Dietary modulation of the progression of nephropathy in aging rats: an evaluation of the importance of protein[1-3]

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ABSTRACT Chronic nephropathy involving glomerular sclerosis markedly progresses in severity with age in male Fischer 344 rats fed ad libitum. Restricting food intake by 40% almost totally prevents progression of these lesions. Restricting food intake by 40% without restricting protein intake is also highly effective although somewhat less so than food restriction that includes protein restriction. These findings indicate that reducing the intake of protein is not the major reason for the retardation by food restriction of the age-associated progression of nephropathy in rats. *Am J Clin Nutr* 1989;49:1217-27.

KEY WORDS Nephropathy and diet, nephropathy and dietary protein, food restriction and nephropathy, renal failure and diet, aging and nephropathy

Introduction

Most strains of rats fed the usually used commercial chows or semisynthetic diets ad libitum exhibit an age-associated progressive chronic nephropathy (1). In our studies (2) with male Fischer 344 rats fed our standard semisynthetic diet a marked progression of chronic nephropathy was noted, with renal failure appearing to be an important contributor to the spontaneous death of most rats. The initial lesions in these Fischer rats were localized to the glomerular basement membrane and mesangial matrix but as the nephropathy progressed, tubulointerstitial injury also occurred. Restricting the food intake of these Fischer rats by 40% almost totally prevented the occurrence of severe renal lesions whereas restricting protein intake to the same extent without restricting caloric intake decreased the severity of the lesions but much less effectively.

An increase in glomerular sclerosis occurs with age in humans (3, 4) but its rate of progression is usually not rapid enough to result in renal failure unless the kidney has also suffered an acute insult (5). However, if an acquired, acute disease has caused an abrupt reduction in the number of functioning nephrons, progression of glomerular sclerosis will often result in patients suffering renal failure long after the acquired disease has ceased to be active. In their review Brenner et al (5) related these renal lesions in humans to those commonly seen in aging rats. Indeed, progression of glomerular sclerosis was explored experimentally by those investigators (6) utilizing the rat ablation model, in which 80-90% of the renal mass was removed or destroyed. They found that hypertrophy of the kidney remnant and alterations in its glomerular structure occurred within a few weeks and they attributed this to marked elevations in glomerular pressure and glomerular flow per unit of kidney mass. Marked increases were found in single-nephron flow rate, glomerular plasma flow, and glomerular capillary pressure in the rat ablation models fed commercial laboratory chow but not in those fed a diet with only a 6% protein content. The alterations in glomerular structure were also prevented by the low-protein diet. On the basis of these studies with the rat ablation model and other data in the literature, Brenner et al (5) concluded that the protein-rich diets consumed by people in the modern Western societies induce chronic glomerular hyperfiltration and hyperperfusion, resulting in age-associated functional and structural deterioration of the kidney.

Because our studies with aging male Fischer 344 rats (2) did not implicate protein as the major dietary factor involved in the progression of chronic nephropathy, we felt it important to undertake the research reported here, aimed at evaluating the importance of dietary protein in this disease process. We also sought to assess the influence of diets that differed in amounts of protein and vitamins on a spectrum of other pathologic processes and on longevity.

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[2] Supported in part by grant AG01188 from the National Institute on Aging of the National Institutes of Health.

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Received April 12, 1988.

Accepted for publication July 19, 1988.

TABLE 1
Composition of the diets*

Component	Diet A	Diet B	Diet D
		g/100 g diet	
Casein (vitamin free)	21	21	35
D,L-Methionine	0.15	0.15	0.25
Dextrin	43.65	42.19	28.09
Sucrose	15	15	15
Corn oil	10	10	10
Vitamin mix	2	3.33	3.33
Choline chloride	0.2	0.33	0.33
Mineral mix	5	5	5
Fiber†	3	3	3

* The physiologic caloric value of diets A, B, and D are 4.10, 4.04, and 4.05 kcal/g, respectively.

† Solka Floc®, James River Corp., Berlin, NH.

Materials and methods

Rat maintenance and dietary procedures

The male Fischer 344 rats for this study were purchased as weanlings (aged 26–30 d) from the Kingston, NY, plant of the Charles River Laboratories. The care and use of the rats were in accord with the guidelines of the University of Texas Health Science Center. To maintain the specific pathogen-free conditions, the rats were transferred on arrival into a barrier facility where they were housed singly in plastic cages with wire-mesh floors suspended on the Hazleton-Enviro Rack System® (Hazleton Systems, Inc, Aberdeen, MD). The basic operations of the barrier facilities were described previously (7). A cycle of 12-h light and 12-h dark was used.

Upon receipt sentinel rats were killed so that virus antibodies (Sendai, Reo-3, GP-VII, PVM, KRV, H-1, SDA, LCM, and Adeno) and *Mycoplasma* antibodies (in serum sent to Microbiological Associates, Rockville, MD) could be monitored. This monitoring of sentinel rats was repeated every 6 mo and rats were killed monthly for the purpose of *Mycoplasma* culture, *Mycoplasma* ELISA, RCV/SDA ELISA, and Sendai ELISA assessment by the Department of Laboratory Animal Resources, University of Texas Health Science Center, San Antonio. Except for the infrequent appearance of a weak positive reaction for RCV/SDA, all tests were negative.

