Salt Restriction Inhibits Renal Growth and Stabilizes Injury in Rats with Established Renal Disease

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ABSTRACT
Salt restriction inhibits renal growth and stabilizes injury in rats with established renal disease. Male Munich-Wistar rats that underwent right nephrectomy and segmental infarction of two thirds of the left kidney were fed standard chow for 4 wk and then randomly assigned to ingest standard or low-salt chow for an additional 4 wk. Four wk after ablation, rats had systemic hypertension, proteinuria, and glomerular sclerosis. The prevalence of sclerosis, protein excretion rate, and glomerular volume increased between the fourth and eighth week in rats that were fed standard chow, however, in rats that were fed low-salt chow, the increase in glomerular volume and development of further glomerular sclerosis was prevented whereas the protein excretion rate actually declined. Micropuncture studies performed 8 wk after ablation revealed that the glomerular hydraulic pressure was elevated in remnant kidneys and was not affected by salt restriction. This study demonstrates that dietary salt restriction can prevent further glomerular injury and reduce proteinuria even when instituted in rats with established renal disease. These findings are also consistent with the hypothesis that glomerular hypertrophy promotes injury in this model of hypertension and progressive renal disease.

Key Words: Remnant kidney, glomerular sclerosis, hypertrophy, hemodynamics, hypertension

In response to a partial loss of renal function, surviving nephrons undergo adaptive changes that tend to restore kidney function toward normal (1). Among these is compensatory growth, which results in an increase in the size of residual glomeruli and tubules. A number of recent studies (2–8) suggest that this hypertrophic response is, in the long term, deleterious, and leads to progressive glomerular sclerosis and end-stage renal failure. Consistent with this view, maneuvers that suppress renal growth, including dietary protein (9) or salt restriction (5–7), as well as administration of particular antihypertensive agents (2,8), ameliorate experimental renal injury. However, in most studies, therapy was begun simultaneously with the initial injury so that hypertrophy and injury were not reversed but rather prevented. In contrast, many patients with kidney disease present with significant renal functional impairment and, presumably, an established hypertrophic response. In addition, although the factors that initiate the renal growth response are largely unknown, studies in experimental models of renal hyperplasia have implicated changes in the expression in a number of early-response genes and growth factors (10–12). For some early-response genes, the increase in expression develops within minutes and returns to baseline within hours (11,12), a process that could not be altered by the imposition of a dietary maneuver weeks after the initial injury. Therefore, it is unclear whether maneuvers that prevent renal growth will also be effective if instituted in animals with chronic injury and extant renal enlargement. To examine this question, we studied the effects of dietary salt restriction begun 4 wk after renal ablation in rats with remnant kidneys. By that time, rats had systemic and glomerular hypertension, renal and glomerular enlargement, and both morphologic and proteinuric evidence of glomerular injury.

METHODS
Study Design
All studies were performed in male Munich-Wistar rats with initial weights of 200 to 250 g. Three groups of rats underwent right nephrectomy and segmental infarction of approximately two thirds of the left kidney as a single procedure by using previously described techniques (6). All rats were fed standard chow (Purina Mills, Richmond, IN) that contained 23% protein and 0.46% sodium, by weight, for 4 wk. Body weight, awake systolic blood pressure, and urinary protein excretion rates were monitored. One group of rats (CON4) was euthanized 4 wk after ablation. Rats in the remaining two groups were randomly assigned either to ingest standard chow (CON8) or to ingest chow that contained a similar amount of protein (20.5%) but a reduced...
amount of sodium (0.09%; LS8) for an additional 4 wk. The sodium content of the low-salt chow provides approximately the lowest sodium intake that is consistent with normal growth and development. Eight wk after ablation, rats in Groups CON8 and LS8 underwent morphologic and morphometric studies. Subsequently, micropuncture studies were performed 8 wk after ablation in two additional groups of CON8 and LS8 rats.

