Genetic Differences Define Severity of Renal Damage after L-NAME-Induced Hypertension in Rats

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Abstract. Genetic factors are important in determining the susceptibility to renal damage. In a backcross of the hypertensive and proteinuric fawn-hooded Erasmus University Rotterdam (FHH/EUR) rat with the normotensive, nonproteinuric August Copenhagen Irish (ACI/EUR) rat, two genes (denoted \( Rf-1 \) and \( Rf-2 \) were genetically mapped for parameters of functional and structural renal damage. The aim of the present study was to investigate the susceptibility to functional and structural renal damage in heterozygous (FHH × ACI) Fl rats compared with the parental FHH and ACI strains at similar levels of systolic BP (SBP). BP elevation was induced by chronic treatment with \( N^{G2}-\text{nitro-l-arginine methyl ester (L-NAME)} \) in either a low dose (LD, 75 to 100 mg/L) or a high dose (HD, 175 to 250 mg/L) in the drinking fluid. Survival of FHH rats and, to a lesser extent, Fl rats, was adversely affected by L-NAME treatment. All ACI rats except for one ACI-HD animal survived. In all strains, L-NAME caused a dose-dependent increase in SBP. At similar levels of SBP, the increase in functional renal damage, as indicated by the level of albuminuria, was higher in Fl compared with ACI, but lower compared with FHH. The same differences were found for the level of structural renal damage, as indicated by the incidence of glomerulosclerosis. Both the SBP and the average BP burden (SBP-Av), defined as SBP averaged over the period of follow-up, directly correlated with the level of albuminuria and incidence of glomerulosclerosis in all strains. However, the increase in the degree of renal damage per mmHg increase in SBP or SBP-Av was significantly higher in the Fl rats compared with ACI, but lower compared with FHH rats. Values for these Fl rats were closer to the ACI rats than to values for the FHH rats and increased above an SBP level of 180 mmHg. The Fl rats, being heterozygous for \( Rf-1 \) and \( Rf-2 \), as well as for other potential genes responsible for renal disease, were largely, but not completely, protected from hypertension-induced renal damage. It is concluded that complete susceptibility to hypertension-associated renal damage in rats primarily depends on the presence of predisposing genes for renal failure even after a significant increase in BP. (J Am Soc Nephrol 9: 363–371, 1998)

Genetic factors appear to be important in determining the susceptibility to renal damage. In humans, the susceptibility of a hypertensive patient to end-stage renal disease (ESRD) seems to vary with ethnicity. Although African-Americans make up only 12% of the U.S. population, 28% of patients on hemodialysis are African-American (1), and the rate of developing ESRD is four times greater for African-Americans than for Caucasians (2–6). This suggests that differences in susceptibility to renal complications may be genetically determined (5). The increased susceptibility to renal failure in African-American hypertensive patients is not accounted for solely by a higher prevalence of hypertension or severity of hypertension (1).

A dissociation between hypertension and renal disease is also present in animal models of genetic hypertension. The fawn-hooded hypertensive (FHH) rat, an inbred strain with a moderate level of systemic hypertension, develops progressive proteinuria and glomerulosclerosis (GS) at a relatively young age, leading to premature death due to ESRD (7–9). In contrast, the spontaneously hypertensive rat (SHR) develops severe systemic hypertension, but remains without significant renal damage until late in life (10). A striking difference between the two strains is the presence of glomerular hypertension in FHH rats (11), whereas in SHR rats glomerular pressure is normal (12,13).

Genetic factors influencing the development and progression of renal damage have been suggested earlier, but specific genes have not yet been found (14). However, we have recently studied the genetics of hypertension and renal damage in the FHH rat (15). Using a backcross of (FHH × August Copenhagen Irish [ACI]) F1 × FHH rats, we genetically mapped three genes on chromosome 1: one for SBP and two for renal damage in the FHH rat. The gene for SBP, denoted \( B pHt-1 \), maps to the \( S_A \) region, which is also known as an important...
gene in determining SBP in other crosses of genetically hypertensive rats (16–18). The genes for renal damage, denoted Rf-1 and Rf-2, were found to be separate and distinct from each other. The Rf-1 gene with the highest logarithm-of-odds scores appears to be a major gene responsible for the high susceptibility to renal damage in FHH rats.

Cross-breeding the FHH with the ACI rat results in an F1 animal that is heterozygous for all genes, including Rf-1 and Rf-2. In the present study, we wanted to investigate the susceptibility of the heterozygous F1 rat to hypertension-induced renal damage compared with both parental strains. Hypertension was induced by chronic administration of different doses of the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) (19–21).