Until 6 wk of age all rats were fed a semisynthetic diet (diet A) ad libitum (**Table 1**); this diet has been the standard diet of our laboratory for > 10 y. At 6 wk of age rats in group A continued to be fed diet A ad libitum for the rest of their lives; of these, 60 were designated for the longevity study. Group A rats served as the reference standard to which all other dietary groups were compared. These group A rats served as the reference for different kinds of dietary manipulations in two other studies (8, 9). In the present paper rats in group A are used as the reference standard for dietary groups B and D. Rats in group B were fed diet B (Table 1) at ~60% of the mean caloric intake of group A rats from 6 wk until 18 mo of age, with caloric intake thereafter being fixed at the level of intake at 18 mo of age. Sixty rats in group B were designated at 6 wk of age for the longevity study.

Group D rats (60 for the longevity study and 30 for the cross-sectional studies) were fed diet D (Table 1) at ~60% of the mean caloric intake of the group A rats from 6 wk until 18 mo of age, with caloric intake thereafter being fixed at the level of intake at 18 mo of age.

The amount of food ingested by each of the group A rats fed ad libitum was measured twice a week over a 3-d and then a 4-d period. The amount ingested per day was calculated. For rats fed the restricted amount of food (groups B and D), each rat was provided the daily food allotment ~1 h before the start of the dark phase of the 12-h light, 12-h dark cycle. To detect spillage of food readily, antibiotic-treated cage boards (Shepherd Specialty Papers Co, Kalamazoo, MI) were placed under the wire-mesh cage floor in place of usual bedding material. The body weight of each rat was measured at 2-wk intervals.

Procedures for pathologic examination of rats sacrificed for cross-sectional studies

Rats at 6, 12, 18, 24, 27, and 30 mo of age were weighed, anesthetized with ether, and killed by exsanguination. The rats were examined for gross pathological lesions. The brain, pituitary gland, heart, lungs, trachea, esophagus, stomach, small intestine, colon, liver, pancreas, spleen, kidneys, urinary bladder, testes, epididymis, prostate, seminal vesicle, thyroid gland, adrenal gland, psoas muscle, sternum, and ventral abdominal skin were excised. The heart, lungs, kidneys, testes, liver, and spleen were weighed and immediately fixed in 10% neutral buffered formalin. Other tissues and organs were not weighed but were fixed immediately. Any other tissue or organ in which lesions were observed by gross inspection was excised and fixed. The fixed tissues were embedded in paraffin, sectioned at 4 μm, occasionally at 2 μm, and stained with hematoxylin-eosin with additional staining with periodic acid–Schiff's reagent as required.

Procedures for pathologic examination of rats dying spontaneously

All rats in the barrier facilities were inspected at least twice daily (at the start and end of the light phase of the light-dark cycle). All rats that died spontaneously were removed from their cages and either immediately necropsied or refrigerated for a brief period before necropsy. Although autolysis occurred in some cases, it did not prevent grading of lesions.

The brain, pituitary gland, heart, lungs, trachea, aorta, esophagus, stomach, small intestine, colon, liver, pancreas, spleen, kidneys, urinary bladder, prostate, testes, epididymis, seminal vesicles, thyroid gland, adrenal glands, parathyroid glands, psoas and thigh muscles, sternum, femur, and vertebrae were excised and fixed in 10% formalin. The tissues were examined histologically by the methods just described. Any other organ or tissue with gross lesions also was examined histologically.

Grading lesions

The initial lesion of chronic nephropathy involves the glomerular capillary basement membrane and mesangial matrix. The lesion progresses to the tubules with thickening of the tubular basement membrane, obstruction of the tubule lumen with hyaline casts, and interstitial lymphocytic infiltration. Eventually there is fibrosis of the glomeruli, tubules, and interstitial tissue. The grading criteria for this chronic nephropathy were described previously (10). The following is a brief summary: grade 0, no lesions; grade 1, minimal-severity lesions primarily involving glomerular capillary basement membrane and mesangial matrix including an occasional hyaline cast; grade 2, lesions of mild severity involving glomerular basement membrane, mesangial matrix, invariably tubular protein-

aceous casts; grade 3, moderate-severity lesions involving the same structures as grade 2 plus the thickening of Bowman's capsule, lymphocyte infiltration, and mild interstitial fibrosis; grade 4, very severe lesions similar to those of grade 3 but more marked, plus segmental or diffuse sclerosis and frequent adhesion of glomerular tuft to Bowman's capsule; and grade E, end-stage lesions involving widespread glomerular sclerosis, diffuse fibrosis, frequent calcification, and marked tubular dilation with numerous proteinaceous casts. Photographs of kidney sections with each grade of lesion have been published (2). The functional meaning of these lesions was evaluated (2): rats with grades 0, 1, 2, and 3 lesions had similar serum creatinine and blood urea nitrogen (BUN) levels; rats with grade 4 lesions had small but significantly elevated levels of serum creatinine and BUN; and rats with grade E lesions had markedly elevated serum creatinine and BUN levels along with parathyroid hyperplasia, osteodystrophy, and metastatic calcification. Thus, rats with grade E lesions appeared to be in renal failure.

Cardiomyopathy was graded as follows: grade 0, no lesions; grade 1, occasional focal myocardial degeneration plus minimal fibrosis; grade 2, frequent myocardial degeneration with extensive fibrosis; and grade 3, widespread and confluent myocardial degeneration with massive fibrosis and occasional calcification. A photograph of a heart section with grade 3 cardiomyopathy has been published (2).

