. The mean of the 2 determinations was considered the baseline value. Twenty-two nephrectomized dogs fed a commercial diet,^a were selected and allotted to 2 groups that were balanced for mean baseline GFR (0.79 ml/min/kg/group), body weight (approx 12 kg/dog), and gender (4 males and 7 females/group). Group-1 (GP1) dogs maintained HPrI and group-2 (GP2) dogs maintained MPrI during P1 of the study, beginning 8 days after surgery. Diets were then switched between groups at the beginning of P2, so that dogs of GP1 maintained MPrI and dogs of GP2 maintained HPrI. At the end of P1, 4 dogs were randomly selected from each group and were euthanatized by barbiturate overdose. Renal tissue from these dogs was immediately processed as described for control tissue. Renal tissue was also collected and prepared in the same manner from all dogs that survived P2.

Dogs were monitored daily for changes in physical status, activity, appetite, and fecal character. Dogs that became dehydrated were administered replacement fluids, SC or IV. Other supportive treatment was not administered. Dogs that became severely uremic were euthanatized by barbiturate overdose.

MEASUREMENTS

Determination of GFR—After initiation of experimental diets, GFR measurement was made monthly. Food was withheld from dogs for 18 hours, but water was available ad libitum. The GFR measurement was performed with each dog resting comfortably in a Pavlov sling. At time zero, water was given by gavage at 3% (v/w) of body weight and [¹⁴C]inulin was administered via the saphenous vein at priming dosage of 0.12 $\mu\text{Ci/kg}$. Thereafter, [¹⁴C]inulin was infused with saline solution (2 ml/min) to deliver approximately 0.002 $\mu\text{Ci/kg/min}$. An indwelling urethral catheter was passed into the urinary bladder to facilitate urine collection. At 40 minutes, the bladder was emptied

^c Inulin [¹⁴C]carboxylic acid, Amersham Corp, Arlington Heights, Ill.

Values from grading of lesions by both reviewers were averaged for each dog.

Analysis of data—All GFR measurements were standardized by dividing measured values by initial body weight; reported values are mean \pm SEM. Statistical analyses were done, using a commercial statistics software package.^o The effect of treatment on repeated measurements was assessed by two-way factorial analysis of variance. Measurements of growth were compared either between or within groups by one-way analysis of variance. Arc transformation of percentage values was used for percentage increase-over-control data and a paired Student *t* test was used where appropriate. Differences indicated by analysis of variance were evaluated for significance by use of the Tukey multiple-range test. Qualitative data were analyzed, using the nonparametric Mann-Whitney 2-sample comparison test. A *P* value < 0.05 was considered significant.

Results

Diets used in this study were highly palatable. Intermittent periods of anorexia were occasionally observed in dogs of both groups; however, caloric intake and body weight did not vary significantly between or within groups throughout the study. Daily protein intake per kilogram was approximately 2.4 and 5.0 g for dogs maintaining MPrI and HPrI, respectively.

Two dogs from each group were euthanatized during P1 because of severe uremia. In each case, clinical and necropsy findings were consistent with decreased renal function (GFR < 0.3 ml/min/kg) without other nonrelated systemic diseases. Before euthanasia, these 4 dogs required administration of replacement fluids; all other dogs remained clinically stable. The study was completed with 9 dogs in each group during P1 and 5 dogs in each group during P2.

Functional effects of diet—Glomerular filtration rate increased in dogs of both groups during P1, but to differing degrees (Fig 1). The effect of protein intake on GFR

^o Statgraphics 2.6, STSC Inc, Rockville, Md.

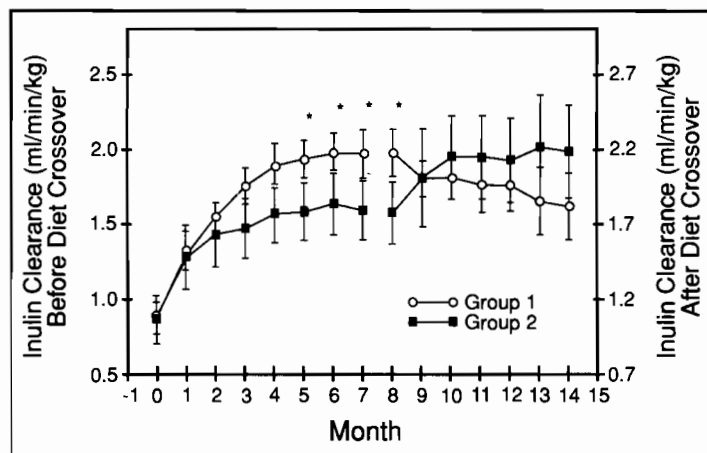


Figure 1—Glomerular filtration rate in group-1 and group-2 dogs during the first and second 7 months of the study. Values are reported as mean \pm SEM; n = 9/group during 0 to 7 months (P1) and 5/group during 8 to 14 months (P2). Significant difference (*P* < 0.05): * Difference between groups.

became evident during the fifth month of study when significantly higher renal clearance of inulin developed and persisted in GP1 dogs maintaining HPrI. Mean GFR values (ml/min/kg) for GP1 and GP2 dogs at the end of P1 were 1.96 and 1.59, respectively. During P2, significant difference in GFR between groups was rapidly lost (1 month). Subsequently, clearance values decreased in dogs switched from HPrI to MPrI (GP1) and increased in dogs switched from MPrI to HPrI (GP2). High-protein intake was associated with comparable GFR values at 7 months in GP1 dogs and at 14 months in GP2 dogs. Additionally, MPrI was associated with comparable GFR values at 7 months in CP2 dogs and at 14 months in GP1 dogs.

