Long-Term Consequences of Uninephrectomy in Male and Female Rats

Isabel Rodríguez-Gómez, Rosemary Wangensteen, Rocío Pérez-Abud, Andrés Quesada, Raimundo G. del Moral, Antonio Osuna, Francisco O’Valle, Juan de Dios Luna, Félix Vargas

Abstract—We investigated the effects of uninephrectomy (UNX) in 6-week-old male and female rats on blood pressure (BP), renal sodium handling, salt sensitivity, oxidative stress, and renal injury over 18 months postsurgery, studying control sham-operated and UNX-operated rats at 6, 12, and 18 months postsurgery, evaluating their renal sodium handling, BP, urinary isoprostanes, N-acetyl-β-D-glucosaminidase, and proteinuria before and after a 2-week high-salt intake period. At 18 months, plasma variables were measured and kidney samples were taken for the analysis of renal morphology and tissue variables. BP was increased at 6 months in male UNX rats versus controls and at 12 and 18 months in both male and female UNX rats and was increased in male versus female UNX groups at 18 months. UNX did not affect water and sodium excretion under basal conditions and after the different test in male and female rats at different ages. However, the renal function curve was shifted to the right in both male and female UNX rats. High-salt intake increased BP in both UNX groups at 6, 12, and 18 months and in the female control group at 18 months, and it increased proteinuria, N-acetyl-β-D-glucosaminidase, and isoprostanes in both UNX groups throughout the study. Renal lesions at 18 months were more severe in male versus female UNX rats. In summary, long-term UNX increased the BP, creatinine, proteinuria, pathological signs of renal injury, and salt sensitivity. Earlier BP elevation was observed and morphological lesions were more severe in male than in female UNX rats. (Hypertension. 2012;60:XX-XX.) ● Online Data Supplement

Key Words: uninephrectomy ■ renal sodium handling ■ renal injury ■ blood pressure ■ rat

Living kidney donation has achieved excellent survival rates for recipients and has been considered to pose a low risk to donors.1 However, enthusiasm for living donation waned somewhat in the early 1980s with experimental reports of hyperfiltration after uninephrectomy (UNX), and fears were expressed that living kidney donation could cause proteinuria, hypertension, and eventual glomerulosclerosis.2 Indeed, it is possible that the impact of donation on blood pressure (BP) and renal function may have been underestimated because of the difficulties in their measurement, and potential vulnerability factors in donors have not been well explored. Nevertheless, the limited availability of organs from deceased donors and the growing demand for transplantation have produced a marked increase in living kidney donation worldwide over recent years.3 As a result, thousands of healthy individuals are becoming uninephric every year, generating some concerns about the long-term consequences of UNX.3,4

Data on the relationship between nephron loss and the risk of increased BP or proteinuria have not been consistent. It has been reported that the initial loss of renal function is, in part, compensated for producing a mean glomerular filtration rate that is 70% to 75% of the pre-UNX value.5,6 Some researchers have found an increase in BP after kidney donation,7,8 and studies comparing donors with siblings or potential kidney donors have shown conflicting results for the impact of donation on BP, renal function, and proteinuria.7-9 It is complicated to dissociate the effects of the aging process from the influence of UNX, because both renal function and BP are influenced by age. Thus, in studies of age- and sex-matched populations, some9,11,12 but not all9 authors found differences in BP between kidney donors and the general population. Rats undergoing UNX at 3 weeks of age were reported to show hypertension and reduced glomerular filtration rate at 6 to 8 weeks in comparison with controls,13 and Zheng et al14 performed UNX in mice and found a more advanced diabetic nephropathy in diabetic mice but almost no effects in nondiabetic mice.

Studies on human kidney donors have been limited, because invasive testing is ruled out in the clinical setting and it is scarcely more feasible to recruit living donors for the long-term follow-up of key renal functional and morphological parameters. With this background, the objective of the present study was to examine the consequences of UNX in male and female rats.
female rats, as a model of donor nephrectomy, on BP, renal sodium handling, salt sensitivity, oxidative stress, and renal injury over 18 months and on renal pathology variables gathered at 18 months.

Methods

Animals
Male and female Wistar rats born and raised in the experimental animal service of the University of Granada were used. Experiments were performed according to European Union guidelines for the ethical care of animals. At 6 weeks of age, the rats were divided into 4 groups (n=25 in each), 1 male and 1 female group for UNX and 1 male and 1 female group for sham operation (controls). In the UNX group, total extirpation of the left kidney was performed, leaving the adrenal gland intact. Sham-operated animals were prepared in the same manner, and the left kidney was handled but not removed. Postsurgery, all animals had access to standard chow and tap water ad libitum.