Hepatic bile duct hyperplasia was graded as follows: grade 0, no lesions; grade 1, increased number of bile ducts in a few portal areas (< 10%); grade 2, increased number of bile ducts in 10–30% of portal areas plus occasional fibrosis and lymphocytic infiltration; and grade 3, increased number of bile ducts in > 30% of portal areas plus extensive fibrosis. A photograph of a liver section with grade 3 bile duct hyperplasia has been published (2).

Hepatic fatty change was graded as follows: grade 0, no lesions; grade 1, a few small fat droplets in hepatocytes near portal area; grade 2, many moderate-sized fat droplets in hepatocytes near portal area and in midzonal region; and grade 3, many large fat droplets in hepatocytes throughout liver. A photograph of a liver section with grade 3 lesions has been published (2).

Lesions were routinely graded microscopically by one of the pathologists without reference to the rat's code number. A second pathologist reviewed the slides from randomly selected animals. In the case of the chronic nephropathy, a test for the reproducibility of the grading was made by submitting to both pathologists a collection of slides from 20 rats representing different grades of lesions. The slides were identified by a code unknown to the pathologists. They were examined and the slides were scored independently. The two pathologists gave the same grade to slides from 17 of the 20 rats; in the case of the other three rats there was a one-grade disagreement in the six-grade system.

Statistical analysis

The survival curves were estimated by product-limit estimates and were compared using a Wilcoxon test (11). The median and 10th-percentile survival times of the dietary groups were compared using the quantile test (12). Food intake, body weight, and organ weights were compared for dietary group and age differences by analyses of variance and linear contrasts (13).

When disease state was assessed by multiple ordered categories, the progression of disease animals that were killed was analyzed by ridit analysis (13), with the reference group, reported by Maeda et al (2), as the marginal distribution for rats fed diet A ad libitum. When the disease state was a binary variable, the disease progression was analyzed with a chi-square analysis for trends in proportions (14). For the animals that died spontaneously the total frequency of disease states was analyzed with a chi-square test (15). When the expected frequencies were too small for the chi-square test, the data were analyzed with Fisher's exact test (15) for two-by-two tables.

Results

Food intake

The intake of food of the rats in groups A, B, and D is presented in **Figure 1.** The food intake of the rats in group A did not change appreciably with age. The rats in groups B and D were provided food at ~60% of the mean intake of the rats in group A and both groups consumed almost all food provided.

Body and organ weight

The body weights of the rats in groups A, B, and D are recorded in **Figure 2.** The weights of all rat groups were similar at age 6 wk. The rats in group A gained weight until ~70 wk of age; their weight then leveled off until ~100 wk of age, after which it decreased. The rats in groups B and D gained weight until ~120 wk of age; their weight then leveled off until ~140 wk of age, after which it decreased. The rats in groups B and D had similar body weights through most of the life span and weighed less than the rats in group A at all ages in common after 2 mo of age.

The organ weights (heart, lungs, liver, spleen, and kidneys) of rats in groups A and B killed at 6, 12, 18, and 24 mo of age were similar to those reported for rats fed the same diets in an earlier study (7, 16). The organ weights of groups B and D were similar and less than those of group A (unpublished observations, 1988). When kidney weight was expressed as percent of body weight, similar values were observed for groups A and B at 6 and 12 mo of age and for groups A, B, and D at 18 and 24 mo of age (group D rats were not sacrificed at 6 and 12 mo of age).

Longevity

The survival curves for the rats in groups A, B, and D are shown in **Figure 3.** The curves for the rats in groups B and D are significantly different from that for the rats in group A ($p < 0.0001$). The survival curve for the rats in group B is not different from that of the rats in group D. The median length of life and the age of the 10th-percentile survivors are presented in **Table 2.**

Chronic nephropathy

Data on the severity of chronic nephropathy in rats killed at 6, 12, 18, 24, 27, or 30 mo of age are reported in **Table 3.** Rats in group A exhibited a marked progression in severity of these lesions with increasing age ($p < 0.0001$). Rats in group D also showed an increase in severity of these lesions with age ($p = 0.003$) but one that was less marked than that of rats in group A. Rats in

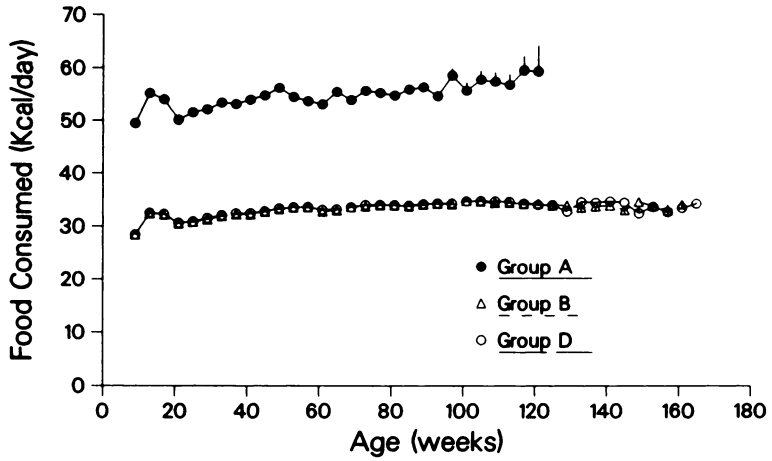

FIG 1. Food intake (kcal per rat per day) by rats in groups A, B, and D of the longevity study. The points are means (bars represent SEM); $n = 60$ for each group at the start of the study and decreased with age.

group B showed a marginal increase in the severity of these lesions with age ($p = 0.05$). At ≥ 12 mo of age the severity of chronic nephropathy was much greater for rats of group A than for rats of groups B and D ($p < 0.0001$). The severity of these lesions in rats of groups B and D were similar at 18, 24, and 27 mo of age but at 30 mo of age the rats of group D had more severe lesions than the rats of group B ($p = 0.014$).