Materials and Methods

Animals

Experiments were conducted in accord with Dutch law on animal experiments. A total of 93 animals was used, 7 wk old at the start of the study. FHH and ACI animals were derived from our own breeding colonies at the Erasmus University Rotterdam (EUR). F1 (FHH × ACI) rats were obtained by cross-breeding FHH/EUR and ACI/EUR rats. Animals were housed in macrolon cages with lights on from 8 a.m. to 8 p.m. Standard commercial rat chow containing 56% carbohydrates, 26% digestible protein, 7% fat, 4% fiber, and 5% minerals (AM II, Hope Farms, Woerden, The Netherlands) and drinking fluid (tap water, acidified to pH 3.0) was provided ad libitum.

Experimental Groups

Animals from each strain were divided at random into three groups, i.e., controls and rats treated with either a low dose (LD) or high dose (HD) (see below). The three ACI groups consisted initially of eight controls, seven LD-treated, and seven HD-treated rats. Data from the ACI rats were obtained from the same groups in a previous study (21). ACI rats that presented with unilateral renal agenesis at autopsy were off-line excluded from data analysis. The three FHH groups initially consisted of nine controls, 17 LD-treated, and six HD-treated rats. From a total of 36 F1 (FHH × ACI) rats, nine were used as controls, 17 were LD-treated, and 10 were HD-treated.

Induction of Hypertension

Hypertension was induced by chronic treatment with L-NAME (Sigma Chemical Co., St. Louis, MO) dissolved in the drinking water at a concentration of either 75 to 100 mg/L (LD) or 175 to 250 mg/L (HD). Age-matched control animals were provided with normal drinking fluid. Absolute L-NAME intake of the individual rats was determined at the time of the metabolic studies. The mean doses, calculated from fluid intake and body weight, are presented in Table 1.

Systolic BP

Systolic BP (SBP) was measured by tail-cuff plethysmography (ITTC Life Science, Woodland Hills, CA) in awake, restrained animals, which were prewarmed for approximately 30 min by ceramic lamps to obtain proper dilation of the tail vessels. Every Thursday between noon and 2 p.m., at least three consecutive measurements were recorded and averaged. To investigate the relationship between SBP over time and renal damage, we calculated the SBP average (SBP-Av) by averaging all SBP data obtained from 3 wk until the end of follow-up.

Metabolic Studies

Measurement of water and food intake and 24-h urine collection were done at 3, 7, and 11 wk of follow-up, by placing the animals in polycarbonate metabolic cages (Tecniplast Gazzada, Buguggiate, Italy). The animals were allowed to adapt over the weekend.

Autopsy

Moribund rats, i.e., rats showing a rapid decrease in body weight over several days to below 300 g, were autopsied at the time of detection, whereas surviving animals were sacrificed after 11 wk of follow-up. A laparotomy was performed under ether anesthesia, and a blood sample was taken from the abdominal aorta. After bleeding the animals, heart and kidneys were removed, washed with saline, and weighed. Rats that died before the end of the follow-up period were weighed and processed if possible, i.e., when obtained shortly after death.

Final GFR

The creatinine clearance rate (Cr) calculated from creatinine concentrations in plasma from blood obtained at autopsy and in urine from the last metabolic measurement shortly before autopsy was used.

