High-salt diet reveals the hypertensive and renal effects of reduced nephron endowment

Leah-Anne M. Ruta,1 Hayley Dickinson,1 Merlin C. Thomas,2 Kate M. Denton,1 Warwick P. Anderson,1 and Michelle M. Kett1

1Department of Physiology, Monash University, Clayton, Victoria; and 2Danielle Alberti Memorial Centre for Diabetic Complications, Baker IDI Heart and Diabetes Institute, Victoria, Australia

Submitted 29 January 2010; accepted in final form 19 March 2010

Ruta LM, Dickinson H, Thomas MC, Denton KM, Anderson WP, Kett MM. High-salt diet reveals the hypertensive and renal effects of reduced nephron endowment. Am J Physiol Renal Physiol 298: F1384–F1392, 2010. First published March 24, 2010; doi:10.1152/ajprenal.00049.2010.—The extent to which a reduced nephron endowment contributes to hypertension and renal disease is confounded in models created by intrauterine insults that also demonstrate other phenotypes. Furthermore, recent data suggest that a reduced nephron endowment provides the “first hit” and simply increases the susceptibility to injurious stimuli. Thus we examined nephron number, glomerular volume, conscious mean arterial pressure (MAP), and renal function in a genetic model of reduced nephron endowment before and after a high-salt (5%) diet. One-year-old glial cell line-derived neurotrophic factor wild-type (WT) mice, heterozygous (HET) mice born with two kidneys (HET2K), and HET mice born with one kidney (HET1K) were used. Nephron number was 25% lower in HET2K and 65% lower in HET1K than WT mice. Glomeruli hypertrophied in both HET groups by 33%, resulting in total glomerular volumes that were similar between HET2K and WT mice but remained 50% lower in HET1K mice. On a normal-salt diet, 24-h MAP was not different between WT, HET2K, and HET1K mice (102 ± 1, 103 ± 1, and 102 ± 2 mmHg). On a high-salt diet, MAP increased 9.1 ± 3.9 mmHg in HET1K mice (P < 0.05) and 5.4 ± 0.9 mmHg in HET2K mice (P < 0.05) and did not change significantly in WT mice. Creatinine clearance was 60% higher in WT mice but 30% lower in HET2K and HET1K mice fed a high-salt diet than in controls maintained on a normal-salt diet. Thus a reduction in nephron number (or total glomerular volume) alone does not lead to hypertension or kidney disease in aged mice, but exposure to high salt uncovers a hypertensive and renal phenotype.

nephron number; congenital nephron deficit; hypertension; unilateral renal agenesis; salt sensitivity

There are large variations in the number of nephrons in human kidneys (18, 50), and it has been suggested that those endowed with few nephrons are at an increased risk of developing hypertension and progressive renal disease (4). A host of animal studies have supported this concept (28, 33, 49), but the maternal or intrauterine insults used in these studies usually result in a wide spectrum of consequences for the offspring (2, 15, 21, 24, 34), in addition to reductions in nephron number. Thus the extent to which a reduction in nephron endowment per se contributes to the development of hypertension or renal insufficiency is unclear.

Glial cell line-derived neurotrophic factor (GDNF), produced by the metanephric mesenchyme, has two important roles in kidney development. First, GDNF is crucial for the initiation of the branching of the ureteric bud from the Wolffian duct, enabling it to enter the metanephric mesenchyme and initiate kidney development (27, 35, 36). In the complete absence of GDNF, the kidneys fail to form and the mice die within 24 h (27, 35, 36). Second, once the ureteric bud enters the metanephric mesenchyme, GDNF induces the branching of the ureteric epithelium and, thus, is a determinant of the final number of nephrons in the adult kidney (8). Because of these distinct roles, GDNF heterozygous (HET) mice are born with two renal phenotypes, allowing for a within-strain study of the correlation between nephron endowment and pathophysiological outcomes. Most GDNF HET mice are born with two smaller kidneys (HET2K) with a moderate (~30%) nephron deficit and a 45% reduction in glomerular capillary surface area and length that is normalized by 30 wk (8, 9, 40). Despite their nephron deficit, HET2K mice do not demonstrate elevated conscious blood pressure, measured by an indwelling catheter (40), or altered glomerular filtration rate (GFR) in old age (3, 9). Approximately 20% of the HET population demonstrate unilateral renal agenesis (URA) at birth (HET1K) (27, 35, 36). The kidney of only one of these HET1K mice (a 30-day-old female) has previously been examined, suggesting a marked 50% deficit in total nephron number (8). It is not known whether these solitary kidneys can fully compensate for the marked congenital deficit in glomerular number and volume, nor is it known whether blood pressure or renal function is affected.