The decrease of GFR in GP1 dogs maintaining MPrI indicated either a nonpathologic response to diet or renal damage attributable to HPrI. Were the latter possible, maximal GFR in response to an oral protein load (filtration capacity) would be expected to be less in GP1 dogs than in GP2 dogs.²² To assess these possibilities, 4 dogs with GFR values closest to their respective group mean GFR value were selected from GP1 and from GP2 at the end of P2 for determination of renal reserve. The GFR value determined after food was withheld for 18 hours was approximately 14% lower in GP1 dogs, relative to GP2 dogs, but the percentage increase in GFR in response to a 10-g/kg protein load was similar: 37.0 \pm 3.47 for GP1 and 36.3 \pm 2.56 for GP2 dogs (Table 2; day 0). To determine whether the chronic feeding of different protein diets had influenced these results, both groups maintained HPrI for 14 days and renal reserve measurements were repeated. The GFR value after withholding food (resting GFR) and peak GFR value (filtration capacity) of GP2 dogs remained unchanged. A marked, but not statistically significant increase in renal reserve was observed in GP1 dogs, relative to GP2 dogs (49.1% vs 35.9%, respectively). This response of GP1 dogs (after a return to HPrI for 2 weeks) was related to a 13% increase in the mean GFR value after food was withheld (1.89 vs 2.14 ml/min/kg) and a statistically significant (paired *t* test) 23% increase in mean filtration capacity (2.59 vs 3.19 ml/min/kg).

Laboratory results for blood and urine samples acquired at study initiation and at the end of P1 and P2 were compared (Table 3). The major abnormalities observed in baseline values (after 7/8 nephrectomy) were mild increases in plasma phosphate, creatinine, and urea nitrogen concentrations. Plasma phosphate concentration became normal in dogs of each group during P1. Mild azotemia persisted in each group of dogs throughout the study, but plasma creatinine concentration fluctuated significantly in association with the level of protein in-

Table 2—Glomerular filtration response to oral administration of 10 g of casein/kg of body weight

Variable	Group 1		Group 2	
	Day 0	Day 14	Day 0	Day 14
Resting GFR (ml/min/kg)	1.89 \pm 0.40	2.14 \pm 0.28	2.15 \pm 0.36	2.17 \pm 0.32
Filtration capacity (ml/min/kg)	2.59 \pm 0.05	3.19 \pm 0.33*	2.93 \pm 0.50	2.95 \pm 0.54
Renal reserve (GFR increase %)	37.0	49.1	36.3	35.9

* Significant (*P* < 0.05) increase in filtration capacity associated with a 14-day return to high-protein intake. Resting GFR = GFR attained after 18-hour withdrawal of food.

Resting GFR and filtration capacity values are reported as group mean \pm SEM, n = 4/group.

Table 3—Hematologic and urinalysis results in dogs of groups 1 and 2 at the beginning (baseline) and end of study periods 1 and 2

Variable	Baseline n = 9		Period 1 n = 9		Period 2 n = 5	
	Gp 1	Gp 2	Gp 1	Gp 2	Gp 1	Gp 2
Blood						
PCV (%)	47 ± 3	46 ± 2	43 ± 2	41 ± 2	39 ± 3	41 ± 3
TS (g/dl)	7.3 ± 0.1	7.5 ± 0.1	6.5 ± 0.1*	6.6 ± 0.2*	6.2 ± 0.3*	6.4 ± 0.2*
Plasma						
Cr (mg/dl)	3.6 ± 0.2	4.0 ± 0.5	1.7 ± 0.1*	2.1 ± 0.2*	1.8 ± 0.1*	1.6 ± 0.2*‡
BUN (mg/dl)	61 ± 6	66 ± 7	30 ± 3*†	22 ± 3*	15 ± 2*‡	20 ± 1*†
Na (mEq/L)	152 ± 1	151 ± 1	153 ± 3	151 ± 1	151 ± 1	150 ± 2
K (mEq/L)	3.9 ± 0.1	4.2 ± 0.1	3.9 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1
Ca (mg/dl)	10.5 ± 0.1	10.6 ± 0.1	9.7 ± 0.1*	10.0 ± 0.2*	10.0 ± 0.3*	10.4 ± 0.1
Mg (mg/dl)	2.0 ± 0.1	2.0 ± 0.1	1.5 ± 0.1*	1.2 ± 0.1*	1.8 ± 0.1	1.8 ± 0.1
Cl (mEq/L)	116 ± 0.7	116 ± 1.0	119 ± 1.0	118 ± 1.0	115 ± 1.0	113 ± 2.0
PO ₄ (mg/dl)	6.8 ± 0.4	7.0 ± 0.5	3.7 ± 0.2*	4.2 ± 0.2*	3.7 ± 0.6*	3.6 ± 0.4*
TCO ₂ (mmol/L)	21 ± 0.9	21 ± 0.9	21 ± 0.4	20 ± 0.4*†	22 ± 1.5	23 ± 0.6*
Urine						
pH	6.0 ± 0.1	6.0 ± 0.2	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.4	6.0 ± 0.2
Sp gr	1.014	1.013	1.023*	1.016	1.011	1.015
P:C	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.3	0.8 ± 0.2	0.41 ± 0.2	0.9 ± 0.4*