Table 1. Calculated L-NAME intake in mg/kg at 3, 7, and 11 wk of treatmenta

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Week 3</th>
<th>n</th>
<th>Week 7</th>
<th>n</th>
<th>Week 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACI-LD</td>
<td>7</td>
<td>8.1 ± 0.5</td>
<td>7</td>
<td>7.0 ± 0.7</td>
<td>7</td>
<td>7.1 ± 0.9</td>
</tr>
<tr>
<td>ACI-HD</td>
<td>7</td>
<td>18.0 ± 7.6</td>
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<td>21.2 ± 5.3</td>
<td>6</td>
<td>18.1 ± 4.7</td>
</tr>
<tr>
<td>Fl-LD</td>
<td>17</td>
<td>9.1 ± 1.9</td>
<td>17</td>
<td>8.4 ± 2.6</td>
<td>17</td>
<td>7.9 ± 2.7</td>
</tr>
<tr>
<td>Fl-HD</td>
<td>10</td>
<td>16.5 ± 1.6</td>
<td>10</td>
<td>19.5 ± 3.7</td>
<td>5</td>
<td>27.2 ± 4.6</td>
</tr>
<tr>
<td>FHH-LD</td>
<td>17</td>
<td>10.8 ± 2.2</td>
<td>15</td>
<td>8.9 ± 4.2</td>
<td>1</td>
<td>16.2</td>
</tr>
<tr>
<td>FHH-HD</td>
<td>6</td>
<td>22.5 ± 5.9</td>
<td>4</td>
<td>15.4 ± 7.4</td>
<td>No survivors</td>
<td></td>
</tr>
<tr>
<td>P1/P2/P3</td>
<td>NS/NS/NS</td>
<td>NS/NS/NS</td>
<td>NS/NS/NS</td>
<td>NS/-/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4/P5/P6</td>
<td>NS/5/S</td>
<td>NS/NS/NS</td>
<td>NS/NS/NS</td>
<td>S/-/-</td>
<td></td>
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</tr>
</tbody>
</table>

*Values are given as mean ± SD. L-NAME, Nω-nitro-L-arginine methyl ester; ACI, August Copenhagen Irish; LD, low dose; HD, high dose; FHH, fawn-hooded hypertensive; NS, not significant; S, significant. P1 = ACI-LD versus Fl-LD; P2 = ACI-LD versus FHH-LD; P3 = Fl-LD versus FHH-LD; P4 = ACI-HD versus Fl-HD; P5 = ACI-HD versus FHH-HD; P6 = Fl-HD versus FHH-HD. S = P < 0.05; NS = P > 0.05.
as measure for GFR. $C_{cr} \text{ (ml/min)}$ was calculated using the formula:

$$C_{cr} = \frac{(U_{cr} \times V)/(P_{cr} \times 1.44)}{V}$$

where $U_{cr}$ is the creatinine level in urine ($\mu$mol/L), $V$ is the urine excretion rate (ml/24 h), and $P_{cr}$ is the plasma creatinine level ($\mu$mol/L). To correct for differences in body weight between strains, $C_{cr}$ was also calculated per 100 g body wt ($C_{cr}/100 \text{ g}$).

**Tissue Processing**

Kidneys were fixed by immersion for 48 h in buffered formaldehyde solution (3.6% M/V, Lommerse Pharma, Oss, The Netherlands, pH 7.4) after longitudinal bisection. Subsequently, pieces were dehydrated in alcohol and blocked in paraffin wax. Sections of 3 mm were stained with periodic acid-Schiff and hematoxylin and eosin counterstain.

The kidney sections were microscopically evaluated by determining the incidence of segmental GS as a semiquantitative injury score. For each animal, 100 glomeruli were examined in both the cortical and juxtaglomerular region, and the number of sclerotic glomeruli were counted, giving the incidence of segmental glomerulosclerosis. Criteria on which glomeruli were designated sclerotic consisted of adhesion of the glomerulus to Bowman’s capsule, thickening of Bowman’s capsule, the presence of increased amounts of periodic acid-Schiff-positive material in the mesangial region, and folding of the glomerular basement membrane with entrapment of amorphous material. Interstitial changes and vascular damage were not assessed at that time. All sections were evaluated without knowledge of the group to which individual rats belonged.

**Analytical Procedures**

Plasma and urine samples were analyzed with the ELAN system (Eppendorf/Merck, Darmstadt, Germany) for the following compounds, using colorimetric assays for total protein with molybdate red, albumin with bromocresol green, and creatinine with the Jaffé method without deproteinization.

**Statistical Analyses**

Data are presented as mean ± SD in tables and as mean ± SEM in figures. Differences in mean values between groups were compared using one-way ANOVA and a Student-Newman-Keuls test to identify the groups that were different. In case of a non-normal data distribution, groups were compared using the Mann-Whitney rank sum test. In all tests, differences were considered statistically significant for $P < 0.05$. The relationship between SBP or SBP-Av and parameters of functional and structural renal damage was assessed by linear regression analysis.

**Results**

**Survival**

All ACI animals except one in the HD group survived throughout the experiment. In contrast, a large number of FHH rats died during follow-up. In the LD group, only three of 15 completed the 11-wk follow-up. In the HD group, all six FHH rats died prematurely due to L-NAME-induced complications. From the F1 LD group, all animals completed the follow-up, but from the HD group, five of 10 rats died prematurely. The L-NAME-induced complications consisted of a decrease in body weight, severe vasoconstriction, and occasionally paralysis of the hind limbs, probably due to constriction of nervous system arteries. In general, animals showed signs of cardiac failure, i.e., constriction of the coronary arteries and scarring of heart muscle.