Data on the severity of chronic nephropathy in rats that died spontaneously are reported in **Table 4.** The severity of these lesions in rats of group A was markedly greater than in rats of groups B and D ($p < 0.0001$) even though the rats of groups B and D died much older. The severity of these lesions in rats of group D was greater than in rats of group B ($p = 0.0001$).

Cardiomyopathy

The data on the severity of cardiomyopathy of rats killed at various ages are reported in **Table 5.** An increase in severity of these lesions with age occurred in all three groups (group A, $p < 0.0001$; group B, $p < 0.0001$; group D, $p = 0.013$).

Data on the severity of cardiomyopathy in rats that died spontaneously are reported in **Table 6.** The severity of these lesions in rats of group A was greater than in rats of groups B and D ($p < 0.0001$). At the time of spontaneous death the severity of cardiomyopathy in rats of group B was similar to that in rats of group D.

Gastrointestinal lesions

The data on gastrointestinal lesions in rats that died spontaneously are recorded in Table 6. Rats in groups B and D had a lower frequency of gastric hyperkeratosis ($p < 0.0001$) and of gastric ulcers ($p < 0.0005$) and a higher frequency of intestinal obstruction by hair balls ($p < 0.008$) than did rats in group A. Rats in group D had a lower frequency of esophageal hyperkeratosis than did those in group A ($p = 0.014$).

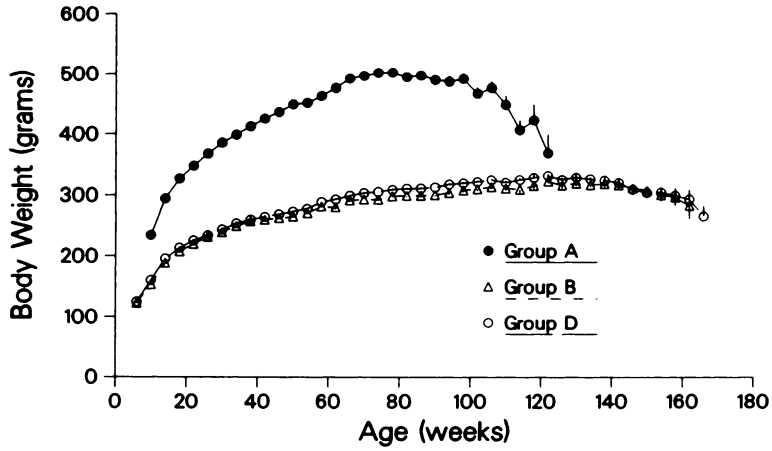

FIG 2. Body weight of rats in groups A, B, and D of the longevity study. The points are means (bars represent SEM); $n = 60$ for each group at the start of the study and decreased with age.

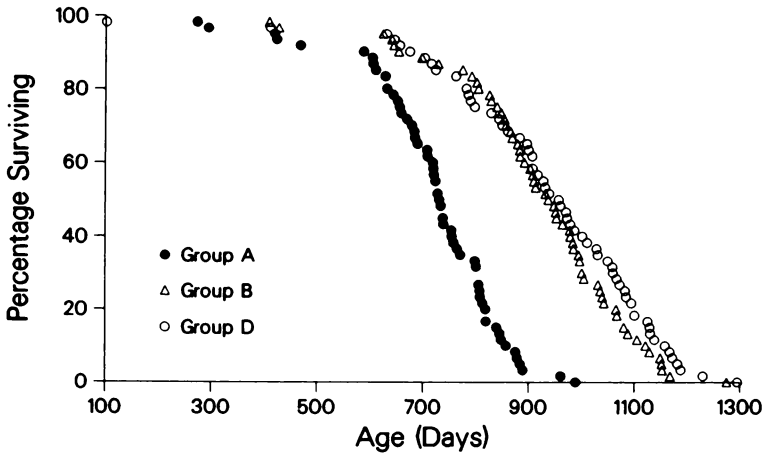

FIG 3. Survival curves of rats in groups A, B, and D. $n = 60$ at the start of the study.

Hepatic lesions

The data on hepatic lesions in rats that died spontaneously are presented in Table 6. Rats in group D had more severe bile duct hyperplasia than did rats in groups A and B ($p \leqq 0.03$). Rats in group A had more severe hepatic fatty change than did rats in groups B and D ($p \leqq 0.001$). The frequency of periductal fibrosis was lower in rats of group B than in rats of groups A and D ($p < 0.0001$).

Reproductive lesions

The data on reproductive lesions occurring in rats killed at various ages are reported in Table 5. The prevalence of testicular interstitial cell tumors increased with age in each rat group (group A, $p < 0.0001$; group B, $p = 0.028$; group D, $p = 0.006$). The magnitude of this age-associated increase was greater for rats of group A than for those of groups B and D ($p \leqq 0.05$). The prevalence of atrophy of seminiferous tubules also increased with age in all rat groups ($p \leqq 0.002$).