**Protein Excretion and Blood Pressure Measurements**

Rats were placed in metabolic cages and their urine was collected over 24 h. Protein concentration was measured by precipitation with 3% sulfosalicylic acid and turbidity was determined by measuring absorbance at 595nm with a spectrophotometer (Spectronic 501; Milton Roy, Rochester, NY). Awake systolic blood pressure (SBP) was measured by using a photoelectric tail cuff device (Model 29; IITC Inc., Woodland Hills, CA) attached to a recorder (Model SE 120; IITC Inc., Woodland Hills, CA). For each rat, the value recorded represented the mean of four to six measurements obtained at a single sitting.

**Morphologic Studies**

Rats were anesthetized with Inactin (Andrew Lockwood & Associates, Sturtevant, WI) (100 mg/kg, ip). A polyethylene catheter (PE50) was inserted into the femoral artery and catheters were inserted into the jugular veins, femoral artery, and ureter for infusion of solutions and collection of samples. The kidney was exposed via a subcostal incision, placed on a polymerized methyl methacrylate holder, and illuminated with a fiberoptic light. All rats received an initial infusion of isoncotic plasma equal to 10 mL/kg body wt followed by a sustained infusion of plasma at approximately 0.5 mL/h adjusted to maintain a stable hematocrit level. Rats also received a 0.5 mL iv bolus followed by a sustained infusion (0.5 mL/h) of [3H]methoxyalinulin. Timed collections of tubular fluid were obtained by micropuncture for determination of inulin content by scintillation counting. Urine and plasma content of inulin were measured by the same method and whole-kidney and single-nephron GFR were calculated by using standard formulas. The technique of Viets et al. (14) was used to measure the protein concentration of the efferent arteriolar blood samples obtained by micropuncture. Systemic protein concentration was determined by refractometry and plasma oncotic pressure calculated with the Landis-Papenhelmer equation. Hydraulic pressure in glomerular capillaries, cortical tubules, and efferent arterioles was measured directly by using the servo-null micropipette technique (9). The determinants of ultrafiltration and segmental vascular resistances were calculated with formulas published by Baylis et al. (15).

**Morphometric Studies**

Morphometric measurements of glomerular tuft volume \( V_g \) were made by a single observer in the same kidney sections that were examined for morphologic changes. Slides that were stained with hematoxylin and eosin were examined with the Zeiss Interactive Digital Analysis System (ZIDAS; Carl Zeiss Inc., Thornwood, NY), and calculations were made on the basis of standard stereologic principles, as reviewed by Elias et al. (13). For the determination of glomerular volume, the mean cross-sectional tuft area was obtained by tracing the outlines of capillary tufts of approximately 75 glomeruli per rat. In this method, it is assumed that the glomerulus is spherical, and that the glomerular tuft cross-sectional areas represent random sections through a population of spheres that are distributed in a statistically predictable pattern. The area of each section is dependent on the diameter of the sphere and the distance of the section from the center of the sphere. From the mean cross-sectional area \( A_g \), \( V_g \) can be calculated by the equation:

\[
V_g = B/k(A_g)^{3/2}
\]

where \( B = 1.38 \), the shape coefficient for spheres, and \( k \) is the size distribution coefficient.