Experimental Protocol
The same experimental protocol was repeated in the same 8 rats from each study group at 6, 12, and 18 months after surgery in the following consecutive procedures: (1) for baseline, animals were housed in metabolic cages (Panlab, Barcelona, Spain) with food and tap water available ad libitum for a 4-day period (2 days for adaptation+2 experimental days), during which food and fluid intakes were measured and urine samples were collected; (2) for dehydration, animals were water deprived for 24 hours; (3) for isotonic saline load, animals received an intraperitoneal injection of 0.9% NaCl at 3 mL/100 g of body weight; urine samples were taken at 5 hours after the injection, applying light suprapubic pressure before and after each urine collection to ensure that the bladder was emptied; (4) for hyperionic saline load, animals received an intraperitoneal injection of 3% NaCl at 3 mL/100 g of body weight; and (5) for high-salt intake, animals received 1% NaCl saline solution as sole drinking liquid for 2 weeks. Tail systolic BP was measured before and after 2 weeks of 1% NaCl solution administration, using tail-cuff plethysmography in unanesthetized rats (LE 5001-Pressure Meter, Letica SA, Barcelona, Spain). Data were gathered on the urine volume and total urinary sodium and potassium at all these procedures and on creatinine, proteinuria, N-acetyl-β-D-glucosaminidase (index of renal injury), and isoprostanes (as index of oxidative stress) under baseline conditions and after 2 weeks of increased saline intake.

At 18 months, after completion of the above sodium-handling study, all rats were anesthetized with ethyl ether, and blood samples were taken by aortic puncture to determine plasma values of urea, creatinine, total proteins (index of volume expansion), electrolytes (sodium and potassium), total cholesterol, high-density lipoprotein, and low-density lipoprotein. Finally, the rats were killed by exsanguination under ethyl ether anesthesia, and the kidneys and ventricles were removed and weighted. Kidney samples were taken for the pathology study and to measure tissue levels of transforming growth factor-β, type III collagen, nitrate/nitrite, and 8-isoprostane. Analytical procedures, histopathologic analysis, and statistical analysis are described in the online-only Data Supplement.

Results

Morphological Variables
Body weight was similar between control and UNX groups at 6 and 12 months but was significantly reduced in both male and female UNX groups at 18 months. Absolute kidney and heart weights were significantly lower in female than in male rats, and the kidney weight/body weight ratio was higher in the UNX groups than in the control groups, as expected. Compensatory renal hypertrophy was greater (P<0.05) in male (159.4±5%) than in female (137.7±4%) UNX rats. Heart weight,body weight and left ventricular weight/body weight ratios were higher in the female groups than in the male groups, and the left ventricular weight/body weight ratio was higher in female UNX rats than in female controls (Figure S1 and Table S1 in the online-only Data Supplement).

Blood Pressure
Figure S1 (right) in the online-only Data Supplement shows that the tail systolic BP at 6 months after UNX was higher in the male UNX group than in the male control group, whereas no significant difference was found between the female UNX group and female control group. At 12 and 18 months postsurgery, the systolic BP was higher in the male and female UNX groups than in the respective control groups at 12 and 18 months, and it was higher in the male versus the female UNX group and in the male versus the female control group at 18 months.

Renal Sodium Handling
Baseline water and sodium excretion levels did not differ between UNX and control groups of either sex. No difference in acute renal sodium handling was observed between UNX and control groups of either sex at any time point. Diuresis

![Figure 1](image-url)

Figure 1. Renal function curves during high- and low-sodium conditions, illustrating the relationship between systolic blood pressure (SBP) and urinary sodium output. All values are expressed as mean±SEM (n=8, each group). The slopes of the relationship between SBP and natriuresis were significantly (*P<0.05) blunted in uninephrectomized rats vs male or female controls.
Table. Histopathologic Variables and Morphometrical Analysis of Renal Fibrosis at the End of the Experiment