Significant difference ($P < 0.05$): * from baseline; † between groups of the same period; ‡ between periods within the same group.
 TS = total solids; Cr = creatinine; TCO₂ = total CO₂; P:C = protein-to-creatinine ratio.
 Urine specific gravity values are reported as group mean, all other values are reported as group mean ± SEM.

take. During P1, dogs maintaining HPrI (GP1) had significantly reduced mean plasma creatinine concentration, relative to dogs maintaining MPrI (GP2). When GP2 dogs were switched to HPrI during P2, plasma creatinine concentration decreased significantly. Urea nitrogen concentration was consistently higher in dogs maintaining HPrI, but remained close to normal. Proteinuria, as assessed by the urinary protein-to-creatinine ratio,²³ gradually increased in GP2 dogs with time. However, this ratio did not vary significantly between groups at any time of the study, and was not significantly different in GP2 dogs between P1 and P2.

Structural effects of diet—Measurements of compensatory renal growth in dogs of each group paralleled changes observed in filtration function (Table 4). Dogs maintaining HPrI (GP1) during P1 developed significantly greater increase in GFR, hypertrophy index, and increase in glo-

merular, proximal tubule, and thick ascending limb of Henle planar area, relative to GP2 dogs. During P2, significant difference between groups was not observed in these variables. However, values for these variables were significantly different in GP2 dogs during P2, relative to P1. Glomerular, proximal tubule, and thick ascending loop of Henle planar areas were significantly greater in remnant kidneys, relative to control tissue in both G1 and G2 throughout the study (Fig 2). Percentage increase in proximal tubule planar area was markedly different between groups during P1 and between phases in GP2 dogs. Dogs maintaining HPrI had a 105% increase in proximal tubule planar area relative to the 38% increase in dogs maintaining MPrI during P1. When protein intake was increased in GP2 dogs during P2, proximal tubule planar area increased by 124% over control (Fig 2).

Table 4—Comparison of functional, histologic, and morphometric results compiled during periods 1 and 2

Variable	Period 1		Period 2	
	Gp 1	Gp 2	Gp 1	Gp 2
Baseline GFR (ml/min/kg)	1.10 ± 0.27	0.94 ± 0.38	0.91 ± 0.17	0.82 ± 0.18
GFR increase (ml/min/kg)	1.07 ± 0.19*	0.40 ± 0.11	0.85 ± 0.33	1.12 ± 0.20†
GLM tuft score (qualitative)	216 ± 50	140 ± 62	195 ± 31	207 ± 37
Kidney weight (g/kg)	2.58 ± 0.22*	1.86 ± 0.14	2.67 ± 0.24	3.09 ± 0.28†
Hypertrophy index	1.07 ± 0.08*	0.80 ± 0.03	1.13 ± 0.06	1.20 ± 0.07†
GLM area increase (μm ²)	1,102 ± 351*	385 ± 99	921 ± 100	925 ± 157†
PT area increase (μm ²)	180 ± 22*	55 ± 6	22 ± 27	186 ± 30†
TAL area increase (μm ²)	107 ± 25*	36 ± 16	61 ± 23	73 ± 10†

Significant difference ($P < 0.05$): * between groups of the same period; † between periods within the same group.
 GFR = glomerular filtration rate; GLM = glomerular; PT = proximal tubule; TAL = thick ascending limb of Henle.

Hypertrophy index was calculated as the ratio of remnant kidney weight to control kidney weight. All values are reported as group mean ± SEM; n = 4/group during period 1, n = 5/group during period 2. Functional data represent only dogs from which morphologic assessments were made.

