Gerontology *28:* 168–175 (1982)

Effects of Caloric Intake and Dietary Composition on the Development of Proteinuria, Age-Associated Renal Disease and Longevity in the Male Rat[1]

Arthur V. Everitt, Barbara D. Porter, Jennifer R. Wyndham

Department of Physiology, University of Sydney, Australia

Key Words. Male rat · Caloric intake · Dietary protein · Proteinuria · Renal lesions · Kidney weight · Life duration

Abstract. The development of age-associated proteinuria and renal disease was studied in groups of male Wistar rats fed 12.5, 25, 50 and 75 kcal of food/day, respectively, and in calorie-restricted (40 kcal/day) rats receiving diets rich in fat, protein or carbohydrate. Proteinuria developed faster, kidneys were larger and the incidence of glomerular lesions and proteinaceous casts was greater in rats eating high calorie diets of 50 kcal/day or more. High protein diets, even when calorie-restricted, increased protein excretion and the incidence of glomerular lesions. In old rats acute food restriction (25 kcal/day) decreased protein excretion by 40% in 1 week, with no further reduction in the 2 week. Life duration was greatest in rats fed 50 kcal/day.

With increasing age in the male rat, there is a rise in protein excretion [*Saxton and Kimball,* 1941; *Everitt,* 1958; *Beauchene* et al., 1970; *Alt* et al., 1980]. Age-associated renal lesions have been described by several authors [*Andrew and Pruett,* 1957; *Bras,* 1969; *Hirokawa,* 1975].

Food restriction inhibits the development of age-associated renal disease [*Saxton and Kimball,* 1941; *Berg and Simms,* 1960; *Bras and Ross,* 1964; *Tucker* et al., 1976] in the rat. Although caloric intake appears to be the main factor in the development of renal lesions, dietary protein has also been implicated [*Newburgh and Curtis,* 1928; *Bras and Ross,* 1964; *Lalich* et al., 1970].

The present study investigates the quantitative relationship between calorie intake and

[1] This work was supported in part by grants from the Consolidated Medical Research Fund of the University of Sydney and the University Research Grant.

the development of renal disease, and the role of dietary constituents in its development. A preliminary report on the effects of different diets on proteinuria has been made [*Everitt and Porter,* 1976].

Materials and Methods

The quantitative relationship between caloric intake and the development of proteinuria and renal disease was studied in four groups of 25 male Wistar rats fed 3.5, 7, 14 and 21 g food/day (corresponding to 12.5, 25, 50 and 75 kcal/day, respectively. A further 8 rats were maintained on 18 g food/day. Studies were commenced at age 28 days. Experimental details have been described previously in a collagen ageing study [*Everitt,* 1971]. The composition of the commercial rat chow was protein 20.1%, fat 7.1%, carbohydrate 53.1%, fibre 5.5%, water 6.2%, NaCl 0.8%, CaO 3.0% and P_2O_5 3.7%. Protein excretion was measured at ages 70, 320, 460, 730 and 900 days. Urine was collected for 24 h from rats placed individually in metabolism cages, without previous adaptation; in place of food and water each rat was provided with 50 ml of 10% sucrose. Urinary protein was estimated by the trichloroacetic acid turbidimetric method of *Henry* et al. [1956]. Serum protein was determined in tail blood by a modification of the *Lowry* et al. [1951] method. Blood urea nitrogen was measured in tail blood using a variation of the diacetyl monoxime method of *Baker and Silverton* [1976]. Systolic blood pressure was recorded using the indirect tail-cuff procedure with a pneumatic pulse transducer (Narco Biosystems, Houston, Tex.) to detect pulse. Data are reported as means ± SE.

Studies in calorie-restricted rats (40 kcal/day) investigated the effects of high levels (60% of calories) of dietary fat, protein and carbohydrate, respectively, on the development of proteinuria. 40 male Wistar rats (8 on each of 5 diets), between ages 85 and 250 days, were housed individually in metabolism cages and fed a high fat, high protein, or high carbohydrate diet at 40 kcal or a 'standard' diet (isocaloric for fat, protein and carbohydrate) at 40 and 80 kcal/day. The detailed composition of these diets is described elsewhere in a collagen ageing study [*Everitt* et al., 1981]. Protein excretion was measured at ages 100 and 250 days.