The data on reproductive lesions in rats that died spontaneously are presented in Table 6. Rats in group B had a lower prevalence of testicular interstitial cell tumors than did rats in groups A and D ($p \leqq 0.002$). The prevalence of calcium deposits in the testes of rats of group D was less than that of rats of group A ($p = 0.03$) and the prevalence of prostatitis was less in the rats of group B than in those of group A ($p = 0.02$).

TABLE 2
Summary of longevity findings*

Diet group	n	Median length of life	Age of 10th percentile	Maximum length of life
		d		
A	60	730 (708–764)	857 (819–961)	989
B	60	936 (883–984)†	1121 (1080–1168)‡	1275
D	60	956 (906–1028)§	1158 (1125–1230)‖¶	1295

* Numbers in parentheses are 95% confidence intervals.
† B different from A ($p \leq 0.0001$).
‡ B different from A ($p \leq 0.0006$).
§ D different from A ($p \leq 0.0001$).
‖ D different from A ($p \leq 0.0006$).
¶ D marginally different from B ($p = 0.056$).

Neoplastic disease

Data on neoplastic lesions other than testicular interstitial cell tumors in rats killed at various ages are reported in Table 5. The prevalence of neoplastic lesions increased with age in all groups (group A, $p < 0.0001$; group B, $p = 0.028$; group D, $p = 0.001$) but the increase in prevalence with age was greater for rats in group A than for those in groups B and D ($p \leqq 0.05$).

Data on neoplastic lesions other than testicular interstitial cell tumors in rats that died spontaneously are presented in Table 6. The total tumor prevalence was similar in all three groups but, of course, rats in groups B and D were older than those in group A. Rats in group B had a lower prevalence of benign tumors ($p \leqq 0.02$) than did rats in groups A and D and a lower prevalence of malignant epithelial tumors than rats in group A ($p = 0.037$). Rats in group B had a lower prevalence of pituitary adenoma than did rats in groups A and D ($p \leqq 0.02$). Rats in groups B and D had a higher prevalence of lymphoma and leukemia than did rats in group A ($p \leqq 0.002$) and rats of group D had a higher prevalence of adrenal cortical tumors than did rats of groups A and B ($p \leqq 0.032$).

Miscellaneous nonneoplastic lesions

Table 6 contains data on nonneoplastic lesions in rats that died spontaneously. Rats in group A had a much higher prevalence of osteodystrophy than did rats in groups B and D ($p < 0.0001$); 30% of the rats in group A had this lesion compared with < 2% of the rats in group D and 0% of the rats in group B. A much higher percentage of rats in group A had Ca deposits in the heart, kidney, and skeletal muscle than did rats in groups B and D ($p \leqq 0.004$). Rats in group D had a higher prevalence of Ca deposits in the heart and skeletal muscle than did rats

TABLE 3
Severity of chronic nephropathy in rats sacrificed at different ages: number of rats with lesions of various grades*

	Age														
	6 mo		12 mo		18 mo			24 mo			27 mo			30 mo	
Lesion grade	Group A (n = 18)	Group B (n = 12)	Group A (n = 10)	Group B† (n = 12)	Group A (n = 15)	Group B† (n = 16)	Group D‡ (n = 7)	Group A (n = 10)	Group B† (n = 10)	Group D‡ (n = 10)	Group A (n = 8)	Group B† (n = 4)	Group D‡ (n = 4)	Group B (n = 10)	Group D§ (n = 8)
0	3	6	0	6	0	0	0	0	0	0	0	0	0	0	0
1	15	6	2	6	0	16	7	0	10	9	0	3	2	7	2
2	0	0	6	0	2	0	0	1	0	1	0	1	1	3	4
3	0	0	2	0	10	0	0	5	0	0	2	0	1	0	2
4	0	0	0	0	1	0	0	4	0	0	4	0	0	0	0
E	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0

* Progression of lesions with age in all groups ($p \leq 0.05$).
† B different from A ($p \leq 0.05$).
‡ D different from A ($p \leq 0.05$).
§ D different from B ($p \leq 0.05$).

in group B ($p \leqq 0.03$). Rats in group A had a higher prevalence of pancreatic lobular atrophy than did rats in group B ($p = 0.03$) and a higher prevalence of thyroid C-cell hyperplasia than did the rats in groups B and D ($p \leqq 0.004$).

Discussion

The findings of this study show that reducing the intake of protein by male Fischer 344 rats is not the major reason for the retardation of age-associated nephropathy by food restriction. Restriction of food intake including protein (group B) almost totally prevented the progression of chronic nephropathy, which is in agreement with many earlier studies (17). However, the progression of this disease process was also markedly retarded by food restriction that did not include the restriction of protein intake (group D). At the time of spontaneous death, only 8% of the rats in group D had grade 4 or E lesions compared with 65% of the rats fed ad libitum (group A). This is particularly remarkable in light of the fact that the intake of dietary protein per unit body mass by the rats in group D was ~1.7 times greater than that of the rats in group A and that at the time of spontaneous death the rats in group D were much older than the rats in group A.

Clearly, restricting protein intake is not the major factor in the retardation of age-associated nephropathy by food restriction. Nevertheless, protein restriction does retard this disease process, as shown by the finding that group D rats killed at age 30 mo as well as those dying spontaneously had somewhat more severe renal lesions than did the group B rats. Also, a previous study (2) found that rats fed ad libitum on a reduced protein intake showed a small but significant reduction in the severity of renal lesions at the time of spontaneous death compared with rats on the higher protein intake of our standard semisynthetic diet.