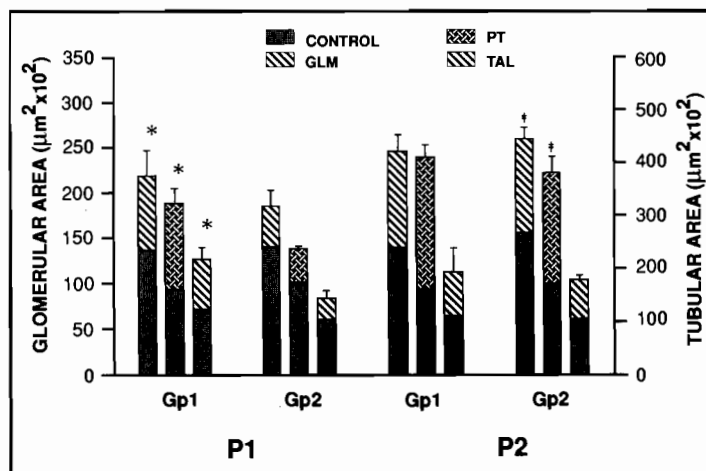


Figure 2—Increase in glomerular and tubule planar area during periods 1 and 2 of the study. Area measurements are reported as mean ± SEM; n = 4/group during period 1 and 5/group during period 2. In both groups and both periods, area increase over control values was significantly different. Significant difference ($P < 0.05$): * Difference in percentage increase over control between groups during period 1; † difference within group 2 between periods 1 and 2 (P1 and P2). GLM = glomerular area; PT = proximal tubule area; TAL = thick ascending limb of Henle area.

Renal growth, as assessed by protein-to-DNA ratio, was predominantly hypertrophic during P1. Protein-to-DNA ratio was comparable in control kidneys of GP1 (22.1 ± 1.0) and GP2 (22.8 ± 1.1) dogs. High-protein intake was associated with a 34% ratio increase (GP1, 29.6 ± 1.6) and MPrI was associated with an 18% ratio increase (GP2, 26.8 ± 1.3). Increase over control was significantly different in each group, but remnant kidney protein-to-DNA ratio was not significantly different between groups. Significant difference did not exist between groups in measurements of CRG during P2; kidneys obtained during P2 were not analyzed for protein-to-DNA ratio.

In control tissues, tubulointerstitial and glomerular structures were histologically normal. Mild increase in interstitial fibrosis, cellular infiltration, and tissue mineralization was observed in remnant kidneys. Mean group scores for these categories were consistently < 1.2 (qualitative grade) when tissue from dogs euthanized because of uremia were excluded. Tubulointerstitial changes in survivors were not significantly different between groups for either phase of the study or between phases within the same group. Group-1 and GP2 dogs that were euthanized during P1 had moderate to severe (qualitative score, 2 to 3) diffuse renal lesions. Pathologic change in glomeruli was evident after nephrectomy, but the severity of glomerular lesions was not significantly different between groups at either phase or between phases in either group. This lack of difference existed when tissues from dogs euthanized because of uremia were either included or excluded during group comparison. Increased mesangial cellularity and matrix deposition were the predominant glomerular pathologic changes observed. The development of capsular adhesions and periglomerular fibrosis was not common during P1, except in the 2 dogs from each group that were euthanized because of severe uremia. During P1, mild proximal tubule hyperplasia was evident in 4 of 6 GP1 dogs and in 2 of 6 GP2 dogs, and during P2, in 1 of 5 GP1 dogs and in 3 of 5 GP2 dogs. The amount of proximal tubule hyperplasia observed in GP1 dogs during P1 may have been responsible for the lack of group difference in protein-to-DNA ratio during this phase of the study.

Relationships between structural and functional change—Association between glomerular size and function was evaluated to determine whether increased glomerular mass was linearly related to increased GFR. Values in GP2 dogs during P2 were not included in these calculations because filtration rate decreased after cessation of HPrI. Correlation of Δ glomerular area (terminal mean area minus control mean area) to Δ GFR (terminal value minus baseline) was poor, and conversion of glomerular area to glomerular volume (volume = shape coefficient/size distribution coefficient \times (area)^{3/2})²⁴⁻²⁶ did not improve the correlation (data not shown). The potential negative effect of glomerular lesions on the correlation of size with function was considered. Summed values for increased mesangial cellularity and matrix deposition were determined for each dog (glomerular tuft score) and were compared with Δ GFR. Only these 2 glomerular lesions were used because it was assumed that their close proximity to the glomerular filtration barrier would have greater effect on filtration rate than would more distal lesions such as periglomerular fibrosis, thickening of Bowman's

capsule, and capsular adhesion. When 4 dogs with glomerular tuft score > 225 were removed from linear regression analysis of Δ glomerular volume with Δ GFR, marked improvement in correlation was noted ($r = 0.72$; $P < 0.05$). Glomerular tuft scores > 225 represent the upper 10% of glomerular tuft scores recorded, and this cut-off was chosen arbitrarily. The correlation of increased glomerular filtration was increased glomerular mass could be further improved ($r = 0.85$) by elimination of 7 dogs with score > 155 . These correlation improvements indicate that glomerular tuft lesions may have a substantial effect on the relationship between glomerular mass and filtration function.

In a recent study of dogs with chronic renal failure, development of glomerular lesions was associated with increased GFR.⁹ This finding was not confirmed in our study. When assessed in all dogs during P1 and in GP2 dogs during P2, poor correlation was attained between glomerular tuft lesions and Δ GFR or percentage increase in GFR. Use of total glomerular score gave nearly identical results (data not shown). In this study, development of glomerular pathologic changes was more closely associated with the initial (baseline) GFR ($r = -0.66$) and with change in glomerular planar area ($r = 0.78$). The latter was presumed to be attributable to contribution of mesangial cell proliferation and matrix deposition to increased glomerular volume.