**Body Weight**

Body weights, shown in Table 2, progressed normally over time in the ACI groups and showed no effect of L-NAME treatment. In F1 rats, there was mild growth retardation but no deterioration in body weight in the HD-treated group over time. Body weight in control and LD-treated rats progressed normally. Body weights of the L-NAME-treated FHH rats were significantly reduced compared with controls, although the decrease in body weight was only mild in the LD-treated group.

**SBP and Albuminuria**

Values for SBP and albuminuria (UaV) obtained at 3, 7, and 11 wk are summarized in Table 2. In all three strains, a dose-dependent increase in SBP was observed. All SBP values in the L-NAME-treated rats were significantly different from the control ACI, F1, and FHH rats throughout the entire experiment. In the F1-HD group, two of five rats could not be measured at week 11 due to vasoconstriction. In the FHH rats, measurement of SBP became extremely difficult during the later part of the follow-up due to the weak or even lack of pulsation in the tail arteries caused by severe L-NAME-induced vasoconstriction. At week 7, only eight of 15 in the FHH-LD group and none in the HD group could be measured. At week 11, only two of the three remaining FHH-LD animals could not have their SBP measured for the same reason. In Table 2 and Figures 3 and 4, we have used the last measured SBP, usually obtained 1 to 2 wk earlier, to calculate the mean values and the regression equations.

Urinary protein excretion was measured simultaneously with UaV in all rats. Because changes in both parameters were almost identical, for brevity only the UaV data will be presented. All strains differed greatly in the effects of L-NAME treatment on UaV. The LD-treated ACI rats showed hardly any increase in UaV, whereas at the end of the follow-up in the HD-treated rats, UaV had increased to a mean value of 13 mg/d. In the F1 rats, UaV was moderately elevated to 30 mg/d at week 7 and further increased to 94 mg/d at week 11. In contrast, a very marked increase in UaV was observed in FHH rats already after 7 wk of follow-up. At that time, the mean UaV in the LD- and HD-treated FHH rats had increased to more than 200 mg/d. After 11 wk of follow-up, the three surviving FHH-LD rats had a mean UaV level of 269 mg/d.

**Autopsy Findings**

Body and organ weights of autopsied animals are summarized in Table 3. In the ACI rat, treatment with L-NAME had little effect on body weight and wet kidney weight. Both absolute and relative heart weight in the ACI-HD group were significantly increased when compared with those of the ACI-LD and ACI control rats. Total kidney weight was about the same in all three groups. In the F1 rats, body weights were significantly lower in HD versus LD and controls. The same applies for the relative total kidney weight. The relative heart
Table 2. Body weight (g), systolic BP (mmHg), and albuminuria (mg/d) in ACI, F1, and FHH rats at 3, 7, and 11 wk of follow-up

