Effect of dietary protein restriction on the development of renal failure after subtotal nephrectomy in rats

A. M. EL-NAHAS, H. PARASKEVAKOU, S. ZOOB, A. J. REES AND D. J. EVANS
Department of Medicine and Pathology, Royal Postgraduate Medical School, Hammersmith Hospital, London

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Summary

1. We have examined the effect of a low protein diet on the development of glomerular sclerosis and progressive renal failure after subtotal nephrectomies in rats.

2. Two groups of male Sprague-Dawley rats were studied after five-sixths nephrectomy; group 1 were maintained on a normal diet (13.5 g day⁻¹ kg⁻¹ body weight) and group 2 were fed with a low protein diet (6 g day⁻¹ kg⁻¹ body weight).

3. Rats maintained on a low protein diet survived for longer, and had significantly less glomerular sclerosis and significantly greater glomerular filtration rates when the experiment ended after 7 months.

4. We conclude that dietary protein influences favourably the development of glomerular scarring and renal failure after subtotal nephrectomy in rats.

Key words: glomerular filtration rate, glomerular sclerosis, low protein diet, proteinuria, subtotal nephrectomy.

Introduction

Chronic renal failure is always accompanied by glomerular and interstitial scarring, but it is not known to what extent this scarring is the direct consequence of the original injury or whether other factors contribute towards its progression. In some patients there is evidence for progression after the original cause of injury has subsided [1].

Materials and methods

Animals

All experiments were performed on male Sprague-Dawley rats, weighing 200–250 g at the start of the experiment. They were housed up to six rats to a cage, but once weekly were placed in individual metabolic cages for collection of 24 h urine samples.

Nephrectomies

Five-sixths nephrectomies were performed in two stages, under ether anaesthesia. Initially two-thirds of the left kidney was removed by ligation.
and resection of the upper and lower poles. Two weeks later the right kidney was removed. At each stage care was taken to leave the adrenal glands intact.

**Diets**

Two weeks after completion of the nephrectomies, the rats were randomly allocated to one of two groups.

Group 1, consisting of 10 rats, were maintained on a normal protein diet (13.5 g day\(^{-1}\) kg\(^{-1}\) body weight); group 2, consisting of 11 rats, were fed a low protein diet (6 g day\(^{-1}\) kg\(^{-1}\) body weight). Extra starch and phosphorus were added to the low protein diet so that diets were isocaloric and contained identical amounts of calcium and phosphorus. Details of the composition of the diets are shown in Table 1.

Animals were given a fixed amount of food each day (20 g/animal) but had free access to tap water. Two additional control groups (four rats in each) were studied to determine the effect of the experimental diets on normal rats.

**Assessment**

Weekly measurements were made of body weight, blood pressure, serum albumin and creatinine clearance, proteinuria, albuminuria, urinary creatinine and 24 h creatinine clearance.

**TABLE 1. Composition of diet**

The minerals, trace elements and vitamin contents of the two diets were identical. ME, Metabolizable energy.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Protein content (% by wt.)</th>
<th>Low</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize starch</td>
<td></td>
<td>62.5</td>
<td>53.5</td>
</tr>
<tr>
<td>Corn oil</td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Solkafloc</td>
<td></td>
<td>17.0</td>
<td>15.3</td>
</tr>
<tr>
<td>Casein</td>
<td></td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Minerals</td>
<td></td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Chalk/limestone</td>
<td></td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td></td>
<td>3.4</td>
<td>2.9</td>
</tr>
</tbody>
</table>

**Calculated analysis**

<table>
<thead>
<tr>
<th>Percentage composition</th>
<th>Low (%)</th>
<th>Medium (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.85</td>
<td>0.85</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td>2680</td>
<td>2680</td>
</tr>
</tbody>
</table>

Rats were bled, under ether anaesthesia, from the tail artery. Creatinine was measured by standard autoanalyser technique, total protein by the biuret method after precipitation with 20% trichloroacetic acid and albumin by immunoelectrophoresis. The mean arterial blood pressure was measured by an indirect tail-cuff method [8] and considered abnormal when it exceeded 130 mmHg in non-anaesthetized animals.

**Renal morphology**

Renal tissue was taken when animals died or when they were killed at the end of the experiment. The kidney was weighed and separate specimens were preserved in neutral buffered formalin for light microscopy, cacodylate-buffer glutaraldehyde for electron microscopy and snap frozen in liquid nitrogen for immunofluorescent examination by standard techniques. The proportion of sclerosed glomeruli was calculated after studying 50 or more glomeruli. Tubular atrophy was assessed on an arbitrary 0 to 3 scale. The morphological assessment was made without knowledge of the animals' experimental group.