Discussion

Compensatory renal growth is observed in rodents with intact kidneys during high-protein intake and after renal mass reduction when dietary protein intake is not altered.²⁷⁻²⁹ When both stimuli are combined, the positive effect of dietary protein on rat CRG is additive to that of mass reduction and is rapidly reversible when protein intake is reduced.^{4,12}

Several studies in dogs have characterized growth³⁰⁻³³ and functional^{30,31,34-38} changes associated with renal mass reduction under constant level of protein intake, and others have evaluated the acute functional effects of short-term increase in protein intake.³⁹⁻⁴¹ To our knowledge, no prior study of dogs has characterized additive effects of dietary protein and renal mass reduction on kidney growth, or simultaneously studied nephron growth and histologic change in response to dietary protein intake.

Functional effects of diet—During the first 7 months (P1) of the study, dogs maintaining MPrI increased their GFR by 91% above baseline after being seven eighths nephrectomized and recovered 80% of their contralateral kidney weight. This increase in GFR was greater than the 55% increase observed in 2 of 3 nephrectomized dogs at 8 weeks,³¹ probably because of the longer duration of our study and the greater reduction in renal mass. The additive effect of protein on CRG was evident in GP1 (HPrI) dogs that had 144% increase in GFR (53% more than GP2 dogs) and 107% recovery of contralateral renal weight (27% more than GP2 dogs) during P1. Additionally, dogs maintaining HPrI attained comparable percentage increase in GFR, regardless of whether HPrI was given during the first 7 months (GP1, 144%) or last 7 months of study (GP2, 147% increase from initial baseline). This result, and lack of difference in morphologic measure-

ments between GP1 dogs during P1 and GP2 dogs during P2, indicate that GP2 dogs of this study retained the ability to respond maximally to increased protein intake, even after GFR had stabilized at a lower protein intake. In a response analogous to that of rats,¹² GFR of GP1 dogs decreased rapidly after reduction of protein intake (P2). Inulin clearance for GP1 dogs at the end of P2 was only slightly greater than that for GP2 dogs at the end of P1 (1.85 vs 1.60 ml/min/kg), indicating that high-protein intake must be maintained to retain this nutrient's stimulation of resting GFR in dogs.

Peak GFR attained after acute protein challenge is considered a measure of the maximal filtration capacity of the glomerular population.⁴² The measured difference between resting GFR (GFR attained after 18 to 24 hours without food) and maximal filtration capacity has been used to represent the filtration reserve of glomeruli, which is frequently referred to as renal reserve.⁴² The clinical relevance of renal reserve in human patients and dogs remains to be clarified. For human patients with reduced renal mass, renal reserve has been proposed to be an indirect measure of glomerular hyperfiltration, with lower reserves representing a greater degree of glomerular hyperfiltration.²² It has been reported that low-protein diet may augment renal reserve (ie, decrease glomerular hyperfiltration) in human beings with renal injury,²² presumably by lowering resting GFR without simultaneously reducing filtration capacity. A similar response was not observed in the dogs of our study. The effect of protein intake was not selective, but rather caused parallel changes in resting GFR and filtration capacity, resulting in nearly identical renal reserve measurements for GP1 and GP2 dogs (Table 2; day 0). Because of a 14% difference between resting GFR values in GP1 and GP2 dogs, results indicate that renal reserve may not be a sensitive measure of canine glomerular hyperfiltration owing to the effect of dietary protein on filtration capacity in this species. The dog's ability to alter glomerular ultrafiltration coefficient (k_f) in response to protein intake may have been partially responsible for the changes in filtration capacity observed during renal reserve measurements.

During the 14 days of reexposure to HPrI, mean resting GFR in GP1 dogs increased from 1.89 to 2.14 ml/min/kg (13% increase) and mean GP1 filtration capacity increased from 2.59 to 3.19 ml/min/kg (23% increase). These results imply that GP1 dogs, which initially had HPrI after nephrectomy, had remarkable ability to increase their resting GFR in a short period and had disproportionately greater ability to increase filtration capacity when reexposed to HPrI. Although functionally dissimilar, this glomerular response of GP1 dogs resembles the transport memory response of hypertrophied proximal tubules observed by others.^{43,44} When evaluated by time, these same 4 GP1 dogs required 3 months of HPrI to increase their resting GFR from 1.9 to 2.15 ml/min/kg during P1, compared with only 14 days at the end of P2. This response implies a potential benefit of HPrI during the acute phase of CRG, in that rapidly growing nephrons may have been conditioned by this diet during P1 and, thus, were able to respond more rapidly to the second exposure to a high-protein intake. The potential importance of this memory response to our understanding of dietary protein effects on renal physiologic mechanisms warrants confirmation of these results with additional studies.