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 3</th>
<th></th>
<th></th>
<th></th>
<th>Week 7</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Week 11</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>BW</td>
<td>SBP</td>
<td>UaV</td>
<td>n</td>
<td>BW</td>
<td>SBP</td>
<td>UaV</td>
<td>n</td>
<td>BW</td>
<td>SBP</td>
<td>UaV</td>
<td></td>
</tr>
<tr>
<td>ACI-CON</td>
<td>8</td>
<td>229 ± 17</td>
<td>130 ± 6</td>
<td>5.1 ± 1.2</td>
<td>8</td>
<td>254 ± 22</td>
<td>119 ± 5</td>
<td>4.8 ± 1.4</td>
<td>8</td>
<td>276 ± 24</td>
<td>128 ± 7</td>
<td>3.8 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>ACI-LD</td>
<td>7</td>
<td>235 ± 8</td>
<td>159 ± 7</td>
<td>4.0 ± 0.7</td>
<td>7</td>
<td>259 ± 12</td>
<td>169 ± 9</td>
<td>5.2 ± 1.1</td>
<td>7</td>
<td>278 ± 15</td>
<td>165 ± 9</td>
<td>6.3 ± 2.3</td>
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</tr>
<tr>
<td>ACI-HD</td>
<td>7</td>
<td>213 ± 6</td>
<td>170 ± 7</td>
<td>5.2 ± 0.9</td>
<td>7</td>
<td>242 ± 7</td>
<td>172 ± 9</td>
<td>10.1 ± 3.7</td>
<td>6</td>
<td>264 ± 70</td>
<td>206 ± 18</td>
<td>12.7 ± 9.0</td>
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<tr>
<td>P1/P2/P3</td>
<td>NS/S/S</td>
<td>S/S/S</td>
<td>NS/NS/NS</td>
<td>NS/NS/NS</td>
<td>NS/NS/NS</td>
<td>S/S/S</td>
<td>NS/NS</td>
<td>NS/NS</td>
<td>NS/NS/NS</td>
<td>S/S/S</td>
<td>NS/NS</td>
<td>NS/NS</td>
<td></td>
</tr>
<tr>
<td>F1-CON</td>
<td>9</td>
<td>284 ± 28</td>
<td>126 ± 2</td>
<td>5.5 ± 1.0</td>
<td>9</td>
<td>315 ± 41</td>
<td>125 ± 5</td>
<td>5.6 ± 1.3</td>
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<td>356 ± 31</td>
<td>134 ± 5</td>
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<td>F1-LD</td>
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<td>287 ± 24</td>
<td>155 ± 7</td>
<td>6.6 ± 2.8</td>
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<td>327 ± 26</td>
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<td>359 ± 25</td>
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<tr>
<td>F1-HD</td>
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<td>274 ± 22</td>
<td>172 ± 4</td>
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<td>10</td>
<td>300 ± 19</td>
<td>201 ± 16</td>
<td>30 ± 25</td>
<td>5</td>
<td>299 ± 21</td>
<td>210 ± 16b</td>
<td>94 ± 31</td>
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<tr>
<td>P1/P2/P3</td>
<td>NS/NS/NS</td>
<td>S/S/S</td>
<td>NS/NS/NS</td>
<td>NS/NS/NS</td>
<td>NS/NS/NS</td>
<td>S/S/S</td>
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<tr>
<td>FHH-CON</td>
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<td>284 ± 26</td>
<td>144 ± 9</td>
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<td>9</td>
<td>330 ± 25</td>
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<td>151 ± 5</td>
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<td>282 ± 23</td>
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<td>FHH-HD</td>
<td>6</td>
<td>267 ± 31</td>
<td>187 ± 12</td>
<td>46 ± 45</td>
<td>4</td>
<td>241 ± 15</td>
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<td>223 ± 27</td>
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</tr>
<tr>
<td>P1/P2/P3</td>
<td>NS/NS/NS</td>
<td>S/S/S</td>
<td>NS/NS/NS</td>
<td>NS/NS/NS</td>
<td>NS/NS/NS</td>
<td>S/S/S</td>
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<td>NS/NS</td>
<td>S/S/S</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are given as mean ± SD. BW, body weight; SBP, systolic BP; UaV, albuminuria; CON, control. Other abbreviations as in Table 1. P1 = LD versus CON; P2 = HD versus CON; P3 = LD versus HD; P4 = ACI-LD versus F1-LD; P5 = ACI-LD versus FHH-LD; P6 = F1-LD versus FHH-LD; P7 = ACI-HD versus F1-HD; P8 = ACI-HD versus FHH-HD; P9 = F1-HD versus FHH-HD; P10 = ACI-CON versus F1-CON; P11 = ACI-CON versus FHH-CON; P12 = F1-CON versus FHH-CON. S = P < 0.05; NS = P > 0.05.

b At week 11, SBP could be measured in two rats only; for the other three rats, the last measured SBP obtained 1 wk earlier was used.

c At week 7, SBP could be obtained in eight rats, only; for the other seven rats, the last measured SBP (1 to 2 wk earlier) was used.

d At week 11, SBP could be obtained in one rat only; for the other rats, the last measured SBP obtained 3 wk earlier was used.

e At week 7, SBP could not be measured; data obtained at week 6 were used.
weight was significantly increased in both treated groups compared with controls. In the FHH, body weight was severely reduced by L-NAME treatment. Relative kidney weight was significantly increased in both treated groups compared with controls. In combination with the lower body weights of the treated rats, the differences in relative heart weight were even more pronounced.

**Glomerulosclerosis**

The incidence of GS presented in Table 3 indicates a dose-dependent increase in structural renal damage in all strains. However, the increase in L-NAME-treated ACI rats was much less marked than that observed in both groups of treated Fl and FHH rats. The most severe structural damage was observed in the treated FHH rats. It must be stated that GS of FHH controls was also higher compared with ACI and Fl controls.