A concept that is emerging in the literature is that a reduced nephron number or filtration surface area (FSA) alone may not be sufficient to produce overt nephropathies or hypertension but, instead, may act as the primary insult or “first hit” on the kidney, thereby increasing susceptibility to a secondary insult or “second hit” (28, 31). Genetics, obesity, and diet may play crucial roles in moderating susceptibility to hypertension and renal disease in those born with fewer nephrons. The GDNF mouse provides a robust model in which to study the implication of a high dietary salt insult on a background of reduced nephron endowment. We have studied aged mice who lived for 1 yr with two degrees of reduced nephron endowment, moderate (HET2K) and marked (HET1K), and compared them with wild-type (WT) littermates. Subsets of each group were then fed a high-salt diet. Our hypothesis was that mice born naturally with a low nephron number would not develop hypertension and renal disease under normal controlled conditions, even at 1 yr of age, but that blood pressure would be elevated and renal function reduced when these mice are challenged with a high-salt diet.
METHODS

Experiments were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and approved in advance by the Monash University School of Biomedical Sciences Animal Ethics Committee. The GDNF mouse colony was established through matings between male GDNF HET mice (hybrid 129Sv and C57BL/6 background) and C57BL/6 females. Tails of GDNF wild-type (WT) mice, HET mice born with 2 kidneys (HET2K), and HET mice born with 1 kidney (HET1K) were clipped approximately one-third of the way from the base of the tail and a 2-mm thick glycolmethacrylate plug was used to seal the wound. GDNF HET mice born with 1 kidney (HET1K) were clipped on the left flank. After recovery (10 days), arterial pressures and heart rate were continuously recorded, with the probe body subcutaneously on the right flank. After recovery (10 days), arterial pressures and heart rate were measured for 4 days by sampling for 10 s every 10 min.

Basic measurements. At 48–52 wk of age, trained male WT (n = 20), HET2K (n = 10), and HET1K (n = 11) mice were placed in metabolic cages for a 24-h period (47). Food and water consumption and urine and fecal production were recorded, and urine samples were collected and frozen (−20°C) for subsequent analysis.

Mice were then anesthetized (isoflurane in 40% O2-60% N2; 4.5% induction, 2.2–2.6% maintenance; Rhodia Australia) for implantation of the radiotelemetry transmitter catheter (TAl11PA-C10, Data Sciences International) into the left carotid artery and probe body subcutaneously on the right flank. After recovery (10 days), arterial pressures and heart rate were measured for 4 days by sampling for 10 s every 10 min.

Effect of high-salt diet. After baseline measurements, approximately half of the WT (n = 13), HET2K (n = 5), and HET1K (n = 6) mice were randomly allocated to receive a high-salt [5% (wt/wt) NaCl] diet (SF05-023, Specialty Feeds, Australia) for 7 days, while the remaining WT (n = 7), HET2K (n = 5), and HET1K (n = 5) mice continued to receive the normal-salt diet, acting as time controls. Arterial pressures and heart rate were continuously recorded, with the final 4 days of recording analyzed. All mice then underwent a second 24-h urine collection. These values were compared with baseline values obtained on normal salt, allowing within-animal comparisons in the three groups. Mice were then anesthetized (isoflurane), and a terminal blood sample was taken. Kidneys and heart were excised, weighed, and immersion-fixed in 10% buffered formalin.

Urinary and plasma analysis. Urine and plasma samples were analyzed for Na⁺ (Synchron CX5, Beckman Coulter) and osmolality (freezing-point depression; Advanced Osmometer 2020, Advanced Instruments), and urinary albumin concentration was measured (Abuwell M ELISA, Exocell). Urinary and plasma creatinine concentration were measured by HPLC, and creatinine clearance (CrCl) was determined as an estimate for GFR (5, 13). Plasma renin concentration (PRC) was measured by radioimmunoassay (32). CrCl and PRC measurements were obtained from the six groups only at the conclusion of the experiment, and not under baseline conditions.

Estimating glomerular number and glomerular volume. Kidneys of mice maintained on the normal-salt diet were processed for stereological analysis. The whole right kidney of WT (n = 6) and HET2K (n = 5) mice was compared with the sole kidney of HET1K mice (n = 5; 3 left and 2 right). The unbiased stereological physical disector-fractionator technique was used to estimate single kidney glomerular number and volume from 20-μm-thick glycolmethacrylate sections stained with periodic acid-Schiff reagent as previously described (8, 9, 22). Values for whole animal nephron number and total glomerular volume were obtained by multiplying the values obtained for one kidney by 2 for WT and HET2K mice, to account for the presence of two kidneys.