**Statistical analysis**

The Mann-Whitney test was used to estimate the significance of differences between the two experimental groups. Survival was calculated by the life table method and difference between groups by log rank test [9]. A probability of 5% was used as the criterion of significance.

**Results**

**General observations**

Irrespective of the dietary protein content all rats recovered after nephrectomy, appeared healthy and grew normally during the course of the experiment (Fig. 1). The normal growth emphasizes that rats on the low protein diet were not malnourished. After nephrectomy the mean arterial blood pressure rose from 110 ± 5 to 130 ± 5 mmHg (Fig. 2). It was similar in both groups of rats and tended to increase further during the course of the study.

**Protein excretion**

At the start of the experiment protein excretion was 25 ± 5 mg/24 h in normal rats on the experimental diets and did not increase after nephrectomy in both groups of experimental rats; at day 60, rats in group 1 had a mean protein
Dietary protein in chronic renal failure

**FIG. 1.** Weights (means ± SE) of control rats on a normal diet (○—○), and experimental rats on a normal diet (●—●) and a low protein diet (▲—▲). Abbreviations used in this Figure and Figs. 2–4: L3 Nx, left two-thirds nephrectomy; RNx, right nephrectomy.

**FIG. 2.** Mean arterial blood pressure (means ± SE) of control rats on a normal diet (○—○), and experimental rats on a normal diet (●—●) and a low protein diet (▲—▲). Significance of differences between control and experimental animals: *P < 0.005; **P < 0.005.
FIG. 3. Twenty-four hour urinary excretion (means ± SE) in control rats on a normal diet (○---○), and experimental rats on a normal diet (●---●) and a low protein diet (▲---▲). Significance of differences between the two experimental groups: * $P<0.005$; ** $P<0.0125$.

FIG. 4. Serum creatinine (means ± SE) of control rats on a normal diet (○---○), and experimental rats on a normal diet (●---●) and a low protein diet (▲---▲). Significance of difference between the two experimental groups: * $P<0.0125$; ** $P<0.025$. 
excretion of 37 ± 10 mg/24 h, which increased to 217 ± 41 mg/24 h by day 210. At 60 days proteinuria in rats from group 2 was comparable with that in group 1 rats and increased only slightly during the subsequent course to reach 83 ± 24 mg/24 h at day 210 (Fig. 3). This difference in proteinuria is significant ($P < 0.0125$, Mann-Whitney test).

**Renal function**

After the nephrectomies the mean serum creatinine rose from 45 to 100 μmol/l (Fig. 4) and was similar in the two groups when the diets were introduced on day 21. It remained so up to day 150, after which serum creatinine rose sharply in group 1 animals; there was no comparable rise in group 2 rats. At the end of the experiments the difference in plasma creatinine between the groups was statistically significant ($P < 0.0025$, Mann-Whitney test). These differences in plasma creatinine were attributable to changes in glomerular filtration rate assessed by 24 h creatinine clearance (Fig. 5).

**Renal morphology**

At death from renal failure, or after killing at the end of the experiment, the kidneys from animals in both groups showed a wide range of glomerular and tubular lesions. Some glomeruli from early animals in both groups were totally sclerosed whilst others had segmental scars. The glomerulosclerosis was much more severe in group 1. In this group at least 25% of glomeruli were totally sclerosed in all nine rats examined whereas only three rats in group 2 had this degree of sclerosis (Fig. 6). This difference is significant ($P < 0.048$, Mann-Whitney test). There were comparable differences in the degree of tubular atrophy (Fig. 6), which was present in seven of nine rats in group 1 compared with three of ten rats in group 2. In group 1, four rats had a small proportion of glomerular tufts surrounded by crescent; none was observed in group 2. No immunoglobulins were detected by immunofluorescence in glomeruli in rats from either group.

On electron microscopical examination the predominant findings in group 1 were hypertrophy of the epithelial and mesangial cells together with wrinkling and breaks in the basement membrane. Hypertrophic changes were less marked in group 2 animals.

**Survival**

Six of ten rats in group 1 died of renal failure before the end of the experiment, compared with only two of 11 in group 2; this difference is highly significant ($P < 0.0028$, log rank test) (Fig. 7).

No differences in growth, blood pressure, proteinuria, renal function and histology were observed between the two control groups.