For histological studies kidneys were obtained from 20 old (893–1,302 days) and 40 young adult (250 days) rats. Kidneys were fixed in formol saline, embedded in paraffin and 7-μm section stained with periodic acid Schiff's reagent. Two indices of renal disease used were the number of proteinaceous casts per square millimeter and the percentage of necrotic glomeruli in a sample of 100–200 glomeruli; 16 random fields of kidney cortex were examined covering an area of 13 mm^2.

Results

Protein Excretion

Quantitative Chronic Effect of Caloric Intake. In ad libitum-fed rats eating 21 g food (75 kcal)/day, protein excretion rose progressively throughout life from a mean of 4 mg/day at 70 days to 88 mg/day at 900 days (fig. 1). The steep rise in protein excretion with age was abolished by severe food restriction to 3.5 g (12.5 kcal) or 7 g (25 kcal)/day, and markedly inhibited even at 14 g (50 kcal)/day.

Chronic Effect of Dietary Composition in Calorie-Restricted Rats. Compared with the replete group caloric restriction by one half markedly reduced the protein excretion in rats on high fat, high carbohydrate and isocaloric standard diet (table I). Thus excess fat and carbohydrate did not appear to affect the development of proteinuria. However, the high protein diet, even when calorically restricted, increased protein excretion. For all of these groups a plot of protein excretion versus protein intake showed a highly significant correlation ($r = 0.96$, $p < 0.01$.).

Acute Effect of Caloric Restriction in Old Rats. When 4 old rats aged 820 days were placed on 25 kcal of commercial food/day,

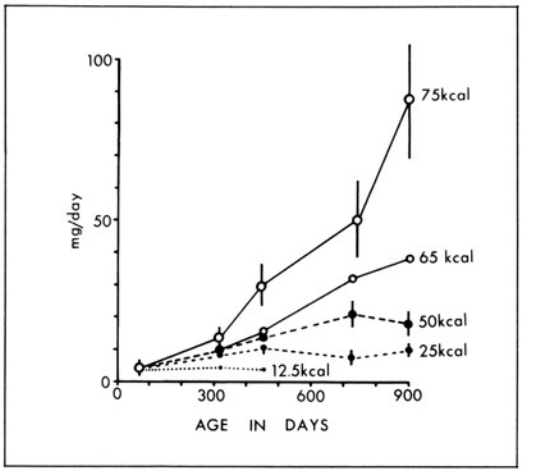

Fig. 1. The long-term effect of caloric intake on the development of age-associated proteinuria. The curves plot mean protein excretion ± SE at different ages for rats living more than 900 days. There were 4 rats on 75 kcal/day, 1 on 65, 5 on 50 and 5 on 25. The 12.5-kcal data are from 1 rat living more than 500 days.

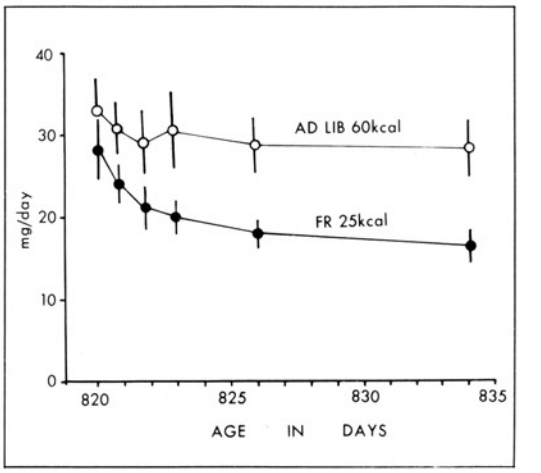

Fig. 2. The short-term effect of severe food restriction (FR; 25 kcal/day) in lowering protein excretion in 4 old male rats. Data are means ± SE.

protein excretion decreased by 40% in 1 week, but was not reduced significantly during the 2nd week (fig. 2); body weight decreased 28%. During this period ad libitum-fed controls underwent a 15% reduction in protein excretion and 9% loss in body weight; the senescent loss of weight was probably exaggerated by the stress of isolated housing in metabolism cages. 2 weeks after refeeding these restricted rats had a protein excretion (27.1 ± 2.8) similar to their pre-restriction level (28.2 ± 3.7).