Statistical analysis. Values are means ± SE. Statistical analysis of baseline data between groups was performed using a one-way ANOVA with Bonferroni’s post hoc test. The impact of the high-salt diet was assessed by a two-way ANOVA, with diet (D) and group (G) as fixed factors. P ≤ 0.05 was considered statistically significant.

RESULTS

Neophrin number and glomerular volume. Neophrin number of the right kidney was ~25% lower in HET2K than WT mice (9.576 ± 560 vs. 12.694 ± 350, P < 0.001). Neophrin number of the single kidney of HET1K mice was 8.952 ± 507, ~30% lower than that of the right kidney of WT mice (P < 0.001) but not significantly different from that of the right kidney of HET2K mice (P = 0.9). When these values were used to calculate whole animal nephron number (WT and HET2K multiplied by 2 to account for the presence of 2 kidneys), neophrin number was 65% lower in HET1K mice and 25% lower in HET2K mice than in WT mice (Fig. 1A).

Compensatory glomerular hypertrophy was evident, with mean individual glomerular volumes of HET2K and HET1K mice significantly greater than WT mice (Fig. 1B). However, mean individual glomerular volumes of HET2K and HET1K mice were not different from each other. Single-kidney glomerular volume (mean glomerular volume × single kidney nephron number) was not different between the three groups (3.14 ± 0.08, 3.08 ± 0.18, and 2.98 ± 0.17 × 10⁻⁴ mm³ for WT, HET2K, and HET1K, respectively). However, in the absence of a second kidney in HET1K mice, this resulted in an overall total glomerular volume for HET1K mice that was half of that for WT and HET2K mice (Fig. 1C). Total glomerular volume of HET2K mice was not significantly different from WT mice (Fig. 1C).

Gross histological examination revealed no evidence of renal disease, such as glomerulosclerosis, interstitial fibrosis, tubular dilatation/degeneration, or glomerular obsolescence, in the three groups of mice (Fig. 2). Glomerular capillaries appeared normal in shape, with no evidence of giant or irregular capillary loops in any of the groups.

Blood pressure and renal function on normal salt. On normal salt, there were no significant differences between WT (n = 20), HET2K (n = 10), and HET1K (n = 11) mice in 24-h MAP (Fig. 1D) or heart rate (548 ± 9, 540 ± 14, and 573 ± 12 beats/min, respectively). Circadian MAP (95 ± 1, 97 ± 1, and 95 ± 1 mmHg during the day and 109 ± 2, 108 ± 2, and 109 ± 2 mmHg at night for WT, HET2K, and HET1K, respectively) were not different between groups, nor were 24-h or circadian systolic and diastolic blood pressures different between groups (data not shown).

Water intake and urinary excretion were not different between HET2K and WT mice, but both were significantly greater in HET1K mice (Fig. 1, E and F). Food intake was greater in HET1K (152 ± 12 μg·24 h⁻¹·g body wt⁻¹, P < 0.05) but not HET2K (123 ± 12 μg·24 h⁻¹·g body wt⁻¹) mice than in WT mice (115 ± 8 μg·24 h⁻¹·g body wt⁻¹). Urinary Na⁺ and osmolar excretion were also significantly greater in HET1K (P < 0.05) than WT and HET2K mice (Fig. 1, G and H). Urinary osmolality was significantly lower in HET1K mice than HET2K and WT littermates (1,948 ± 73, 2,540 ± 134, and 2,768 ± 91 mosmol/kgH₂O, respectively, P < 0.001). WT and HET2K mice did not differ in any of these parameters. Baseline 24-h urinary albumin excretion was not different between groups (Fig. 1).

Effects of high-salt diet on blood pressure and renal function. Characteristics of WT, HET2K, and HET1K mice fed the high- or normal-salt diet for 7 days are shown in Table 1. The diets did not significantly affect body weight in any group.
The sole kidney of HET1K mice fed the normal-salt diet was 15% larger than single kidneys of WT mice and 34% larger than single kidneys of HET2K mice. However, total kidney weight was 43% lower in HET1K mice and 15% lower in HET2K mice than in WT mice (Table 1). Kidney weights were significantly greater in mice fed the high-salt diet than in those fed the normal-salt diet (Table 1). No differences were seen in left ventricular weight on normal salt, although left ventricular weight-to-body weight ratios were higher in HET1K than WT mice (P < 0.05).