**Discussion**

Although the process of glomerular scarring is poorly understood it is well known that, in both rats [10] and humans [11], there is a progressive increase in the proportion of sclerosed glomeruli with age. In rats glomerulosclerosis is so extensive as to cause death from renal failure. Such sclerosis occurs more rapidly after unilateral nephrectomy [3] and can be greatly exacerbated by subtotal nephrectomy [2]. Lately evidence has been presented to suggest that the rate of glomerular sclerosis may also be increased by reduction in renal mass in man; patients with unilateral renal agenesis have an increased incidence of glomerular sclerosis [5] and glomerular sclerosis frequently complicates renal transplant [12]. These observations raise the questions whether a similar mechanism of scarring might contribute to the development of renal failure after other types of renal injury, such as glomerulonephritis or after vesico-ureteric reflux, had acutely reduced the
glomerular filtration rate. If so, such sclerosis might proceed independently of the primary disease, well after it had resolved. For this reason we used five-sixths nephrectomy in rats to study some factors that influence the rate of glomerular sclerosis.

Substantial adaptive changes take place immediately after the nephrectomy [13]. These consist of hypertrophy of the kidney, which is largely complete after 2 weeks [14], and which is accompanied by a massive increase in renal blood flow [4] and single nephron glomerular filtration rate [15]. In the ensuing 3 months nephrectomized rats develop extensive glomerular sclerosis in their remnant kidney. It is uncertain, however, to what extent the sclerosis is predetermined by the degree

![Graph 1](image1.png)

**Fig. 6.** Renal morphology at time of death of the two experimental groups. Significance of differences for glomerular sclerosis: \( P < 0.048 \); for tubular atrophy: \( P < 0.025 \).

![Graph 2](image2.png)

**Fig. 7.** Survival of experimental rats on a normal diet (●) and on a low protein diet (▲). Significance of differences: * \( P < 0.0028 \) (log rank test).
of the early adaptation, and to what extent it depends on persistence of the increased blood flow or other compensatory changes. In the latter case, the rate of progressive scarring might be reduced by appropriate, although delayed, therapeutic interventions.

We chose to study the effect of protein restriction on glomerular sclerosis because it has already been shown to delay spontaneous scarring and the development of renal failure in the ageing rat [7]. Our results clearly show that moderate protein restriction also delays the onset of proteinuria and decreases the incidence of glomerular sclerosis as well as renal failure after five-sixths nephrectomy.

It is probable that this protection is directly attributable to the protein restriction. Previously, other dietary changes, and in particular phosphate restriction, have been shown to limit the development of renal failure in rats after partial nephrectomy [16] and after nephrotoxic nephritis [17]. Our diets contained identical quantities of calcium and phosphorus and differed only in the protein and starch content (Table 1). Similarly the results cannot be explained by the general effect of malnutrition on the inflammatory response, as the rats in both groups were not malnourished and grew normally during the course of the experiments. Hypertension could not explain the results; although blood pressure increased after the nephrectomies, it did not differ significantly between nephrectomized rats fed with either diet.

Even though protein restriction appeared to be directly responsible for the protection, from our experiments we can only speculate on the mechanisms involved. Severe protein restriction has been shown to reduce renal hypertrophy [18] as well as the increment in renal blood flow [6] and single nephron glomerular filtration rate [19] after subtotal nephrectomy. We were interested principally in the late development of scarring and therefore delayed starting of diets until 14 days after the completion of the nephrectomy, by which time most of the adaptive changes would have occurred. This, together with the fact that serum creatinine in both groups remained the same for a further 20 weeks, suggests that the protection from the glomerular sclerosis that we observed was not predetermined by the extent of the acute adaptive response. However, the protective effect of the diet could be mediated by long-term changes of renal blood flow or single nephron glomerular filtration rate as well as by fluid and solute excretion.

It is important to remember that protein restriction also has several effects on metabolism and causes a reduction in plasma triglyceride concentration [20] and of thyroid function [21]; theoretically, either or both of these could protect from the development of glomerular sclerosis.

In conclusion, we have shown that glomerular scarring after five-sixths nephrectomies in rats can be significantly reduced by moderate restriction of dietary protein. This proves that the rate of scarring in this model is not solely predetermined by the extent of acute adaptation but can be influenced by subsequent treatment. If similar mechanisms influence the rate of glomerular scarring after an acute injury in man, these observations carry important implications for the prevention of end-stage renal failure in patients with progressive renal disease.

References