**Micropuncture Studies**

Micropuncture studies were performed 8 wk after ablation in two groups of rats that had ingested standard chow for 8 wk (CON8; \( N = 8 \)) or standard chow for 4 wk followed by low-salt chow for 4 wk (LS8; \( N = 8 \)). Rats were anesthetized with 100 mg/kg of Inactin, and prepared in the standard fashion for micropuncture (5). A tracheotomy was performed and polyethylene catheters were inserted into the jugular veins, femoral artery, and ureter for infusion of solutions and collection of samples. The kidney was exposed via a subcostal incision, placed on a polymerized methyl methacrylate holder, and illuminated with a fiberoptic light. All rats received an initial infusion of iso-oncotic plasma equal to 10 mL/kg body wt followed by a sustained infusion of plasma at an approximately 0.5 mL/h adjusted to maintain a stable hematocrit level. Rats also received a 0.5 mL iv bolus followed by a sustained infusion (0.5 mL/h) of [3H]methoxyalinulinulin. Timed collections of tubular fluid were obtained by micropuncture for determination of inulin content by scintillation counting. Urine and plasma content of inulin were measured by the same method and whole-kidney and single-nephron GFR were calculated by using standard formulas. The technique of Viets et al. (14) was used to measure the protein concentration of the efferent arteriolar blood samples obtained by micropuncture. Systemic protein concentration was determined by refractometry and plasma oncotic pressure calculated with the Landis-Papenhelmer equation. Hydraulic pressure in glomerular capillaries, cortical tubules, and efferent arterioles was measured directly by using the servo-null micropipette technique (9). The determinants of ultrafiltration and segmental vascular resistances were calculated with formulas published by Baylis et al. (15).

**Statistics**

Statistical analysis was performed by using a personal computer running SigmaStat software (Jandel Scientific Software, San Rafael, CA). Whole-kidney clearance, and morphologic and morphometric data in Groups CON4, CON8, and LS8 were analyzed by one-way analysis of variance followed by multiple pair-wise comparisons according to the Student-Newman-Kuels method. Micropuncture data were analyzed by unpaired t test. Statistical significance was defined as \( P < 0.05 \). Data are reported as the mean \( \pm \)SE.

**RESULTS**

**Whole Animal Data**

The mean values for initial serum creatinine (Scr) determined 3 days after renal ablation were similar in the three groups but elevated compared with normal rats \((4 \pm 0.1 \text{ mg/dL})\) (Table 1), indicating that the extent of renal ablation was approximately equivalent in the groups. All rats gained weight from time of ablation to time of euthanization. Eight wk after ablation, there were no significant differences in body weight between rats fed the standard or low-salt chow. Systolic blood pressure measured in awake rats was elevated 4 wk after ablation.
ization, there was no significant difference in SBP in rats assigned to either the normal or low-salt chow. The MAP determined in anesthetized rats revealed the presence of marked hypertension 4 wk after ablation. MAP did not change significantly in rats that had been fed standard chow between 4 and 8 wk after ablation. There was a tendency toward lower blood pressure in rats fed the low-salt chow, however, the effect was inconsistent and this difference did not reach statistical significance.

A range of morphologic abnormalities developed in the glomeruli of rats with remnant kidneys, including retraction and sclerosis of one or more segments, adhesion to Bowman’s capsule, and enlargement of Bowman’s space. Figure 1 displays the mean values quantifying the extent of glomerular injury in the three groups. Sclerosis was observed in 11.1 ± 4.6% of the glomeruli in CON4. Eight wk after ablation, the prevalence of sclerotic glomeruli in rats that had been fed standard chow (CON8) had increased significantly, to 24.0 ± 1.6%. In contrast, additional injury was prevented in rats fed the low-salt chow. LS8 rats had only 10.1 ± 3.1% sclerotic glomeruli, a mean value not different from that observed at 4 wk and significantly less than that in CON8 rats that had been fed standard chow. Thus, once instituted, dietary salt restriction completely prevented further morphologic evidence of injury in remnant-kidney rats. Notably, tubulointerstitial changes were generally commensurate with glomerular changes.

Protein excretion rates in the three groups are shown in Figure 2. Four wk after ablation, pathologic proteinuria was present in all three remnant groups and the mean protein excretion rates in the three groups were not statistically different. In rats that continued on standard chow, proteinuria progressively increased at 6 and 8 wk, reaching a mean value of 84.2 ± 8.1 mg/24 h immediately before euthanization. In low-salt rats, proteinuria actually declined to a value of 32.4 ± 6.3 mg/24 h by the eighth week after ablation. The protein excretion rate was significantly lower in LS as compared with CON rats at both 6 and 8 wk.