The results of the present study focus attention on the need to determine which of the components of the diet

TABLE 4
Severity of chronic nephropathy in rats dying spontaneously: number of rats with lesions of various grades

	Age																	
	< 12 mo			12 through 17 mo			18 through 23 mo			24 through 30 mo			> 30 mo			Total		
Lesion grade	Group A (n = 4)	Group B (n = 1)	Group D (n = 1)	Group A (n = 9)	Group B (n = 3)	Group D (n = 2)	Group A (n = 60)	Group B (n = 16)	Group D (n = 7)	Group A (n = 36)	Group B (n = 32)	Group D (n = 18)	Group A (n = 2)	Group B (n = 37)	Group D (n = 35)	Group A (n = 111)	Group B* (n = 89)	Group D†‡ (n = 63)
0	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	3	1
1	3	0	0	3	2	2	0	14	7	0	31	11	0	23	6	6	70	26
2	1	0	0	6	0	0	6	1	0	1	1	4	0	9	17	14	11	21
3	0	0	0	0	0	0	15	0	0	4	0	2	0	5	8	19	5	10
4	0	0	0	0	0	0	14	0	0	11	0	0	1	0	1	26	0	1
E	0	0	0	0	0	0	25	0	0	20	0	1	1	0	3	46	0	4

* B different from A ($p \leq 0.05$).
† D different from A ($p \leq 0.05$).
‡ D different from B ($p \leq 0.05$).

TABLE 5
Number of killed rats with lesions other than chronic nephropathy

Type of lesion	Age														
	6 mo		12 mo		18 mo			24 mo			27 mo			30 mo	
	Group A (n = 18)	Group B (n = 12)	Group A (n = 10)	Group B (n = 12)	Group A (n = 15)	Group B (n = 16)	Group D (n = 7)	Group A (n = 10)	Group B (n = 10)	Group D (n = 10)	Group A (n = 8)	Group B (n = 4)	Group D (n = 4)	Group B (n = 10)	Group D (n = 8)
Cardiomyopathy*															
Grade 0 lesion	11	9	3	9	3	11	3	0	4	0	0	1	0	0	0
Grade 1 lesions	7	3	7	3	10	5	4	6	5	8	2	2	2	7	5
Grade 2 lesions	0	0	0	0	2	0	0	3	1	2	4	1	2	3	3
Grade 3 lesions	0	0	0	0†‡	0	0†‡	0	1	0†‡	0‡§	2	0†‡	0	0	0
Reproductive system															
Testicular interstitial cell hyperplasia	0	0	0	0	9	3	2	8	6	7	6	1	3	4	3
Testicular interstitial cell tumors*	0	0	0	0	11	1	0	9	3	6	8	2	3	5	5
Atrophy of seminiferous tubules	1	0	1	2	3	7	0	9	5	6	8	3	3	8	6
Testicular calcium deposits	0	0	2	0	2	4	1	3	3	4	6	2	0	3	0
Acute or chronic prostatitis	0	0	0	0	1	0	1	0	1	0	5	0	1	3	1
Tumor‖															
Benign	0	0	0	0	8	1	1	7	4	4	5	0	1	2	2
Malignant	0	0	0	0	0	0	0	1	1	4	3	0	1	5	3
Total	0	0	0	0	8	1	1	7	4	6	6	0	2	6	5
Epithelial tumors¶															
Benign	0	0	0	0	2	0	0	0	1	1	2	0	0	0	0
Malignant	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mesenchymal tumors¶															
Benign	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
Malignant	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0
Selected nonendocrine-specific tumors															
Preputial gland	0	0	0	0	1	0	0	3	0	0	0	0	0	0	0
Lymphoma or leukemia	0	0	0	0	0	0	0	0	0	1	2	0	0	2	2
Mesothelioma	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1
Endocrine-gland tumors															
Pituitary adenoma	0	0	0	0	3	0	0	3	1	1	4	0	0	2	1
Thyroid C-cell tumors	0	0	0	0	0	1	1	2	1	2	2	0	1	2	1
Thyroid follicular tumors	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Adrenal cortical tumors	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Pheochromocytoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pancreas islet cell tumors	0	0	0	0	1	0	0	4	0	0	1	0	0	1	0

* Progression of lesions with age in all groups ($p \leq 0.05$).
† B different from A ($p \leq 0.05$).
‡ Statistical analysis based on group lesion score determined by ridit analysis.
§ D different from B ($p \leq 0.05$).
‖ In tumor-bearing rats, tumors other than testicular interstitial cell tumors.
¶ Excluding selected nonendocrine-specific tumors and endocrine-gland tumors.