**Creatinine Clearance**

The final plasma creatinine and calculated \( C_{\text{Cr}} \) values, as measured for GFR, are presented in Table 4. The mean \( C_{\text{Cr}} \) values in L-NAME-treated ACI rats were not significantly different from controls. In contrast, mean \( C_{\text{Cr}} \) was higher in control and treated Fl rats compared with ACI. However, \( C_{\text{Cr}} \) in the Fl-HD was severely decreased compared with control and LD rats, indicative of a deteriorating GFR. In the FHH controls, \( C_{\text{Cr}} \) was higher compared with Fl and ACI control and treated rats. FHH rats treated with L-NAME all showed a significantly lower \( C_{\text{Cr}} \) compared with controls and ACI and Fl control and treated rats. Differences are even greater when \( C_{\text{Cr}} \) per 100 g body wt (\( C_{\text{Cr}}/100 \ g \), Table 4) is considered.

**Comparison of Groups with Similar SBP Levels**

Because we wanted to relate the development of renal damage in groups with approximately the same BP levels, we compared the data UaV and GS in the ACI-HD, Fl-HD, and FHH-LD groups. All three groups developed hypertension, the final SBP level being approximately 210 mmHg. The ACI rats, however, showed a more gradual rise in SBP than the Fl and FHH rats (Table 2).

Figure 1 shows the changes in UaV in the three groups. FHH-LD rats already developed UaV after 3 wk of treatment, progressing with time to approximately 270 mg/d at week 11. ACI-HD rats developed no increase in UaV during the study. Fl rats showed intermediate responses. At week 7, there was an increase from 30 to 95 mg/d at week 11.

Figure 2 shows that at autopsy, the incidence of GS is significantly higher in the FHH-LD rats compared with the ACI and Fl-HD rats. Hypertensive ACI-HD rats showed only a moderate degree of GS, with Fl-HD rats being intermediate. This indicates that the structural renal damage at similar SBP levels is also more pronounced in FHH than in ACI and Fl rats.

**Comparison of the Three Strains by Regression Analysis**

We also compared the three strains by examining the relationships between SBP and the parameters for functional (i.e., UaV) and structural (i.e., incidence of GS) renal damage. Figures 3 and 4 show the scatter plots of the relationship between SBP and UaV in the three strains at weeks 7 and 11 of the follow-up, respectively. In the ACI rats, the correlation

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**Table 3.** Body, kidney, and heart weights and incidence of glomerulosclerosis at autopsy after 11 wk of follow-up or at premature death.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW (g)</th>
<th>TKW (mg)</th>
<th>TKW/100 g</th>
<th>GS (%)</th>
<th>HW (mg)</th>
<th>HW/100 g</th>
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</thead>
<tbody>
<tr>
<td>ACI-CON</td>
<td>8</td>
<td>279 ± 12</td>
<td>1953 ± 100</td>
<td>701 ± 27</td>
<td>0.9 ± 0.6</td>
<td>736 ± 34</td>
<td>265 ± 14</td>
</tr>
<tr>
<td>ACI-LD</td>
<td>7</td>
<td>272 ± 16</td>
<td>1784 ± 74</td>
<td>658 ± 21</td>
<td>3.7 ± 1.3</td>
<td>756 ± 42</td>
<td>279 ± 11</td>
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<tr>
<td>ACI-HD</td>
<td>7</td>
<td>261 ± 18</td>
<td>1856 ± 136</td>
<td>717 ± 88</td>
<td>7.0 ± 1.5</td>
<td>835 ± 37</td>
<td>321 ± 17</td>
</tr>
<tr>
<td>P1/P2/P3</td>
<td>8</td>
<td>NS/NS/NS</td>
<td>S/NS/NS</td>
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<td>S/NS/NS</td>
<td>NS/NS/NS</td>
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<tr>
<td>F1-CON</td>
<td>9</td>
<td>362 ± 30</td>
<td>2597 ± 127</td>
<td>719 ± 31</td>
<td>2.0 ± 1.6</td>
<td>942 ± 57</td>
<td>261 ± 11</td>
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<tr>
<td>F1-LD</td>
<td>17</td>
<td>365 ± 32</td>
<td>2538 ± 179</td>
<td>698 ± 33</td>
<td>7.3 ± 4.4</td>
<td>1072 ± 82</td>
<td>295 ± 25</td>
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<tr>
<td>F1-HD</td>
<td>8</td>
<td>265 ± 27</td>
<td>2479 ± 299</td>
<td>940 ± 98</td>
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<td>394 ± 31</td>
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<tr>
<td>FHH-CON</td>
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<td>342 ± 25</td>
<td>2463 ± 183</td>
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<td>4.2 ± 1.5</td>
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<td>FHH-LD</td>
<td>16</td>
<td>245 ± 24</td>
<td>2582 ± 81</td>
<td>1016 ± 42</td>
<td>31.4 ± 7.0</td>
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<td>FHH-HD</td>
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<td>2688 ± 171</td>
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*Values are given as mean ± SD. TKW, total wet kidney weight; TKW/100 g, TWK per 100 g BW; GS, glomerulosclerosis; HW, wet heart weight; HW/100 g, wet HW per 100 g BW. Other abbreviations as in Tables 1 and 2.*
Figure 2. The incidence of glomerulosclerosis (GS) in ACI-HD, Fl-HD, and FHH-LD rats. Values are given as mean ± SEM. ACI-HD (■); Fl-HD (□); and FHH-LD (○). Numbers above the bars indicate the number of animals that were evaluated. *P < 0.05 Fl-HD versus ACI-HD; **P < 0.05 FHH-LD versus ACI and Fl HD.