The 7-day high-salt diet led to differential increases in MAP across the groups [Pd < 0.001, \( P_g \times P_d \) \( < 0.05 \); Fig. 3A]. The high-salt diet caused 24-h MAP to increase by 9.1 \( \pm \) 1.9 mmHg in HET1K mice (Fig. 3A; \( P < 0.05 \), paired \( t \)-test comparing within-animal MAP changes from baseline) and 5.4 \( \pm \) 0.9 mmHg in HET2K mice (\( P < 0.05 \)) but had no significant effect in WT mice (1.9 \( \pm \) 0.8 mmHg). The stepwise increase in blood pressure in the WT, HET2K, and HET1K high-salt groups demonstrated a highly significant linear trend (\( P < 0.001 \)). Similar increases in 24-h and circadian systolic and diastolic blood pressure in HET1K and HET2K mice were also seen (data not shown). Mice maintained on the normal-salt diet for 7 days after baseline recordings showed minimal, nonsignificant changes in 24-h MAP (Fig. 3A). Heart rate was unaffected by dietary salt in the three groups of mice.

On the normal-salt diet, \( C_F \) was greater in HET1K and HET2K than WT mice (Fig. 3B; \( P < 0.05 \), 1-way ANOVA with Bonferroni’s post hoc test). The effect of the high-salt diet was also different among the groups. Although \( C_F \) was 60% greater in WT mice fed the high-salt diet than in their normal-salt diet-fed controls, \( C_F \) was 30% lower in HET2K and HET1K mice fed the high-salt diet than in their normal-salt diet-fed controls (\( P_g \times d \) \( < 0.001 \); Fig. 3B). In response to the high-salt diet, 24-h urinary albumin excretion increased significantly in WT, HET2K, and HET1K mice (Fig. 3C; \( P < 0.01 \), \( P < 0.05 \), and \( P < 0.05 \), respectively, by paired \( t \)-test com-
was higher in mice fed the high-salt diet, although the values were similar across the groups ($P_g = NS$; Table 2). Minimal nonsignificant changes were observed in these variables in mice maintained on the normal-salt diet (Table 2).

**Plasma electrolytes and renin concentration.** Plasma Na$^+$ concentration and osmolality were not significantly different between groups on either diet (Table 2). Hematocrit was significantly lower in HET1K than WT and HET2K mice. On the normal-salt diet, PRC was 50% lower in HET1K than WT mice ($P < 0.05$; Fig. 3D). PRC of HET2K mice was not significantly different from that of WT mice ($P = 0.08$; Fig. 3D). PRC was proportionally lower in all high-salt diet- than normal-salt diet-fed groups ($P_d < 0.001$; Fig. 3D).

**DISCUSSION**

We have shown that, despite moderate and marked levels of nephron deficiency in GDNF HET2K and HET1K mice, there was no relationship between nephron number or total glomerular volume and MAP or renal function in aged mice maintained on a normal-salt diet. MAP and renal function were, however, susceptible to a secondary insult in the form of an acute high-salt diet. For MAP, this susceptibility appeared dependent on nephron dosage, and not apparent FSA. These findings, utilizing a genetic model of reduced nephron endowment, suggest that deficits in nephron number or glomerular volume alone do not always directly translate to hypertension or renal disease, even when examined well into adulthood.

**Nephron number and glomerular volume.** This is the first time a detailed stereological and functional analysis has been performed in any species born with URA. The sole kidney of HET1K mice is identical in nephron number and mean individual glomerular volume to a single kidney of the HET2K mice. However, if the presence of two kidneys in HET2K and WT mice is taken into account, HET1K mice exhibited an overall marked 65% reduction in glomerular number, compared with a moderate 25% reduction in HET2K mice compared with WT mice. HET2K and HET1K mice demonstrated significant glomerular hypertrophy. Mean individual glomerular volume was 33% greater in HET2K than WT mice, offsetting the 25% reduction in nephron number, such that total glomerular volume of HET2K mice was not significantly different from that of WT littermates, consistent with our previous findings (9, 40). Although glomerular hypertrophy was also evident in HET1K mice and of the same 33% magnitude as in HET2K mice, it was not sufficient to fully compensate for the 65% deficit in nephron number, leaving total glomerular volume (and thus, one could argue, total glomerular FSA) 50% reduced compared with WT mice. Thus mice of the GDNF strain are born with a naturally occurring, wide range in nephron number and differences in adult total glomerular volume. This provides a unique, within-strain model with which to study the correlation between nephron endowment and adult pathophysiological outcomes.

**Basal blood pressure.** Neither the moderate (HET2K) nor severe (HET1K) nephron deficit nor the persistent reduction in total glomerular volume in the HET1K mouse impacted on adult blood pressure of 1-yr-old GDNF HET mice maintained on the normal-salt chow. As discussed above, although nephron number was 25% reduced in HET2K mice, mean glomerular volume had increased, such that total glomerular
Fig. 3. MAP and renal function following high-salt (open bars) or normal-salt (solid bars) diet. A: change in 24-h MAP from initial baseline measurements taken on a normal-salt diet. B: absolute creatinine clearance in the 6 groups at the end of the study. C: change in 24-h albumin excretion from baseline. D: absolute plasma renin concentration (PRC). *P < 0.05 describes difference within group from baseline to high salt diet for MAP and albumin excretion (paired t-test). \( P_d \) diet effect; \( P_g \) group effect; \( P_d \times g \) diet \( \times \) group interaction; NS, not significant.