Serum Protein

In ad libitum-fed rats there was no age change in serum protein (96 ± 3 mg/ml at 225 days; 93 ± 4 at 650 days; 94 ± 3 at 1,000 days; n = 4). Only at advanced ages (922–1,117 days) did food-restricted rats (25 kcal/day) have a significantly lower serum protein (81 ± 4) than replete animals ($p < 0.05$).

Blood Urea Nitrogen (BUN)

In ad libitum-fed rats BUN did not change with age (23.0 ± 1.18 mg/100 ml in 6 rats aged 354 days; 25.6 ± 1.48 in 11 rats aged 820 days). 5 chronically food-restricted (25 kcal/day) rats aged 845 days had a significantly lower BUN of 17.3 ± 1.7 ($p < 0.01$).

In apparently healthy old ad libitum-fed rats of mean age 820 days, high proteinuria was not associated with raised BUN. 4 high protein excretors (mean 44.4 mg protein/day) had a BUN of 26.2 ± 2.50 while 6 low protein excretors (mean 3.7 mg/day) had a BUN of 25.2 ± 2.15.

Table I. Effect of dietary composition in low-calorie diets on renal ageing of male rats measured by protein excretion per day

Dietary composition	Number of rats	Energy intake kcal/day	Age days	Protein excretion (± SE) mg/day
Initial high-calorie	8	75	100	8.7 ± 0.5**
High-fat low-calorie	8	40	250	12.1 ± 0.7
High-carbohydrate low-calorie	8	40	250	12.8 ± 1.0
High-protein low-calorie	8	40	250	19.0 ± 1.6**
Standard low-calorie	8	40	250	13.5 ± 1.1
Standard high-calorie	8	80	250	22.8 ± 1.6**

** Significantly different ($p < 0.01$) from the standard low-calorie diet at 250 days.

Systolic Blood Pressure

In ad libitum-fed rats systolic blood pressure did not change with age (109.8 ± 1.5 mm Hg in 14 rats aged 301 days; 108.8 ± 3.5 mm Hg in 23 rats aged 749 days). Chronic food restriction (25 kcal/day) did not lower blood pressure (111.2 ± 6.5 mm Hg in 6 rats aged 713 days). High proteinuria in old age was not associated with raised blood pressure (high protein excretors 112 ± 4.3 mm Hg; low protein excretors 110.6 ± 5.2 mm Hg).

Renal Histopathology

In old age (909–1,194 days) kidneys from 10 ad libitum-fed (60 kcal/day) rats showed varying degrees of glomerular hyalinization, increased mesangium and fibrosis; tubular dilatation and casts, and thickening of the basement membranes of glomeruli, tubules and Bowman's capsule (fig. 3). However, in 10 calorie-restricted rats (25 kcal/day) of similar ages (893–1,302 days), the glomerular and tubular changes were seen only occasionally and basement membrane thickening was much less pronounced. Quantitative studies (table II) showed that compared with food-restricted rats the ad libitum-fed rats had a lower glomerular count (131 vs. 174), a higher percentage of necrotic glomeruli (27.9 vs. 2%) and a higher count of casts (1.45 vs. 0.11/mm^2).

At age 250 days the percentage ± SE of necrotic glomeruli was significantly greater ($p < 0.01$) in ad libitum-fed controls (39.2 ± 4.9%) and ($p < 0.05$) in high protein, low calorie rats (28.4 ± 3.9%) than in standard low calorie rats (16.3 ± 1.6%). The count of casts was too low for reliable estimation.

Renal Weight

The development of renal pathology is associated with increased kidney weight. Kidney weights increased as caloric intake or body weight increased (table III). Compared with kidneys from young rats of similar body weights, there was no significant increase with age in rats on a low caloric intake (25 kcal/day), but significant increases occurred in rats on higher caloric intakes (50 and 75 kcal/day). The absence of an age-related increase in renal weight in rats on low food intake (25 kcal/day) is consistent with the absence of a significant rise in protein excretion with age (fig. 1) and a lower incidence of renal pathology (table II).