FHH rats (Figure 3). Thus, already at week 7, the increase in UaV per mmHg increase in SBP in FHH rats was approximately 50 times higher than in ACI and approximately 10 times higher than in Fl rats. The Fl rats showed an increase in albumin loss five times higher than the ACI rats.

When comparing the equations obtained at week 11, as shown in Figure 4, it is clear that the rise in UaV per mmHg increase in SBP was highest in the FHH rats, i.e., 3.1 mg/d. The rise was only 0.12 in the ACI rats. The Fl rats showed a biphasic distribution pattern. Up to an SBP level of 180 mmHg,
Figure 3. Relationship between systolic BP (SBP) and UaV at week 7. Slopes of the regression lines are indicated at the different lines. ACI (Δ); F1 (□); FHH (∆). ACI (— —); F1 (——); FHH (— —). See also footnotes a and b of Table 2. Equations: ACI, UaV = 0.04 × SBP + 0.8 (n = 22, r = 0.298, P = 0.177); F1, UaV = 0.2 × SBP − 15.9 (n = 36, r = 0.329, P = 0.05); FHH, UaV = 2.0 × SBP − 215 (n = 28, r = 0.660, P < 0.001).

Figure 4. Relationship between SBP and UaV at week 11. Slopes of the regression lines are indicated next to the different lines., ACI (Δ); F1 (□); FHH (∆). ACI (— —); F1 (——); FHH (— —). See also footnotes c and d of Table 2. Equations: ACI, UaV = 0.12 × SBP − 12.4 (n = 21, r = 0.655, P = 0.001); F1, UaV = 0.2 × SBP − 22.0 (first part, n = 21, r = 0.814, P < 0.001), and UaV = 1.7 × SBP − 288 (last part, n = 22, r = 0.0708, P < 0.001); FHH, UaV = 3.1 × SBP − 411 (n = 12, r = 0.896, P < 0.001).

UaV increased only with 0.2 mg per mmHg increase in SBP, which was similar to the situation at week 7. Above the SBP level of 180 mmHg, the increase was eight times more, up to 1.7 mg/d. These data indicate that F1 rats were protected against the development of severe UaV up to an SBP level of 180 mmHg.

Comparing the UaV obtained at week 11 with SBP-Av, i.e., the SBP averaged over the entire follow-up period, showed a similar pattern. For each mmHg increase in SBP-Av, the increase in UaV was 3.4 mg/d in FHH, 0.9 mg/d in F1, and 0.2 mg/d in ACI rats.

At autopsy, the incidence of GS correlated with SBP-Av in all strains. However, the increase in GS per mmHg in FHH (GS = 0.7 × SBP-Av − 92, n = 29, r = 0.886, P < 0.001) was sevenfold larger than in ACI (GS = 0.1 × SBP-Av − 11, n = 22, r = 0.842, P < 0.001) and approximately fourfold larger than in F1 (GS = 0.2 × SBP − 25, n = 34, r = 0.727, P < 0.01). The increase was twice as much in the F1 compared with ACI. Significant correlations were also present between the incidence of GS and UaV at week 11 in FHH (r = 0.851, P < 0.001) and F1 rats (r = 0.836, P < 0.001). Such a correlation was absent in ACI rats (r = 0.321, P = 0.156).