<table>
<thead>
<tr>
<th>Morphological Lesions</th>
<th>Male Control</th>
<th>Female Control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=19)</td>
<td>Uninephrectomized (n=17)</td>
<td>Control (n=16)</td>
</tr>
<tr>
<td>Gomeral sclerosis</td>
<td>0.23±0.43</td>
<td>0.68±0.67</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Mesangium increased</td>
<td>0.23±0.43</td>
<td>0.89±0.31</td>
<td>0.12±0.34</td>
</tr>
<tr>
<td>Synechias</td>
<td>0.94±0.24</td>
<td>0.97±0.26</td>
<td>0.87±0.34</td>
</tr>
<tr>
<td>Cellularity</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>0.62±0.50</td>
</tr>
<tr>
<td>Glomerular cyst</td>
<td>0.58±0.50</td>
<td>0.73±0.87</td>
<td>0.18±0.40</td>
</tr>
<tr>
<td>Hyaline casts</td>
<td>0.82±0.52</td>
<td>1.78±0.90</td>
<td>0.12±0.34</td>
</tr>
<tr>
<td>Tubular cyst</td>
<td>0.00±0.00</td>
<td>0.92±0.75</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Brush border loss</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>Tubular atrophy</td>
<td>0.11±0.33</td>
<td>0.60±0.71</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Inflammatory infiltrate</td>
<td>0.17±0.39</td>
<td>0.92±0.67</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Hyaline arteriolosclerosis</td>
<td>0.41±0.50</td>
<td>1.10±0.45</td>
<td>0.37±0.50</td>
</tr>
<tr>
<td>Renal fibrosis, %</td>
<td>11.92±4.46</td>
<td>19.54±6.07</td>
<td>9.45±2.99</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. NS indicates not significant.
*P<0.05 and **P<0.01 between male and female controls.
†P<0.05 and ††P<0.01 male control and male uninephrectomized.
‡P<0.05 and ‡‡P<0.01 female control and female uninephrectomized.
§P<0.05 and §§P<0.01 male and female uninephrectomized. Test used Mann-Whitney.

Figure 2. Baseline levels and changes in systolic blood pressure (SBP), proteinuria, N-acetyl-β-D-glucosaminidase (NAG), and total excretion of isoprostanes induced by an increased saline intake for 2 weeks (via drinking water, 1% NaCl). Data are mean±SEM (n=8, each group). +P<0.05 vs baseline; *P<0.01 vs male or female controls.
and natriuresis were higher in female versus male groups at all time points (Figure S2 in the online-only Data Supplement), although significance was not reached in some tests.

Figure 1 depicts the pressure-natriuresis relationship (between systolic BP and steady-state sodium excretion) in the groups before and after 2 weeks of high-salt intake. The renal function curve was altered in comparison with controls in the male UNX group at 6 months and in both UNX groups at 12 and 18 months, with a shift to the right of the pressure-natriuresis relationship. In addition, a reduced slope was observed in the UNX female group at 6 months and in both UNX groups at 12 and 18 months in comparison with their respective controls.

Plasma and Renal Variables Measured at the End of the Study

Plasma urea and creatinine levels were higher in both UNX groups. Plasma sodium and potassium levels were similar among all groups, whereas total plasma protein was slightly lower in the UNX groups but without reaching statistical significance. Creatinine clearance was reduced in both UNX groups in comparison with their respective controls. After 2 weeks of increased saline intake, creatinine clearance was similar to baseline values in both male groups but markedly elevated versus baseline in the female groups. Total cholesterol was lower in the female UNX groups than in either male group, whereas high-density lipoprotein and low-density lipoprotein were lower in the female control group than in either male group (Table S2 in the online-only Data Supplement).

Salt-Sensitivity Variables

Figure 2 depicts the salt-sensitivity results in the 4 groups. The 2-week high-saline intake period produced a BP increase in male and female UNX groups at different ages (6, 12, and 18 months) but produced no BP increase in male controls and an increase in female controls only at 18 months. Proteinuria was increased in all groups at 12 and 18 months in comparison with values at 6 months, and the increase was higher in the UNX groups at the end of the experiment. Proteinuria was not significantly modified by the 2-week high-saline intake period in any group at 6 months but was increased versus normal conditions in the male UNX group at 12 months and in all groups at 18 months, when the UNX groups showed the highest values. Urinary N-acetyl-β-d-glucosaminidase was increased by high-salt intake in male and female UNX rats at 6 months and markedly increased at 12 and 18 months; it was also increased by the high-salt intake in male control rats at 12 months and in both male and female controls at 18 months. Under normal conditions, urinary isoprostanate excretion was higher in female groups than in male groups during the entire study period and was higher in the female UNX group than in the other 3 groups at 18 months. 8-Isoprostane values after the high-salt intake period were markedly higher in both UNX groups at 12 and 18 months and were not changed in male or female control groups except for a slight increase at 18 months.