TABLE 6
Number of rats dying spontaneously with lesions other than chronic nephropathy

Type of lesion	Age															Total		
	< 12 mo			12 through 17 mo			18 through 23 mo			24 through 30 mo			> 30 mo					
	Group A (n = 4)	Group B (n = 1)	Group D (n = 1)	Group A (n = 9)	Group B (n = 3)	Group D (n = 2)	Group A (n = 60)	Group B (n = 16)	Group D (n = 7)	Group A (n = 36)	Group B (n = 32)	Group D (n = 18)	Group A (n = 2)	Group B (n = 37)	Group D (n = 35)	Group A (n = 111)	Group B (n = 89)	Group D (n = 63)
Cardiomyopathy																		
Grade 0 lesion	2	1	1	2	2	2	3	7	2	0	6	1	0	1	2	7	17	8
Grade 1 lesion	2	0	0	7	1	0	23	7	4	8	21	10	0	21	18	40	50	32
Grade 2 lesion	0	0	0	0	0	0	25	2	1	18	2	6	1	10	11	44	14	18
Grade 3 lesion	0	0	0	0	0	0	9	0	0	10	3	1	1	5	4	20	8*†	5†‡
Gastrointestinal tract																		
Esophageal hyperkeratosis	0	0	0	5	1	0	31	7	3	24	21	11	2	12	9	62	41	23‡
Gastric hyperkeratosis	1	1	0	0	0	0	28	1	1	22	3	1	1	2	3	62	7*	5‡
Gastric ulcer	0	0	0	0	0	0	21	0	0	19	3	0	1	3	7	41	6*	7‡
Intestinal obstruction by hair balls	3	1	0	6	3	2	2	7	1	0	6	0	0	9	13	11	26*	16‡
Hepatic																		
Bile duct hyperplasia																		
Grade 0 lesion	4	1	1	3	2	1	0	1	1	0	2	0	0	3	1	7	9	4
Grade 1 lesion	0	0	0	5	1	1	28	8	4	14	13	3	0	12	8	47	34	16
Grade 2 lesion	0	0	0	1	0	0	21	7	1	15	12	9	2	13	14	39	32	24
Grade 3 lesion	0	0	0	0	0	0	11	0	1	7	5	6	0	9	12	18	14	19†‡§
Fatty change																		
Grade 0 lesion	1	1	1	2	0	2	14	10	6	8	15	3	1	15	19	26	41	31
Grade 1 lesion	3	0	0	6	3	0	32	6	1	20	14	14	0	16	8	61	39	23
Grade 2 lesion	0	0	0	1	0	0	8	0	0	5	3	1	0	3	6	14	6	7
Grade 3 lesion	0	0	0	0	0	0	6	0	0	3	0	0	1	3	2	10	3*†	2†‡
Periductal fibrosis	0	0	0	0	0	0	38	3	5	31	6	13	1	16	21	70	25*	39§
Cystic space	1	0	0	0	0	0	6	1	0	8	3	1	1	2	3	16	6	4
Reproductive system																		
Testicular interstitial cell hyperplasia	0	0	0	1	0	0	28	4	1	14	9	9	0	3	2	43	16*	12‡
Testicular interstitial cell tumors	0	0	0	0	0	0	46	3	1	35	13	14	2	28	32	83	44*	47§
Atrophy of seminiferous tubules	0	0	0	1	1	0	35	5	3	32	15	13	2	29	23	70	50	39

Testicular calcium deposits	0	0	0	0	0	0	23	1	0	18	13	11	1	14	3	42	28	14‡
Acute or chronic prostatitis	3	0	0	1	0	1	14	1	0	10	2	2	2	9	12	30	12*	15
Tumor‖																		
Benign	0	0	0	2	0	0	34	2	0	24	9	9	2	19	24	62	30*	33§
Malignant	0	0	0	1	0	0	24	3	4	16	16	12	0	19	15	41	38	31
Total	0	0	0	3	0	0	46	4	4	31	23	14	2	28	30	82	55	48
Epithelial tumors¶																		
Benign	0	0	0	0	0	0	4	1	0	3	1	0	1	2	2	8	4	2
Malignant	0	0	0	0	0	0	5	0	0	3	1	0	0	0	2	8	1*	2
Mesenchymal tumors¶																		
Benign	0	0	0	0	0	0	2	0	0	0	1	2	0	1	2	2	2	4
Malignant	0	0	0	1	0	0	4	2	2	0	3	3	0	2	1	5	7	6
Selected nonendocrine-specific tumors																		
Preputial gland	0	0	0	0	0	0	11	1	0	3	0	0	0	1	0	14	2*	0‡
Lymphoma or leukemia	0	0	0	0	0	0	5	1	2	2	7	5	0	11	8	7	19*	15‡
Mesothelioma	0	0	0	0	0	0	4	0	0	0	1	0	0	0	1	4	1	1
Endocrine tumors																		
Pituitary adenoma	0	0	0	1	0	0	15	1	0	10	2	3	1	4	10	27	7*	13§
Thyroid C-cell tumors	0	0	0	1	0	0	8	0	0	8	2	4	0	5	3	17	7	7
Thyroid follicular tumors	0	0	0	0	0	0	2	0	0	2	0	0	0	0	1	4	0	1
Adrenal cortical tumors	0	0	0	0	0	0	3	0	0	1	0	2	0	4	7	4	4	9†§
Pheochromocytoma	0	0	0	0	0	0	2	0	0	5	1	2	1	4	1	8	5	3
Pancreas islet cell tumors	0	0	0	0	0	0	5	0	0	6	4	3	1	8	3	12	12	6
Selected nonneoplastic																		
Osteodystrophy	0	0	0	0	0	0	16	0	0	16	0	1	1	0	0	33	0*	1‡
Calcium deposits in																		
Heart	0	0	0	0	0	0	12	0	0	13	0	1	1	0	3	26	0*	4†§
Kidneys	4	0	0	2	1	0	34	5	4	29	14	5	1	9	10	70	29*	19‡
Skeletal muscles	0	0	0	0	0	0	21	1	1	20	1	4	2	2	4	43	4*	9†§
Pancreatic lobular atrophy	0	0	0	2	0	0	18	4	1	7	5	4	0	2	7	27	11*	12
Thyroid C-cell hyperplasia	0	0	0	0	0	0	21	2	1	15	5	3	0	3	4	36	10*	8‡

* B different from A ($p \leq 0.05$).