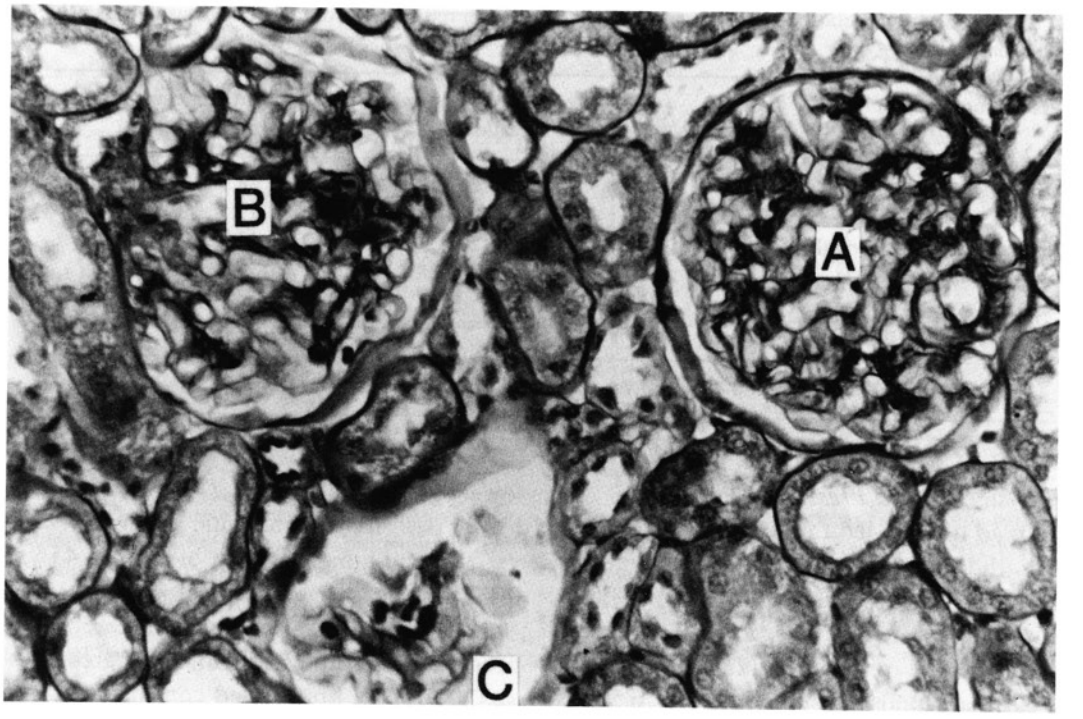

Fig. 3. Section of the kidney cortex in a male ad libitum-fed rat, 1,114 days old, showing three glomeruli in different stages of degeneration. × 250. **A** Glomerulus which is essentially normal. Only a small increase in mesangial cell fibrosis and basement membrane thickness is visible. **B** Glomerulus showing signs of necrosis. There is increased mesangial fibrosis, thickened basement membrane in the glomerulus and Bowman's capsule. A tubular cast is just visible below the glomerulus. **C** A necrotic glomerulus, where most of the capillaries have degenerated – only one patent capillary lumen is present. Bowman's capsule is markedly thickened and casts are visible within the necrotic glomerulus.

Table II. The incidence of necrotic glomeruli and proteinaceous casts in the kidneys of old rats aged 893–1302 days which were fed either ad libitum or food restricted from early life at age 28 days (mean ± SE)

Group	Number of rats	Glomeruli in 13 mm²			Casts/mm²
		total	necrotic	% necrotic	
Ad libitum (60 kcal/day)	10	131 ± 8.4	35.1 ± 1.9	27.9 ± 2.6	1.45 ± 0.31
Food restricted (25 kcal/day)	10	174 ± 8.8**	3.4 ± 0.69**	2.0 ± 0.46**	0.11 ± 0.02**