Renal Tissue Inflammatory and Oxidative Stress Variables

Renal transforming growth factor-β, type III collagen, nitrate/nitrite, and 8-isoprostane values at the end of the experiment (18 months) were similar among all groups (data not shown).

Histopathologic and Quantitative Interstitial Fibrosis Results

Some structural morphological changes were found in the control groups of rats at 18 months, observing mild increased mesangial matrix with glomerular sclerosis and tuft synechiae, cystic appearance of some glomeruli with mild tuft atrophy, and the presence of tubular atrophy (Figure S3). Only tubular casts, mild glomerular cyst, and glomerular hypercellularity were significantly increased in 18-month-old male control rats in comparison with female control rats. Overall, glomerular, tubulointerstitial, and vascular lesions were significantly more severe in male UNX rats than in female UNX rats or male control rats at 18 months of age (Table). Histopathologic examination of renal slices from male UNX rats showed major alterations, including abundant tubular casts, marked dilation of tubules and incipient tubular atrophy, and focal inflammatory infiltrate (Figure S3). In comparison with the other groups, male UNX rats showed more intense glomerular sclerosis, mesangial matrix, tubular atrophy, and the presence of tubular casts, with images of thyroidization and thickening of the basement membrane in both tubular and capsular structures and more severe chronic inflammatory infiltrate and hyaline arteriopathy.

Quantitative interstitial fibrosis analysis revealed a higher degree of fibrosis in both UNX groups versus their respective controls and in male versus female UNX rats, as shown in the Table. The percentage of interstitial connective tissue in the male control rats was higher than the 5% to 8% generally reported in young rats (2–4 months), indicating the development of age-related fibrosis in the kidney of normal rats.

Discussion

The main findings of this study were that UNX after completion of nephrogenesis was accompanied in the long-term by increased BP, proteinuria, and biochemical and pathological signs of renal injury in male and female rats. UNX rats also showed increased salt sensitivity after 2 weeks of an increased saline intake, manifested by augmented BP and an increase in proteinuria and urinary N-acetyl-β-d-glucosaminidase (both indexes of renal injury) and isoprostanes (index of oxidative stress). However, UNX did not affect the acute renal sodium handling after saline loads. Moreover, the increased BP appeared earlier and the morphological renal lesions were greater in male than in female UNX rats.

The plasma levels of urea and creatinine were higher and the creatinine clearance was lower in both UNX male and female groups versus controls at 18 months, consistent with their reduced renal mass and in agreement with reports in rats at 6 to 8 weeks and cats at 2 to 5 years after UNX. Creatinine clearance after 2 weeks of increased saline intake was similar to baseline values in both male groups but was markedly elevated in the female groups. This phenomenon has
also been reported in rats after a longer period of increased saline intake.\textsuperscript{13,16}

Brenner et al\textsuperscript{17} proposed an inverse relationship between nephron number and the risk of hypertension in later life. However, controversial data have been reported on the effects of nephrectomy on BP for live kidney donation, with some authors finding no increase in the prevalence of hypertension\textsuperscript{15,18} and others reporting an increase in BP.\textsuperscript{8,9,20}\textsuperscript{23} Our results show that UNX produces a long-term BP increase that is sex related, with male rats being more sensitive to renal mass reduction. Our data agree with previous observations of a relationship between reduced nephron number and elevated BP in animal models\textsuperscript{21,22} and in humans.\textsuperscript{8,19,20}\textsuperscript{23} Our data also agree with the sex differences in BP regulation documented in several animal models.\textsuperscript{23} Although the present study did not explore how this sexual dimorphism is produced, one possibility is a different regulation of vascular function between the sexes. Thus, it is known that the responsiveness to vasoconstrictors in the renal vasculature is greater in male groups than in female groups.\textsuperscript{24}

Most studies have revealed an increased proteinuria after UNX,\textsuperscript{7,11,19} although the amount of urinary protein is usually small. A meta-analysis that included >5000 donors reported a slight increase in proteinuria in comparison with controls,\textsuperscript{20} whereas several studies of donors\textsuperscript{7,11,19} showed greater proteinuria in the men than in the women. Our study shows that proteinuria was increased in all groups with age and that UNX increased proteinuria in male groups at 12 months, with the greatest increase being observed in both UNX groups at the end of the experiment. Our results agree, in part, with the above data in UNX humans, although we did not find a greater proteinuria in the male groups. It was reported that proteinuria in rats at 2 to 5 years after UNX did not significantly differ from that in age- and sex-matched controls,\textsuperscript{15} suggesting species-related differences.