Table 1. Weights of 52-wk-old GDNF WT and heterozygous mice after 7 days on a normal- or high-salt diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Salt (n = 7)</th>
<th>Normal Salt (n = 5)</th>
<th>Normal Salt (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body wt, g</td>
<td>End diet, g</td>
<td>Δ</td>
</tr>
<tr>
<td>Before diet, g</td>
<td>32.5 ± 1.1</td>
<td>35.6 ± 1.2</td>
<td>35.2 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td></td>
<td>32.6 ± 1.1</td>
<td>35.6 ± 1.5</td>
<td>31.7 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td></td>
<td>0.1 ± 0.4</td>
<td>0.0 ± 0.5</td>
<td>-0.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td></td>
<td>-1.1 ± 0.4</td>
<td>-1.2 ± 0.5</td>
<td>0.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Kidney wt, mg</td>
<td>205 ± 6</td>
<td>176 ± 6</td>
<td>243 ± 10*</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 5)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td></td>
<td>220 ± 15</td>
<td>186 ± 9</td>
<td>272 ± 13†</td>
</tr>
<tr>
<td></td>
<td>(n = 6)</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td></td>
<td>425 ± 16</td>
<td>363 ± 14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td></td>
<td>478 ± 12</td>
<td>378 ± 25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Kidney wt-to-body wt ratio, mg/g</td>
<td>13.1 ± 0.4</td>
<td>10.3 ± 0.7</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td></td>
<td>13.6 ± 0.4</td>
<td>11.1 ± 0.5</td>
<td>8.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td></td>
<td>3.3 ± 0.2</td>
<td>3.3 ± 0.1</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td></td>
<td>3.4 ± 0.1</td>
<td>3.6 ± 0.2</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
</tbody>
</table>

Values are means ± SE. GDNF, glial cell line-derived neurotrophic factor; WT, wild-type; HET2K, heterozygous mice born with 2 kidneys; HET1K, heterozygous mice born with 1 kidney; LV, left ventricle; NS, not significant. Kidney weights for HET1K mice are placed in total kidney weight. *n = 3 for left kidney and n = 2 for right kidney. †P = 4 for left kidney and n = 1 for right kidney. Data were analyzed using 2-way ANOVA with diet (\( P_d \)) and group (\( P_g \)) as fixed factors.
Table 2. Change in 24-h urinary excretion profile and plasma values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal salt</th>
<th>High salt</th>
<th>Normal salt</th>
<th>High salt</th>
<th>Normal salt</th>
<th>High salt</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔWater intake, ml/24 h</td>
<td>-0.5 ± 0.4</td>
<td>3.8 ± 0.7</td>
<td>-0.1 ± 0.7</td>
<td>5.3 ± 0.7</td>
<td>-0.2 ± 0.6</td>
<td>8.1 ± 1.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ΔUrinary excretion, ml/24 h</td>
<td>-0.2 ± 0.1</td>
<td>3.5 ± 0.5</td>
<td>0.0 ± 0.1</td>
<td>4.8 ± 0.7</td>
<td>0.2 ± 0.1</td>
<td>8.0 ± 1.4</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>ΔFood intake, g/24 h</td>
<td>-0.1 ± 0.6</td>
<td>0.1 ± 0.4</td>
<td>-0.3 ± 0.9</td>
<td>0.0 ± 0.6</td>
<td>0.5 ± 0.6</td>
<td>0.8 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>ΔUrinary Na⁺ excretion, μmol/24 h</td>
<td>-35 ± 25</td>
<td>2,470 ± 255</td>
<td>11 ± 17</td>
<td>2,112 ± 237</td>
<td>6 ± 45</td>
<td>3,052 ± 453</td>
<td>NS</td>
</tr>
<tr>
<td>ΔUrinary osmolar excretion, mosM/24 h</td>
<td>-494 ± 238</td>
<td>5,199 ± 695</td>
<td>207 ± 203</td>
<td>4,006 ± 697</td>
<td>214 ± 306</td>
<td>6,931 ± 1,808</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FE Na⁺, %</td>
<td>0.123 ± 0.026</td>
<td>1.235 ± 0.195</td>
<td>0.129 ± 0.021</td>
<td>1.379 ± 0.121</td>
<td>0.140 ± 0.015</td>
<td>1.490 ± 0.464</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma Na⁺, mmol/l</td>
<td>145.2 ± 1.3</td>
<td>144.2 ± 1.2</td>
<td>143.8 ± 1.8</td>
<td>143.4 ± 1.4</td>
<td>146.0 ± 3.4</td>
<td>148.8 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma osmolality, mosmol/kg H2O</td>
<td>336 ± 6</td>
<td>323 ± 5</td>
<td>325 ± 2</td>
<td>327 ± 4</td>
<td>337 ± 7</td>
<td>333 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>45.3 ± 0.7</td>
<td>44.7 ± 0.7</td>
<td>44.9 ± 1.3</td>
<td>41.5 ± 1.6</td>
<td>42.3 ± 1.1</td>
<td>41.7 ± 1.4</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. Δ, change from initial baseline measurements taken on normal-salt diet; FE, fractional excretion. *n = 4 and 7 for normal- and high-salt WT, respectively; n = 4 and 4 for normal- and high-salt HET2K, respectively; n = 5 and 4 for normal- and high-salt HET1K, respectively. Data were analyzed using 2-way ANOVA, with diet (P₁) and group (P₂) as fixed factors.