** Significant at the 1% level.

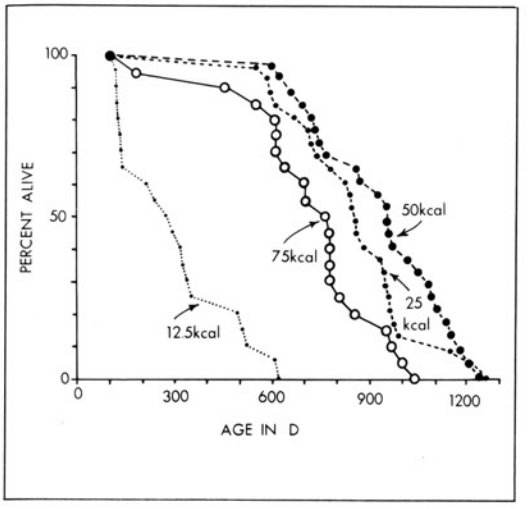

Fig. 4. The survival curves of rats fed differing levels of calories from age 28 days until natural death. Rats which died before age 100 days were discarded from the study. All rats were housed individually.

Life Duration

The ad libitum (75 kcal/day) group had a mean life duration of 728 ± 46 (SE) days. Severe caloric restriction (12.5 kcal) shortened life duration to 295 ± 43 days ($p < 0.01$), while mild restriction (50 kcal) prolonged life to 936 ± 39 days ($p < 0.01$) as did moderate restriction (25 kcal) to 859 ± 38 ($p < 0.01$). Survival curves are shown in figure 4.

Discussion

This study clearly demonstrated the role of calories in the development of age-associated proteinuria, age-associated renal lesions, renal hypertrophy and life duration in the male Wistar rat.

Table III. Weight of both kidneys at autopsy in old male rats, aged 700 days or more, which during life were either fed ad libitum (75 kcal/day) or food restricted (FR, 25 or 50 kcal/day)

	Number of rats	Mean age days	Mean body weight, g	Mean (± SE) weight of two kidneys g	t	p
Young ad libitum	10	42	122	1.34 ± 0.04	1.56	NS
Old FR (25 kcal/day)	10	864	124	1.44 ± 0.05		
Young ad libitum	6	55	191	1.72 ± 0.17	3.81	**
Old FR (50 kcal/day)	8	891	184	2.43 ± 0.10		
Young ad libitum	8	75	281	2.17 ± 0.17	6.37	**
Old ad libitum (75 kcal/day)	10	875	288	3.61 ± 0.15		

Comparisons are made with the kidney weights of young rats of similar body weights.
NS = Not significant.
** Significant at the 1% level.

Age-associated renal lesions and proteinuria, although rarely accompanied by major impairment of kidney function, are usually associated with a reduced life expectancy. Uraemia and hypertension were seen in less than 10% of apparently healthy old rats and were not associated with high levels of proteinuria. No direct measurements of renal function were made in the longevity study since such tests would involve sacrifice of the animals. However, the comparable study of *Alt* et al. [1980] on old male Wistar rats aged 38 months (1,150 days), with a similar degree of proteinuria, showed a 30% reduction in glomerular filtration rate compared with young adult rats aged 5.5 months (165 days).

Raising the intake of protein in the diet increased the level of proteinuria and the incidence of renal lesions as reported earlier by *Bras and Ross* [1964]. The mechanism is still obscure. The proteinuric effect of calories and protein is not associated with the serum protein level, but may be due to the action of amino acids [*Hardy* et al., 1960], urea [*Rumsfield,* 1956] or phosphate [*Haut* et al., 1980] on the kidney. High phosphate diets (2–3% phosphate) induce renal injury in partially nephrectomized rats [*Haut* et al., 1980]. In such animals phosphate restriction prevents proteinuria, renal histological changes and deterioration of kidney function [*Ibels* et al., 1978]. Thus low calorie and low protein diets may prevent the development of age-related renal pathology due to their low phosphorus content. The commercial diet used in the present study was relatively high in phosphate (1.6%) and thus may have contributed to the rise in protein excretion with age.

In addition to the long-term effects of calories there is also a short-term effect, since caloric restriction in old rats with moderate proteinuria produced a 40% fall in protein excretion within a week (fig. 2). However, this level of proteinuria is significantly higher than the level found in rats fed a low calorie diet (25 kcal/day) throughout life. This suggests that there is an acute effect of calories on proteinuria in addition to a long-term pathological effect.