UNX did not affect water and sodium excretion in our male and female rats under baseline conditions or after the different tests at the different ages. However, a reduced natriuretic response to volume expansion was observed in UNX rats at 10 and 26 weeks after surgery,\textsuperscript{25} whereas Carlström et al\textsuperscript{13} reported an increased diuresis in UNX male rats at 6 to 8 weeks after the operation. These discrepancies may be because of the different post-UNX periods considered.

The kidneys play a key role in the homeostatic regulation of body fluid volume and therefore in long-term BP control.\textsuperscript{26} In all forms of chronic hypertension, the renal pressure-natriuresis mechanism is abnormal.\textsuperscript{27} Analysis of the relationship between the BP and steady-state sodium excretion in the groups before and after 2 weeks of high-salt intake showed a shift to the right and a reduced slope, resulting in a blunted pressure natriuresis, in the male UNX rats at 6 months and in both UNX groups at 12 and 18 months. These data agree with previous reports\textsuperscript{13,14} in UNX male rats after a shorter postoperative period (5–8 weeks). The mechanisms underlying this phenomenon and the salt-sensitive hypertension in the UNX rats have not been addressed in this study, but a role may be played by a decreased dopamine-induced natriuresis\textsuperscript{25} or an altered adaptive change of the renal sodium transporters to the increased salt intake of the UNX rats.\textsuperscript{28}

We examined the effects of UNX at a young age, but after completion of nephrogenesis, on BP after 2 weeks of increased saline intake, finding that BP was increased in both male and female UNX groups at 6, 12, and 18 months but in female controls only at 18 months. Carlström et al\textsuperscript{13} also observed that high-salt diet increased the BP in male groups after a short period of UNX (6–8 weeks). Our study clearly demonstrates that aging in female rats and a reduction in nephron number in male or female rats cause salt-sensitive hypertension. Moreover, variables related to salt-induced renal injury, such as proteinuria, N-acetyl-b-D-glucosaminidase, and iso- prostanes,\textsuperscript{16,29} were markedly increased by the high-salt intake in both UNX groups, indicating that UNX increases renal salt sensitivity.

Several studies have extensively detailed morphological changes in the kidney during aging.\textsuperscript{30,31} The normal aging process leads to slightly more pronounced changes in kidney morphology in male versus female rats.\textsuperscript{31} Old intact male rats develop glomerulosclerosis, but castrated male rats are protected from this injury.\textsuperscript{31} The present results are compatible with previous descriptions of age-related kidney changes and with the sexual dimorphism pattern of these morphological features.\textsuperscript{30,31} Our data also reveal more intense histopathologic tubulointerstitial renal lesions and a higher degree of interstitial fibrosis in both UNX groups versus controls and in the male versus female UNX rats.

In summary, this study indicates that UNX in young male and female rats over the long term is associated with increased BP, augmented plasma levels of urea and creatinine, proteinuria, and morphological signs of renal injury. UNX is also associated with salt sensitivity, manifested by an elevated BP, increased oxidative stress, and signs of renal injury in response to an increased saline intake. However, UNX did not affect the acute renal sodium handling. BP was increased earlier and renal parenchyma injury was greater in male versus female UNX rats.

**Perspectives**

The marked increase in living kidney donation worldwide means that thousands of healthy individuals become uninephric every year. The observations reported in this study contribute experimental data on the long-term consequences of UNX in a rat model of donor nephrectomy. UNX at a young age produced a long-term increase in BP and in biochemical and morphological signs of renal injury in both male and female rats, with an earlier BP elevation and more severe morphological lesions in male versus female UNX rats. This study also showed that UNX causes salt-sensitive hypertension in both sexes. From a clinical and public health perspective, this study indicates that UNX increases BP and aggravates age-related renal injury, especially in male individuals, suggesting that salt restriction should be recommended to attenuate these alterations.

**Acknowledgments**

We thank R. Arcas, M. Quintana, and M. D. Rodríguez for their expert technical assistance and are grateful to R. Davies for help with the English version.
Source of Funding

This study was supported by grants from the Ministry of Education and Science (SAF2009-12294), from an Excelencia project (CTS-6704) of the Regional Government of Andalucía, and from the Carlos III Health Institute of the Spanish Ministry of Health and Consumer Affairs (Red de Investigación Renal, REDinREN RD06/0016/0017 and D07/0016/2008). FEDER una manera de hacer Europa.