Reduced nephron endowment argue strongly that the documented hypertension in intrauterine programming models is due to factors other than simply nephron number or FSA. Consistent with this argument, several studies have demonstrated a lack of correlation between nephron number and hypertension. Ortiz et al. (33) injected pregnant rats with dexamethasone for distinct 48-h periods during gestation and found a reduced nephron endowment only in offspring that received the injections over days 15–16 and 17–18 of gestation. However, blood pressure was elevated not only in these groups of rats, but also in males injected with dexamethasone on days 13–14 that had normal nephron numbers (33). Furthermore, none of the female groups, even those with a reduced nephron endowment, demonstrated hypertension at 6 mo. Similar disparity between nephron number and hypertension has also been demonstrated in low-protein models (20, 23, 49).

**Basal renal function.** Hypertrophy and hyperfiltration, necessitated by a lower total FSA, are thought to cause damage to glomeruli and facilitate progression to glomerulosclerosis and renal failure (4, 7, 48, 49). In the present study, there was no evidence of albuminuria or glomerulosclerosis, and whole animal 24-h GFR of nephron-deficient HET2K and HET1K mice was not reduced but, rather, was higher than in WT mice, achieved by an increased single-nephron GFR (SNFG). Calculated SNFG (GFR divided by nephron number) of HET2K mice was double that of WT mice and nearly fourfold higher in HET1K than WT mice. These findings are supported by our previous study of 60-wk-old anesthetized HET2K mice (9) and the findings of Boger et al. (3), who also could not find a reduction in renal function in aged HET2K mice.

The higher Ccr values in the nephron-deficient HET mice may appear counterintuitive to what one would expect with a life-long nephron deficit; however, similar findings have been reported by Sanders et al. (37, 38) in nephron-deficient rats. They (37) suggest that the compensatory tubular hypertrophy that occurs in nephron-deficient animals may be likened to that described by Vallon, Blantz, Thomson, and colleagues (44–46) in early diabetes and facilitates hyperfiltration. This tubulocentric principle suggests that hypertrophy of the proximal tubule leads to increased proximal tubule Na⁺ reabsorption, thereby reducing Na⁺ delivery to the distal nephron and macula densa. The decreased Na⁺ load to the macula densa reduces tubuloglomerular feedback (TGF)-dependent afferent arteriole tone, thereby increasing GFR (37, 44–46). Consistent with this tubulocentric principle for the control of GFR hypothesis, we do not see higher conscious or anesthetized GFR in young HET2K or HET1K mice prior to compensatory hypertrophy (unpublished findings). This principle may also explain why Ccr of 1-yr-old HET1K mice is as elevated as that of HET2K mice. Structurally, the only difference noted between a kidney of an HET1K and HET2K mouse was the 34% greater kidney, and thus one could argue tubular, mass of HET1K mice. If this increase in tubular mass was primarily proximal, it may contribute to the greater SNFG of HET1K than HET2K mice. It is interesting to note that Dagan et al. (10) found increased proximal tubule transport in the dexamethasone model of reduced nephron endowment, in agreement with this proposal. Detailed comparisons of tubule segment lengths and reabsorption and TGF in the three groups of mice are required to clarify the mechanisms leading to elevated GFR in HET2K and HET1K mice.

Animal and human studies support our findings that even marked nephron deficiency and hyperfiltration do not always lead to renal dysfunction. In particular, the long-term outlook for kidney donors, children with a single kidney, or those born with a single healthy kidney (URA) is good, with stable renal function and blood pressure expected for up to five decades (17, 19, 39). Although most individuals living with one kidney remain unaffected, some develop renal dysfunction and hypertension, although this is usually associated with congenital abnormalities of the solitary kidney (e.g., ureteropelvic junction obstruction in those with URA) or factors such as an elevated body mass index (16, 17).