Structural effects of diet—In this study, a remnant kidney model was chosen to allow well controlled, abrupt surgical reduction in kidney mass. The nephrons that remained were presumed to be undamaged and, therefore, were considered to be capable of maximal hypertrophic response. Regardless of diet or period of study, planar areas of remnant glomeruli and tubules were significantly greater than those of control kidneys, indicating growth in response to renal mass reduction. Similar to that in rats,^{4,12} an additive effect of high-protein intake on CRG was observed in dogs maintaining HPrI during P1. This growth was characterized by consistent greater percentage increase in proximal tubule planar area than either glomerular or thick ascending limb of Henle planar area increase. This finding supports the contention that the proximal tubule is the site of greatest mass increase during CRG.¹ However, because tubule length was not measured in this study, it could not be documented.

Thick ascending limb of Henle hypertrophy in intact rat kidneys has been observed in response to chronic water deprivation, antidiuretic hormone supplementation, and high dietary protein intake.⁴⁵ In the current study, a 40% increase in planar area of the thick ascending limb of Henle was recorded in dogs maintaining MPrI during P1, indicating that this tubular segment hypertrophies in response to renal mass reduction when protein intake is moderate. The almost twofold greater percentage increase in thick ascending limb of Henle planar area of dogs maintaining HPrI (76% relative to 40%) indicates that high-dietary protein has an additive effect on hypertrophy of this tubule segment. Further work will be required to determine whether the increased urine concentrating ability of dogs consuming high-protein^{46,47} diet is mediated through this hypertrophic response within the thick ascending limb of Henle.

Little difference existed between measurements of hypertrophy (Fig 2) in GP1 and GP2 dogs at the end of P2; yet, GFR was observed to decrease rapidly in GP1 dogs during P2 (Fig 1). Although it is possible that reduced protein intake caused reduction in filtration without affecting renal morphologic features, we believe this disparity existed because P2 remnant kidney tissue from GP1 dogs was not acquired until after these dogs had been reexposed to 14 days of HPrI for renal reserve measurements. Because resting GFR in GP1 dogs increased during this 14-day feeding trial, it is logical to assume that reintroduction to HPrI stimulated new hypertrophic growth in the remnant kidney of these dogs. Conservative interpretation of these data, however, does not permit a definitive statement regarding the effect of reduced protein intake on CRG in GP1 dogs. For the same reason, the lack of difference in renal lesions between groups during P2 will not be addressed.

Relationships between structural and functional change—Differences in renal physiologic function between rats and dogs make direct extrapolation of results from one species to another tenuous. Glomerular filtration pressure disequilibrium is a normal finding in dogs, whereas in some breeds of rats, filtration pressure disequilibrium develops only during periods of severe protein restriction.^{48,49} Micropuncture studies indicate that the increase in single nephron GFR of uninephrectomized rats⁵⁰ develops secondary to increased glomerular blood flow and

transcapillary hydraulic pressure with little increase in the K_f , whereas hyperfiltration by remnant canine glomeruli is associated with significant increase in K_f , as well as glomerular flow and transcapillary hydraulic pressure.⁵¹ These physiologic differences indicate that the effect of dietary protein on glomerular function and damage may be different for these 2 species. The dog's ability to increase K_f during hyperfiltration may partially explain why an association between progressive glomerulosclerosis and hyperfiltration is more evident in rats⁵²⁻⁵⁶ than in dogs.⁶⁻⁸

In both groups of the current study, seven-eighths nephrectomized dogs had development of increased glomerular cellularity, matrix deposition, capsular adhesions, and periglomerular fibrosis during P1 (control vs treatment tissue; $P < 0.05$). An important contribution to these pathologic features by high-protein diet was not documented (GP1 vs GP2; $P > 0.05$). However, HPrI was associated with 54% increase in GP1 glomerular tuft score, relative to GP2 during P1 (Table 3). A substantial portion of the difference in mean scores (42%) was attributable to increased mesangial cellularity in GP1 glomeruli. Because glomerular cell hyperplasia develops during CRG as a normal phenomenon, it is unclear whether the increased glomerular cellularity or renal tissue from GP1 dogs during P1 was attributable solely to injury-mediated mesangial proliferation or was partially a consequence of protein-stimulated CRG. If attributable to glomerular injury, the difference in glomerular tuft score between GP1 and GP2 dogs should be viewed with concern and the assumption made that reevaluation with larger study groups might document a significant effect of dietary protein on canine glomerular lesions.

In this study, the severity of glomerular lesions was poorly correlated with either absolute or percentage increase in GFR. This finding gains importance when coupled with the observed significant stimulation of resting GFR by HPrI. Considered together, these results support the conclusion that either the functional effect of HPrI on GFR has little impact on development of glomerular lesions in dogs or that change in GFR over time is a poor indication of the degree of glomerular damage within this species. The cause for the correlation ($r = -0.66$) between low initial GFR and increased development of glomerular lesions in the dogs of this study remains unclear. Some physiologic aberration within the remnant nephrons of severely affected glomeruli may have been responsible for the observed increase in lesions, however, the data obtained do not incriminate increased GFR as the cause. On the basis of these results, it seems inappropriate to view canine glomerular damage as a linear cause-effect relationship between increased glomerular filtration and formation of glomerular lesions as has been suggested for rats.^{50,51}