**Micropuncture Data**

To examine the mechanism by which salt restriction reduced glomerular damage, we used micropuncture techniques to measure glomerular hemodynamics 8 wk after ablation in rats that had been fed low-salt or standard chow for the final 4 wk. Body weight, hematocrit, MAP, and GFR levels, and the flows, pressures, and resistances in glomeruli determined in CON8 or LS8 rats are summarized in Table 2. As we had observed in the groups used in the morphologic stud-

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### Table 1. Whole animal data

<table>
<thead>
<tr>
<th>Group (Number of Animals)</th>
<th>Initial SCr (3 days) (mg/dL)</th>
<th>4-week SBP (mm Hg)</th>
<th>Weightb (g)</th>
<th>MAPb (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON4 (N = 8)</td>
<td>1.01 ± 0.05</td>
<td>NA</td>
<td>247 ± 5</td>
<td>155 ± 11</td>
</tr>
<tr>
<td>CON8 (N = 9)</td>
<td>1.09 ± 0.03</td>
<td>191 ± 10</td>
<td>270 ± 9c</td>
<td>147 ± 8</td>
</tr>
<tr>
<td>LS8 (N = 8)</td>
<td>1.04 ± 0.05</td>
<td>194 ± 7</td>
<td>282 ± 7c</td>
<td>136 ± 10</td>
</tr>
</tbody>
</table>

*Values are presented as mean ± SE. SCr, serum creatinine; SBP, systolic blood pressure; MAP, mean arterial pressure; CON4, rats euthanized 4 wk after ablation; CON8, rats fed standard chow for an additional 4 wk after ablation; LS8, rats fed low-salt chow for an additional 4 wk after ablation; NA, data not obtained.

b Weight and MAP are values determined at the time of euthanization, 4 wk after ablation in CON4, and 8 wk after ablation in CON8 and LS8.

c P < 0.05 versus CON4.

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**Figure 1.** Prevalence of abnormal glomeruli in remnant kidneys, by the (mean ± SE) percentage of abnormal glomeruli observed in rats 4 or 8 wk after ablation. CON4, rats euthanized 4 wk after ablation; CON8, rats fed standard chow for an additional 4 wk after ablation; LS8, rats fed low-salt chow for an additional 4 wk after ablation. * P < 0.05 versus CON4 and LS8. The numbers within the bars correspond to the mean values for the percentage of sclerotic glomeruli observed in each group.

**Figure 2.** Protein excretion rates in rats with remnant kidneys. Closed circle, CON4; squares, CON8; triangles, LS8 (see legend to Figure 1 for definition of groups). * P < 0.001 versus CON8.
les, there was a tendency for MAP to decline in LS rats, however, the effect was variable and did not reach statistical significance. In fact, no significant differences in glomerular hemodynamics were observed between rats on the two diets. In particular, glomerular capillary pressure was similar in the two groups, averaging 65 ± 4 mm Hg in CON8 and 67 ± 1 mm Hg in LS8 rats. Notably, these values are significantly elevated compared with the usual value in normal rats of approximately 50 mm Hg (15), confirming that glomerular capillary pressure is elevated in remnant kidneys. However, as has been reported previously in rats fed a low-salt diet from the time of ablation (5-7), the increase in glomerular pressure in remnant glomeruli is not prevented by dietary salt restriction. Whole-kidney GFR was similar in the two groups, indicating that although CON8 rats had more proteinuric and morphologic evidence of renal damage, this had not as yet resulted in a significant decline in kidney function. Therefore, our data only suggest, but do not prove, that salt restriction would also have been associated with prevention of progression to ESRD.