In contrast to natural URA in GDNF mice that are devoid of renal disease, rats that were uninephrectomized in the neonatal period showed marked glomerulosclerosis, tubular atrophy, interstitial fibrosis, and reduced GFR and hypertension (7, 48). It is unclear whether sheep that are uninephrectomized during period showed marked glomerulosclerosis, tubular atrophy, abnormalities of the solitary kidney (e.g., ureteropelvic junction obstruction in those with URA) or factors such as an elevated body mass index.

The higher CCr values in the nephron-deficient HET mice may appear counterintuitive to what one would expect with a life-long nephron deficit; however, similar findings have been reported by Sanders et al. (37, 38) in nephron-deficient rats. They (37) suggest that the compensatory tubular hypertrophy that occurs in nephron-deficient animals may be likened to that described by Vallon, Blantz, Thomson, and colleagues (44–46) in early diabetes and facilitates hyperfiltration. This tubulocentric principle suggests that hypertrophy of the proximal tubule leads to increased proximal tubule Na⁺ reabsorption, thereby reducing Na⁺ delivery to the distal nephron and macula densa. The decreased Na⁺ load to the macula densa reduces tubuloglomerular feedback (TGF)-dependent afferent arteriole tone, thereby increasing GFR (37, 44–46). Consistent with this tubulocentric principle for the control of GFR hypothesis, we do not see higher conscious or anesthetized GFR in young HET2K or HET1K mice prior to compensatory hypertrophy (unpublished findings). This principle may also explain why Ccr of 1-yr-old HET1K mice is as elevated as that of HET2K mice. Structurally, the only difference noted between a kidney of an HET1K and HET2K mouse was the 34% greater kidney, and thus one could argue tubular, mass of HET1K mice. If this increase in tubular mass was primarily proximal, it may contribute to the greater SNFG of HET1K than HET2K mice. It is interesting to note that Dagan et al. (10) found increased proximal tubule transport in the dexamethasone model of reduced nephron endowment, in agreement with this proposal. Detailed comparisons of tubule segment lengths and reabsorption and TGF in the three groups of mice are required to clarify the mechanisms leading to elevated GFR in HET2K and HET1K mice.

Animal and human studies support our findings that even marked nephron deficiency and hyperfiltration do not always lead to renal dysfunction. In particular, the long-term outlook for kidney donors, children with a single kidney, or those born with a single healthy kidney (URA) is good, with stable renal function and blood pressure expected for up to five decades (17, 19, 39). Although most individuals living with one kidney remain unaffected, some develop renal dysfunction and hypertension, although this is usually associated with congenital abnormalities of the solitary kidney (e.g., ureteropelvic junction obstruction in those with URA) or factors such as an elevated body mass index (16, 17).

In contrast to natural URA in GDNF mice that are devoid of renal disease, rats that were uninephrectomized in the neonatal period showed marked glomerulosclerosis, tubular atrophy, interstitial fibrosis, and reduced GFR and hypertension (7, 48). It is unclear whether sheep that are uninephrectomized during development and also show hypertension and progressive renal dysfunction and proteinuria also demonstrate significant glomerulosclerosis (29, 42). Studies in mice suggest that a genetic susceptibility to developing glomerular sclerosis and kidney
disease may explain why some animals and humans develop renal dysfunction in a setting of reduced nephron number (14, 25). C57BL/6 mice, the background strain of the GDNF colony, are known to be sclerosis-resistant in this regard (14, 25), which, no doubt, renders HET mice capable of maintaining structural integrity of glomerular capillaries in the face of significant and life-long hyperfiltration and glomerular hypertension, protecting them from developing renal dysfunction. The outcomes of the GDNF mutation on a more sclerosis-prone phenotype are unknown.

One-year-old HET1K mice demonstrate a renal phenotype that is distinct from WT mice. Food and water intake, diuresis, and natriuresis are greater and urinary osmolality is decreased in HET1K compared with WT mice. Although tending to be higher, these parameters in HET2K mice are not significantly different from those in WT mice. It is unclear why urine excretion and urine-concentrating ability would be affected by a marked congenital nephron deficit. Indeed, few studies have even looked at 24-h excretions in models of reduced nephron endowment; thus it is unknown whether this is common to any other model. Alteration of the proportions of long- and short-looped nephrons, crucial in the urine-concentrating mechanism, may be involved (1). Changes to water and Na⁺ transporters along nephrons have been demonstrated in offspring of maternal programming and may be involved in this defect in HET1K mice (10, 26). Unfortunately, this could not be examined in the present study, as the sole kidney of HET1K mice was used for stereological analysis.