The functional benefits of compensatory renal growth aid in restabilization of renal homeostasis after loss of kidney mass. Within the confines of this study, we were unable to identify a significant adverse response to dietary protein in remnant kidneys of seven-eighths nephrectomized dogs. Rather, we conclude that HPrI relative to MPrI generated a significant, positive effect on canine CRG and function, without causing additional renal lesions greater than those that developed secondary to the effect of renal mass reduction. In dogs, maintenance of

enhanced glomerular filtration requires sustained high-protein intake. However, if protein intake is limited to a moderate level for several months after renal damage, maximal GFR can be attained by return to continuous high-protein intake. Conversely, if the canine kidney is preconditioned to high-protein intake after acute insult and before protein restriction is instituted, the time required for return to maximal filtration function is markedly reduced when a high-protein diet is restarted. Although results of this study document the benefits of dietary protein on renal growth and function after acute insult, they should not be misconstrued to imply that dietary protein could not have an adverse effect in long-term chronic renal failure in dogs. Additional long-term studies will be required to resolve this issue.

References

1. Fine LG. The biology of renal hypertrophy. *Kidney Int* 1986;29:619-634.
2. Malt RA. Compensatory growth of the kidney. *N Engl J Med* 1968;280:1446-1459.
3. Halliburton IW. The effect of unilateral nephrectomy and of diet on the composition of the kidney. In: Nowinski WW, Goss RJ, eds. *Compensatory renal hypertrophy* New York: Academic Press Inc, 1969;102-128.
4. Fine LG. The role of nutrition in hypertrophy of renal tissue. *Kidney Int* 1987;32:S2-S8.
5. Zeller KR. Effects of dietary protein and phosphorous restriction on the progression of chronic renal failure. *Am J Med Sci* 1987;294:328-340.
6. Finco DR, Crowell WA, Barsanti JA. Effects of three diets on dogs with induced chronic renal failure. *Am J Vet Res* 1985;46:646-653.
7. Bovee KC, Kronfield DS, Ramberg C, et al. Long-term measurement of renal function on partially nephrectomized dogs fed 56, 27, or 19% protein diets. *Invest Urol* 1979;16:378-384.
8. Polzin DJ, Osborne CA, Stevens JB, et al. Influence of modified protein diets on the nutritional status of dogs with induced chronic renal failure. *Am J Vet Res* 1983;44:1694-1702.
9. Polzin DJ, Lenninger JR, Osborne CA, et al. Development of renal lesions in dogs after 11/12 reduction of renal mass. *Lab Invest* 1988;58:172-183.
10. Dijkhuis CM, van Urk H, Malamud D, et al. Rapid reversal of compensatory renal hypertrophy after withdrawal of the stimulus. *Surgery* 1975;78:476-480.
11. Silber S, Malvin RL. Compensatory and obligatory renal growth in rats. *Am J Physiol* 1974;266:114-117.
12. Addis T, Lew W. Protein consumption and the restoration of lost organ tissue. *J Exp Med* 1940;71:563-568.
13. Gittes RF, Rist M, Treves S, et al. Autoregulation in rats with transplanted supernumerary kidneys. *Nature* 1980;284:618-620.
14. Heifets M, Morrissey JJ, Purkerson ML, et al. Effect of dietary lipids on renal function in rats with subtotal nephrectomy. *Kidney Int* 1987;32:335-341.
15. Scharschmidt LA, Gibbons NB, McGarry L, et al. Effects of dietary fish oil on renal insufficiency in rats with subtotal nephrectomy. *Kidney Int* 1987;32:700-709.
16. Klahr S. Role of dietary factors in the progression of chronic renal disease. *Kidney Int* 1983;24:579-587.
17. Ross LA, Finco DR, Crowell WA. Effect of dietary phosphorus restriction on the kidneys of cats with reduced renal mass. *Am J Vet Res* 1982;43:1023-1026.
18. Brown S, Finco D, Crowell WA, et al. Beneficial effect of moderate phosphate restriction in partially nephrectomized dogs on a low protein diet. *Kidney Int* 1987;31:380.
19. Kendall PT, Blaza SE, Smith PM. Comparative digestible energy requirements of adult Beagles and domestic cats for body weight maintenance. *J Nutr* 1983;113:1946-1955.
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-254.
21. La Barca C, Paigen K. A simple, rapid, and sensitive DNA assay procedure. *Anal Biochem* 1980;102:344-352.