Relative heart weight was also directly related to SBP-Av in FHH (r = 0.668; P < 0.001), F1 (r = 0.741, P < 0.001), and ACI (r = 0.751; P < 0.001) rats. For each mmHg increase in SBP-Av, the increase in relative heart weight was 2.5 mg/100 g body wt in FHH, 1.5 mg/100 g body wt in F1, and 0.9 mg/100 g body wt in ACI rats.

Discussion

The present study was performed to compare the development of renal damage in the presence of L-NAME-induced hypertension in two rat strains differing in susceptibility and in the intercross of both strains. The presented data clearly support the hypothesis that in rats, the susceptibility to renal damage depends on genetic background. It was demonstrated that at similar levels of hypertension, FHH rats developed more severe functional and structural renal damage than did the ACI rats. (FHH × ACI) F1 rats showed intermediate responses, genetically closer to ACI than to FHH rats. The ACI rats were largely protected from developing marked UaV up to an SBP level of 225 mmHg. The F1 rats were protected to a level of approximately 180 mmHg, whereas FHH rats were not protected at all. Furthermore, the FHH and F1 rats showed correlations between SBP and UaV or GS that were present earlier and had steeper slopes than those in the ACI rats, with the F1 rats again being intermediate. Using the SBP-Av instead of SBP at each time point, similar differences were observed, indicating that a similar BP burden over the treatment period caused more renal damage in the FHH and the F1 than in the ACI rat.

A major drawback was the high mortality of the L-NAME-treated FHH rats. In previous studies, we observed that unilaterally nephrectomized FHH rats and FHL rats with two kidneys also died prematurely during chronic L-NAME treatment (21,22). Others reported a very high mortality rate in L-NAME-treated SHR rats (23). A decrease in GFR, indicated by a decrease in Cr, was found in the F1-HD and both treated FHH groups. However, none of the screened FHH and F1 rats that died before the end of the follow-up did so because of terminal renal failure. We think that cardiac or central nervous damage...
due to the severe L-NAME-induced vasoconstriction, in combination with the high BP, would be the most likely cause of the premature death. Thus, apart from the kidney, other organ systems of heterozygous rats are also more sensitive to the adverse effects of nitric oxide synthase inhibition. Relative heart weight was increased in treated rats of all strains, but heart weights were higher in treated FHH and F1 rats compared with treated ACI rats, being most pronounced in FHH rats. Cardiac hypertrophy after chronic L-NAME treatment has also been reported by others (24,25), although some authors found the increase in cardiac weight to be relatively mild (26,27).

An increase in SBP appears to be a universal characteristic of chronic L-NAME treatment, because it has been reported to occur in various rat strains (19-33). Micropuncture studies have shown that the elevation of systemic pressure is accompanied by an increase in intraglomerular capillary pressure (19,22,29,33). The effects of the L-NAME-induced hypertension on urinary protein or albumin excretion or on structural renal damage have only occasionally been reported. However, in our previous study in normotensive Fawn-Hooded (FHL) rats, we showed that renal damage occurs upon BP elevation (21). This strain is also susceptible to renal damage, and multiple gene interaction is likely to be involved. A relatively mild increase in proteinuria, UA, or GS, indicating a relatively mild degree of renal damage similar to that observed by us in the ACI rat, has been reported after investigating Munich-Wistar (19,20,29,32) or Sprague Dawley rats (28) with two intact kidneys. However, it should be noted that a study directly comparing various rat strains with regard to the effects of L-NAME-induced hypertension on renal damage, such as the one presented here, has not been reported.

The effects of chronic treatment with L-NAME appear to be more distinct in rats with reduced renal mass or other models of renal damage. It has been reported that L-NAME worsens renal damage in the unilaterally nephrectomized FHH rat (22) and in Munich-Wistar rats after subtotal nephrectomy (33). In addition, it has been reported that in Munich-Wistar rats, sodium excess aggravates both the L-NAME-induced systemic and glomerular hypertension, leading to more severe renal parenchymal injury (29).

In conclusion, F1 rats, which are heterozygous for renal failure genes, are partially protected from hypertension-associated renal damage. At similar levels of hypertension, (FHH × ACI) F1 rats are more vulnerable than ACI rats to L-NAME-induced complications, but less susceptible than the parental FHH rats. The present data support our previous observation that susceptibility to renal damage in rats is genetically determined (15).

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References