Response to an acute high-salt diet. The blood pressure response to the high-salt diet in WT mice was minimal, as expected (37); however, both HET phenotypes demonstrated an increase in blood pressure. The increase in MAP was greatest in HET1K mice (~10 mmHg) followed by HET2K mice (~5 mmHg). This suggests that a reduced nephron endowment may contribute to salt sensitivity of blood pressure in the population. The graded blood pressure response appeared to be related to the degree of nephron deficit of the HET1K (65%) and HET2K (25%) mice, rather than total glomerular volume, which was 50% reduced in HET1K, but not different in HET2K, compared with WT mice. This suggests that factors other than FSA contribute to the rise in blood pressure in HET2K mice and argues strongly for a role for the vascular and/or tubular compartments of the nephron contributing to salt sensitivity of blood pressure in GDNF HET mice. Abnormal tubular salt handling is recognized in human forms of salt sensitivity, secondary to channel defects (6), and has been demonstrated in a few models of reduced nephron endowment (10, 26).

Renal function was also sensitive to the high salt intake, highlighting that fewer nephrons are vulnerable to a secondary insult. After 7 days of high salt intake, Ccr was higher in WT mice, which is consistent with the literature, showing usually no change or an increase in GFR in response to high-salt diets (12, 43). However, Ccr was lower in HET2K and HET1K mice fed the high-salt diet than in their corresponding normal-salt controls. This renal profile was similar to that seen by Sanders et al. (37) in rats with a reduced nephron endowment when fed a high-salt diet. Furthermore, the response is again consistent with the tubulocentric principle of GFR control proposed by Vallon, Blantz, Thomson, and colleagues (44–46) to describe the salt paradox of early diabetes. In early diabetes, a high Na⁺ load leads to suppression of Na⁺ reabsorption in the hypertrophied proximal tubule, leading to activation of TGF and reduction of GFR (44–46). Direct examination of proximal tubule reabsorption, however, in the hypertrophied tubules of HET2K and HET1K mice is needed to confirm its role in the control of GFR in normal- and high-salt states.

Although GDNF HET mice were able to excrete the Na⁺ load, it was at the expense of an increased blood pressure. The mechanisms underlying salt-sensitive hypertension are much debated and, most likely, multifactorial. Measurements in the present study were obtained 7 days after commencement of the high-salt diet, when the mice were in equilibrium, making it impossible to elucidate the mechanisms related to the rise in blood pressure occurring within the first few days. Very small (1–3 mmol/l) increases in plasma Na⁺ have been suggested as part of the mechanisms whereby dietary salt increases blood pressure (11). Plasma Na⁺ concentration was 2.8 mmol/l higher in HET1K mice fed the high-salt diet than in HET1K rats fed the normal-salt diet; however, this rise did not reach significance, nor was there a rise in plasma Na⁺ concentration in HET2K mice. The renin-angiotensin system (RAS) has also been implicated in salt-sensitive hypertension. PRC values were 60–80% lower in mice from all high-salt diet-fed groups than in their corresponding normal-salt diet-fed group, suggesting that the salt-sensitive hypertension was not due to inadequate suppression of the RAS systemically. However, given the known effects of anesthesia on renin release, measurements of PRC need to be obtained in the conscious animal to confirm these findings. Furthermore, examination of intrarenal RAS is also needed, although this was impossible in the present study, given that the sole kidney of HET1K mice was used for stereological analysis. There are no other known effects of GDNF haploinsufficiency that could contribute to the salt-sensitive phenotype, with these mice demonstrating normal noradrenergic, dopaminergic, and motor systems (10, 27, 35).

Conclusion. Our results in GDNF HET mice show for the first time that, under controlled conditions, moderate or severe congenital reductions in nephron number and/or reductions in total glomerular volume alone do not directly translate to hypertension, renal insufficiency, or renal disease, even when observed well into adulthood. Deficits in nephron number in these mice do, however, increase susceptibility of blood pressure and renal function to a secondary insult, in the form of high dietary salt intake. Given that the level of salt intake in Western societies is well in excess of dietary requirements, even moderate nephron deficits that are undetectable in humans may be contributing to elevated blood pressures and renal dysfunction in the community. Our findings are consistent with the hypothesis that it is the presence of a secondary challenge to the kidney, such as salt in the present study or a sclerosis-prone phenotype, that is key to the translation of reduced nephron number into higher blood pressures and renal disease (31).

ACKNOWLEDGMENTS

The authors thank Rebecca Flower for technical assistance.

GRANTS

This work was supported by the National Heart Foundation of Australia (Project Grant G04M 1551 and a fellowship to M. M. Kett), the Foundation for High Blood Pressure Research, and National Health and Medical Research Council.
REFERENCES