† Statistical analysis based on group lesion score determined by ridit analysis.

‡ D different from A ($p \leq 0.05$).

§ D different from B ($p \leq 0.05$).

‖ In tumor-bearing rats, tumors other than testicular interstitial cell tumors.

¶ Excluding selected nonendocrine-specific tumors and endocrine-gland tumors.

other than protein and vitamins influence the age-associated progression of nephropathy. There are data in the literature (18) that indicate that minerals, particularly phosphates, may be involved. We recently reported (9) that when the mineral component of our semisynthetic diet is restricted to the same extent as in group B of the present study but without restricting other dietary components, the progression of nephropathy with age in male Fischer 344 rat was not influenced. In the same study the restriction of the fat component in a similar fashion was found to retard the progression of the nephropathy but much less effectively than the food-restriction regimen. Thus, it appears that the marked retardation by food restriction of chronic nephropathy in male Fischer 344 rats is due either to the restriction of calories or, possibly, of the carbohydrate components. The work of Kleinknecht et al (19) on the rat ablation models indicated that carbohydrates may play a role in the nephropathy of that model. In contrast, Kasiske et al (20) found that intermittent carbohydrate restriction failed to significantly influence the initiation and progression of nephron damage in obese Zucker rats. In our opinion, restriction of calories is probably the most important factor but further work is needed to unequivocally establish this view.

The mechanism by which food restriction retards the progression of chronic nephropathy also remains to be defined. An age-related increase in systolic blood pressure occurs in male Fischer 344 rats but its development is not influenced by food restriction (7). Serum lipids have been implicated in the progression of renal disease (18), and food restriction markedly retards the age-related increase in serum lipids in male Fischer 344 rats (21). Heifets et al (22) reported that chronic exercise training ameliorates the progression of renal disease in the rat abalation model and food restriction retards the age-related decrease in spontaneous motor activity of Fischer 344 rats (7). However, food restriction in rodents retards such a broad spectrum of age-associated physiological processes (17) that only a systematic study will uncover which of those actions influence chronic nephropathy. Another possible mechanism by which food restriction could influence nephropathy is by its effect on the temporal pattern of food intake. This mechanism does not seem likely because Nelson (23) reports that pattern of food intake by food-restricted rodents does not influence longevity. Nevertheless, this possibility remains to be rigorously tested in regard to chronic nephropathy.

An analysis of the spectrum of pathologic lesions that occurred with increasing age in the rats of each of the dietary groups was undertaken to assess the potential interplay between nephropathy and other disease processes. Many age-associated lesions in addition to chronic nephropathy were retarded by food restriction with or without protein restriction (ie, in both groups B and D). Several of these lesions appear to be secondary to the nephropathy, specifically cardiomyopathy, osteodystrophy, gastric ulcers, and Ca deposits in heart, skeletal muscle, and kidneys (*see* Maeda et al [2] for discussion of the relationship between these lesions and nephropathy). Other lesions were more prevalent in groups B and D than in group A, eg, lymphoma or leukemia and obstruction of intestine by hair balls. The greater longevity of groups B and D appears to explain the greater prevalence of leukemia and lymphoma in these groups at the time of spontaneous death compared with the rats of group A. Testicular interstitial cell tumors, periductal fibrosis, and pituitary adenoma at the time of spontaneous death had a higher prevalence in groups A and D than in group B, indicating that dietary protein may play a role in the development of these lesions. Adrenal cortical tumors and bile duct hyperplasia were more prevalent at the time of spontaneous death in group D than in group A or group B. In spite of these differences in pathologic lesions, the rats in groups B and D had similar survival curves.

Indeed, the findings in the literature on the influence of dietary protein on the longevity of rodents are conflicting (24–28). Part of this discrepancy is probably due to the failure in many of the studies to measure or report caloric intake. However, not all of the discrepancy can be so explained. The results of our previous study together with those of this study provide insight into another reason for these conflicting findings. In our earlier study (2) the rats fed ad libitum a diet containing 21% casein had a significantly reduced median length of life and age of 10th-percentile survivors compared with rats fed the 12.6% casein diet isocalorically. The extent to which the reduced protein intake retarded chronic nephropathy appeared to be the major reason for this difference in longevity. In the present study the median length of life of group B rats (food restriction involving both calories and protein) was the same as that of group D rats (food restriction involving calories but not protein). Indeed, the age of the 10th-percentile survivors was marginally greater for group D rats than for group B rats. Neither group B or D rats had sufficiently severe chronic nephropathy for it to play a major role in mortality. It appears that restricting protein increases longevity under dietary conditions in which renal failure is a major contributing factor to death but when renal failure is not a major contributing factor, increasing dietary protein, if anything, increases longevity.

The conclusions to be drawn are *1*) reducing the intake of protein is not the major reason for the retardation by food restriction of the age-associated progression of chronic nephropathy in rats; *2*) although dietary protein can significantly influence the progression of nephropathy, its effects are small compared with other dietary component or components yet to be defined; caloric intake appears most likely to be the major factor; and *3*) if our findings with rats have a bearing on the treatment of humans with chronic renal failure, the current emphasis on restricting the protein intake of these patients (29) should be reassessed.

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