Acknowledgement

The authors are grateful to Dr. *Ian Hutchinson* for his helpful criticisms of this manuscript.

References

Alt, J.M.; Hackbarth, H.; Deerberg, F.; Stolte, H.: Proteinuria in rats in relation to age-dependent renal changes. Lab. Anim. *14:* 95–101 (1980).

Andrew, W.; Pruett, D.: Senile changes in the kidneys of Wistar institute rats. Am. J. Anat. *100:* 51–80 (1957).

Baker, F.J.; Silverton, R.E.: Introduction to medical laboratory technology; 5th ed., pp. 280–282 (Butterworths, London 1976).

Beauchene, R.E.; Roeder, L.M.; Barrows, C.H., Jr.: The inter-relationship of age, tissue protein synthesis and proteinuria. J. Geront. *25:* 359–363 (1970).

Berg, B.N.; Simms, H.S.: Nutrition and longevity in the rat. II. Longevity and onset of disease with different levels of food intake. J. Nutr. *71:* 255–263 (1960).

Bras, G.: Age-associated kidney lesions in the rat. J. infect. Dis. *120:* 131–134 (1969).

Bras, G.; Ross, M.H.: Kidney disease and nutrition in the rat. Toxicol. appl. Pharmacol. *6:* 247–262 (1964).

Everitt, A.V.: The urinary excretion of protein, non-protein nitrogen, creatinine and uric acid in ageing male rats. Gerontologia *2:* 33–46 (1958).

Everitt, A.V.: Food intake, growth and ageing of collagen in rat tail tendon. Gerontologia *17:* 98–104 (1971).

Everitt, A.V.; Porter, B.: Nutrition and aging; in Everitt, Burgess, Hypothalamus, pituitary and aging, pp. 570–613 (Thomas, Springfield 1976).

Everitt, A.V.; Porter, B.D.; Steele, M.: Dietary, caging and temperature factors in the ageing of collagen fibres in rat tail tendon. Gerontology *27:* 37–41 (1981).

Hardy, R.W.F.; Tatengco, V.O.; Bauman, C.A.: Effects of amino acids on the excretion of proteins by the rat. J. Nutr. *70:* 438–446 (1960).

Haut, L.L.; Alfrey, A.C.; Guggenheim, S.; Buddington, B.; Schrier, N.: Renal toxicity of phosphate in rats. Kidney int. *17:* 722–731 (1980).

Henry, R.J.; Sobell, C.; Segalove, M.: Turbidimetric determination of proteins with sulfosalicylic and trichloracetic acids. Proc. Soc. exp. Biol. Med. *92:* 748–753 (1956).

Hirokawa, K.: Characterization of age-associated kidney disease in Wistar rats. Mech. Age. Dev. *4:* 301–316 (1975).

Ibels, L.S.; Alfrey, A.C.; Haut, L.; Huffer, W.E.: Preservation of function in experimental renal disease by dietary restriction of phosphate. New Engl. J. Med. *298:* 122–126 (1978).

Lalich, J.J.; Faith, G.C.; Harding, G.E.: Protein overload nephropathy. Archs Path. *89:* 548–559 (1970).

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J.: Protein measurement with the Folin phenol reagent. J. biol. Chem. *193:* 265–275 (1951).

Rumsfield, H.W., Jr.: The role of dietary protein in normal rat proteinuria. Am. J. Physiol. *184:* 473–478 (1956).

Saxton, J.A., Jr.; Kimball, J.C.: Relation of nephrosis and diseases of albino rats to age and to modifications of diet. Archs Path. *32:* 951–965 (1941).

Tucker, S.M.; Mason, R.L.; Beauchene, R.E.: Influence of diet and feed restriction on kidney function of aging male rats. J. Geront. *31:* 264–270 (1976).

Received: July 5, 1981
Accepted: August 12, 1981

Assoc. Prof. A.V. Everitt, Department of Physiology, F13, University of Sydney, Sydney, NSW 2006 (Australia)