**Morphometric Data**

Although glomerular perfusion was not altered, we did observe an effect of salt restriction on compensatory kidney growth. Morphometric data on kidney weight and glomerular volume are presented in Table 3. To facilitate comparisons between rats euthanized 4 or 8 wk after ablation, and that had different body weights, both kidney weight and glomerular volume are factored for body weight. The kidney weight:body weight ratio was similar in rats that had been fed standard chow and euthanized either 4 or 8 wk after ablation. However, ingestion of the low-salt diet from the fourth to the eighth week was associated with a significant (20%) decline in this ratio. This reduction in kidney weight was associated with a similar inhibitory effect of salt restriction on glomerular enlargement. Unlike kidney weight, glomerular volume actually increased further (by 31%) in remnant-kidney rats between the fourth and eighth week after ablation. This increase was entirely prevented by salt restriction. On average, the glomerular volume in LS8 rats was 33% lower than that observed in CON8 rats. The mean glomerular volume in LS8 rats was even numer-

**TABLE 3. Morphometric data**

<table>
<thead>
<tr>
<th>Group (Number of Animals)</th>
<th>Kidney Weight (g/100 g)</th>
<th>Glomerular Volume (μm² × 10⁴/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON4 (N = 8)</td>
<td>0.624 ± 0.054</td>
<td>0.919 ± 0.052</td>
</tr>
<tr>
<td>CON8 (N = 9)</td>
<td>0.591 ± 0.016</td>
<td>1.289 ± 0.089</td>
</tr>
<tr>
<td>LS8 (N = 8)</td>
<td>0.484 ± 0.017a,b</td>
<td>0.862 ± 0.050b</td>
</tr>
</tbody>
</table>

*P < 0.05 versus CON4.

P < 0.05 versus CON8.
ically less than that in CON4 rats, although the difference was not statistically significant.

**DISCUSSION**

In the study presented here, as has also been repeatedly noted in other studies (2,3,6,8,9), five-sixths nephrectomy was associated with systemic and glomerular hypertension, progressive proteinuria, and glomerular sclerosis. Previous studies (16,17) have implicated an increase in glomerular capillary pressure in the pathogenesis of the glomerular sclerosis in this model. Although marked glomerular hypertension was again observed in our rats, it is clear that a reduction in glomerular pressure did not account for the beneficial effects of dietary salt restriction. Thus, in this study, in which salt restriction was introduced after hypertension and renal disease were well established, glomerular pressure was similarly elevated in both the normal and low-salt diet groups. These findings are consistent with the results of two previous studies (6,7) in the remnant-kidney model and in the uninephrectomized spontaneously hypertensive rat (SHR) (5), in which dietary salt restriction was instituted at an earlier time point, immediately after surgical reduction in renal mass. All three prior studies also failed to find a significant effect of salt restriction on glomerular pressure, although systemic blood pressure declined modestly in some cases.

As reviewed elsewhere (1), in addition to glomerular hypertension, a number of other factors have been associated with glomerular sclerosis in models of hypertension and reduced renal mass. Among these, a considerable body of data supports the hypothesis that compensatory kidney growth promotes glomerular injury. Kidney and glomerular enlargement are characteristic findings in many of the models of progressive kidney failure, including rats with remnant kidneys (6,7), desoxycorticosterone-salt hypertension (9), the uninephrectomized spontaneously hypertensive rat (SHR) (5), and streptozotocin-induced diabetes mellitus (18). Some maneuvers that had originally been thought to lessen renal injury by reducing glomerular pressure, specifically, dietary protein restriction (9) and antihypertensive therapy (2,8,19), have also been shown to inhibit renal growth, and some authors have suggested that it is the antihypertrophic rather than the hemodynamic effects of these maneuvers that retard renal injury (2,3). Analysis of these studies is complicated by the fact that glomerular hypertension and hypertrophy often coexist, making it difficult to evaluate the individual pathogenic significance of each factor. Some insight has been gained from studies in which the hemodynamic and hypertrophic responses to injury have been isolated. These studies suggest that glomerular injury is most severe when glomerular hypertension and hypertrophy coexist.