22. Bosch JP, Lews S, Glabman S, et al. Renal hemodynamic changes in humans: response to protein loading in normal and diseased kidneys. *Am J Med* 1986;81:809-815.
23. White JV. Diagnostic approach to proteinuria. In: Kirk RW, ed. *Current veterinary therapy X*. Philadelphia: WB Saunders Co, 1989;1139-1142.
24. Weibel ER. *Stereological methods: practical methods for biological morphology*. London: Academic Press Inc, 1979;51-57.
25. Hirose K, Osterby R, Nozawa M, et al. Development of glomerular lesions in experimental long-term diabetes in the rat. *Kidney Int* 1982;21:689-695.
26. Dworkin LD, Hostetter TH, Rennke HG, et al. Hemodynamic basis for glomerular injury in rats with deoxycorticosterone-salt hypertension. *J Clin Invest* 1984;73:1448-1461.
27. Smith H. Trophic and other factors related to renal function. In: *The kidney: structure and function in health and disease*. New York: Oxford University Press, 1951;461-491.
28. Addis T, Lee DD, Lew W, et al. The protein content of the organs and tissues at different levels of protein consumption. *J Nutr* 1940;19:199-205.
29. Addis T, Lew W. The restoration of lost organ tissue. *J Exp Med* 1940;71:325-333.
30. Bradley SEE, Chien KH, Coelho JB, et al. Effects of uninephrectomy on glomerulotubular functional structural balance in the dog. *Kidney Int* 1974;5:122-130.
31. Maros TN, Maros TG, Scherfer S, et al. Some characteristics of the renal compensatory hypertrophy after mononephrectomy in dog. *Morphol Embryol* 1983;2:75-85.
32. Allen RB, Bollman JL, Mann FC. Effect of resection of large fractions of renal substance: an experimental study. *Arch Pathol* 1935;19:174-189.
33. Winternitz MC, Waters LL. The effect of hypophysectomy on compensatory renal hypertrophy in dogs. *Yale J Biol Med* 1940;12:705-709.
34. McNay JL, Mujazaki M. Regional increases in mass and flow during compensatory renal growth. *Am J Physiol* 1973;224:219-222.
35. Mitchell AD, Valk WL. Compensatory renal hypertrophy. *J Urol* 1962;88:11-18.
36. Rous SN, Wakim KG. Kidney function before, during, and after compensatory hypertrophy. *J Urol* 1967;98:30-35.
37. Bourgoignie JJ, Gavellas G, Martinez E, et al. Glomerular function and morphology after renal mass reduction in dogs. *J Lab Clin Med* 1987;109:380-388.
38. Carriere S, Gagnan-Brunette M. Compensatory renal hypertrophy in dogs: single nephron glomerular filtration rate. *Can J Physiol Pharmacol* 1977;55:105-110.
39. Allen FM, Cope OM. Influence of diet on blood pressure and kidney size in dogs. *J Urol* 1942;47:751-768.
40. Smith HW. Trophic and other factors related to renal function. In: *The kidney: Structure and function in health and disease*. New York: Oxford University Press, 1951;461-491.
41. Moustgaard J. Variation of the renal function in normal and unilaterally nephrectomized dogs. *Am J Vet Res* 1947;8:301-306.
42. Bosch JP, Saccaggi A, Lauer A, et al. Renal functional reserve in humans: effect of protein intake on glomerular filtration rate. *Am J Med* 1983;75:943-950.
43. Fine LG, Trizna W, Bourgoignie JJ, et al. Functional profile of the isolated uremic nephron: role of compensatory hypertrophy in the control of fluid reabsorption by the proximal straight tubule. *J Clin Invest* 1978;61:1508-1518.
44. Trizna W, Yanagawa N, Bar-Khayim Y, et al. Functional profile of the isolated uremic nephron: evidence of proximal tubular "memory" in experimental renal disease. *J Clin Invest* 1981;68:760-767.
45. Bouby N, Trinh-Trang-Tan MM, Laouari D, et al. Role of the urinary concentrating process in the renal effects of high protein intake. *Kidney Int* 1988;34:4-12.
46. Pitts RF. The effect of infusing glycine and varying dietary protein intake on renal hemodynamics in the dog. *Am J Physiol* 1944;142:355-365.
47. Schneidt-Nielson B, Robinson RR. Contribution of urea to urinary concentrating ability in the dog. *Am J Physiol* 1970;218:1363-1369.
48. Ott CE, Marchand GR, Diaz-Buxo JA, et al. Determinants of glomerular filtration rate in the dog. *Am J Physiol* 1976;231:235-239.
49. Ichikawa I, Purkerkson ML, Klahr S, et al. Mechanism of reduced glomerular filtration rate in chronic malnutrition. *J Clin Invest* 1980;65:982-988.
50. Deen WM, Maddox DA, Robertson CR, et al. Dynamics of glomerular ultrafiltration in the rat. VII. Response to reduce renal mass. *Am J Physiol* 1974;227:556-562.
51. Brown SA, Finco DR, Navar LG. Renal and glomerular hemodynamics in $\frac{3}{4}$ nephrectomized dogs. *Kidney Int* 1989;35:424.
52. Bates SB, Andrews PM. Effect of dietary protein on the normal rat kidney. *Kidney Int* 1986;29:297.
53. Hostetter TH, Meyer TW, Rennke HG, et al. Chronic effects of dietary protein in the rat with intact and reduced renal mass. *Kidney Int* 1986;30:509-517.
54. Hostetter TH, Olson JL, Rennke HG, et al. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am J Physiol* 1981;241:F85-F93.
55. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med* 1982;307:652-659.
56. Kenner CH, Evan AP, Blomgren P, et al. Effect of protein intake on renal function and structure in partially nephrectomized rats. *Kidney Int* 1985;27:739-750.