Yoshida and coworkers (3) examined rats in which renal excretory function was reduced by segmental infarction of the left kidney combined with either resection or ureteral diversion of the right kidney. Rats subjected to infarction plus ureteral diversion developed only glomerular hypertension, whereas both hypertension and hypertrophy were present in the resected group. Only resected rats developed severe glomerular injury. Meyer and Rennke (4) compared rats in which renal mass was reduced by 50% by either surgical resection or segmental infarction. Both groups developed glomerular hypertrophy, however, systemic and glomerular hypertension were only observed in infarcted rats. Once again, injury was largely limited to the group that displayed both glomerular hypertrophy and hypertension. Similar responses have also been observed in studies in which antihypertensive drugs have been used to modify glomerular hemodynamics or growth. These studies suggest that drugs that selectively reduce either glomerular pressure, reported most often with angiotensin-converting enzyme (ACE) inhibitors, or growth, most commonly reported with calcium antagonists, can reduce injury (8,19).

Several questions regarding the relationship of hypertrophy to injury are unresolved. One issue, addressed in the study presented here, is whether maneuvers that inhibit renal growth will be effective if they are initiated after renal disease is well established. In fact, several factors suggest that late therapy might be unsuccessful. Data on kidney weight and DNA synthesis after subtotal renal ablation (20) indicate that the compensatory growth response begins within 24 h after the reduction in renal mass, and actually precedes the increase in renal plasma flow and GFR. In addition, the various signals and steps that initiate and sustain the growth response are largely unknown, as are the sites at which antihypertrophic maneuvers interrupt the process. Beneficial effects of late therapy have been observed with protein restriction (21) and with administration of an ACE inhibitor (19), however, although these maneuvers may inhibit renal growth, they also reduce glomerular pressure. Studies that specifically examined antihypertrophic interventions have generally begun therapy immediately after induction of renal disease (5–8,19).

In the study presented here, rats were followed for 4 wk after five-sixths nephrectomy, by which time systemic hypertension, proteinuria, and glomerular sclerosis were well established. Morphometric analysis revealed that kidney and glomerular enlargement were also present at this stage. Nevertheless, dietary salt restriction was remarkably successful in preventing further injury; proteinuria declined and glomerular sclerosis was halted. Morphometric studies indicated that the beneficial effect of salt restriction was correlated with inhibition of renal growth. Kidney weight declined and further glomerular enlargement was prevented. These findings are consistent with the hypothesis that salt restriction reduced injury by inhibiting compensatory renal growth. Notably, the beneficial effect of salt restriction was as great as has
been reported for dietary protein restriction (21) or administration of an ACE inhibitor (22).

Although the observation that kidney size is directly related to salt intake is an old one (23), the mechanisms by which hypertrophy promotes injury and by which salt restriction limits compensatory growth are unknown and not addressed in the study presented here. In our prior study in SHR rats (5), the effect was not reproduced by the administration of a diuretic, suggesting that it is not related to alterations in total body salt content or activity of the renin-angiotensin system. In fact, angiotensin II levels are predicted to increase in salt-restricted rats, and because angiotensin II is mitogenic for many cells, including glomerular mesangial cells, this change should promote rather than retard renal growth. Because of the marked beneficial effect of ACE inhibitors on experimental renal injury, it has been suggested that activation of the renin-angiotensin system is a crucial event in the pathogenesis of progressive kidney damage. However, the fact that glomerular injury was completely arrested by salt restriction indicates that renal disease can be effectively prevented despite the ongoing activation of this system.

In summary, moderate dietary salt restriction was effective in arresting further glomerular sclerosis and reducing proteinuria in rats with remnant kidneys and established renal disease. The beneficial effect on injury was associated with a reduction in kidney weight and prevention of further glomerular enlargement. These findings are consistent with the hypothesis that hypertrophy promotes injury and that maneuvers that suppress renal growth can lessen damage despite persistence of systemic and glomerular hypertension. Whether the marked effect of dietary salt restriction to arrest experimental renal damage will also be characteristic of human renal disease has not been tested.

ACKNOWLEDGMENTS

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REFERENCES