Disclosures

None.

References


Novelty and Significance

What Is New?

• This study analyzes, for the first time, the long-term consequences of uninephrectomy, as a model of donor nephrectomy, on blood pressure, renal function, and morphology and the influence of sex on these variables.

What Is Relevant?

• These findings on the long-term effects of uninephrectomy may be of clinical relevance, given the marked increase in living kidney donations worldwide.

Summary

• This study indicates that uninephrectomy in young male and female rats produces blood pressure elevation, salt sensitivity, and aggravation of age-related renal injury and that the male groups are more susceptible than the female groups to the increase in blood pressure and renal injury.
Long-Term Consequences of Uninephrectomy in Male and Female Rats
Isabel Rodríguez-Gómez, Rosemary Wangensteen, Rocío Pérez-Abud, Andrés Quesada, Raimundo G. del Moral, Antonio Osuna, Francisco O’Valle, Juan de Dios Luna and Félix Vargas

Hypertension, published online October 15, 2012:
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2012/10/15/HYPERTENSIONAHA.112.198499

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2012/10/17/HYPERTENSIONAHA.112.198499.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
Long-term Consequences of Uninephrectomy in Male and Female Rats

Rodríguez-Gómez et al. Effects of Uninephrectomy in rats

Isabel Rodríguez-Gómez, Rosemary Wangensteen³, Rocío Pérez-Abud², Andrés Quesada³, Raimundo G. del Moral⁴, Antonio Osuna², Francisco O’Valle⁴, Juan de Dios Luna¹ and Félix Vargas¹

Departamento de Fisiología y Departamento de Bioestadística¹, Facultad de Medicina, 18012, Granada, Spain
²Servicio de Nefrología, Unidad Experimental, Hospital Virgen de las Nieves, 18012, Granada, Spain
³Departamento de Ciencias de la Salud, Universidad de Jaén, Spain
⁴Departamento de Anatomía Patológica e Instituto de Biomedicina Regenerativa (IBIMER), Facultad de Medicina, 18012, Granada, Spain
Supplementary Methods

**Analytical procedures.** Plasma and urinary electrolytes and creatinine were measured in an autoanalyzer (Hitachi-912, Roche, Spain). Collagen type III was determined with an ELISA kit purchased from Cusabio Biotech. TGF-beta was measured by Luminex x-MAP technology with a kit purchased from Millipore (Billerica, MA, USA), and 8-isoprostane concentrations in urine and kidney samples were measured by a Colorimetric Assay Kit purchased from Cayman Chemical (Cayman Ann Arbor, MI, USA). Proteinuria was determined with the DC Protein Assay kit (Bio-Rad, Madrid, Spain), and urine NAG levels were determined by means of a colorimetric method purchased from Roche Diagnostics (Barcelona, Spain). Tissue NO$^\text{2}$ and NO$^\text{3}$ (NOx) concentrations were measured by using nitrate reductase and Griess reaction. Renal tissue was homogenized in 1 ml of a solution of 0.1M phosphate buffered saline (pH 7.4), 1mM EDTA, and 0.005% butylated hydroxytoluene (BHT).

**Histopathological analysis.** For conventional morphology, paraffin-embedded longitudinal rat kidney sections in sagittal plane were stained with hematoxylin and eosin, Masson’s trichrome, and periodic acid-Schiff stain (PAS). The morphological study was done in blinded fashion (RGM, FO) on 4-micrometer sections with light microscopy, using the most appropriate stain for each lesion. The severity of glomerular (sclerosis, cysts, synechiae, mesangium proliferation, cellularity), tubular (atrophy, cysts, brush border loss, casts, inflammatory infiltrate), and vascular (hyaline arteriolar sclerosis, fibrinoid necrosis) lesions was calculated semiquantitatively using a 4-point scale (0, absence; 1, mild [<10% of tubules, vessels or glomeruli involved]; 2, moderate [10-25%]; 3, severe [>25%]). A quantitative study of interstitial fibrosis was performed by image analysis using Sirius red stain and the Fibrosis HR® program, as previously described by our group (1)

**Statistical Analysis.** Stata 11.1 was used for the statistical analyses. Differences were considered statistically significant at $p<0.05$. The following statistical analyses were performed. 1) The same four-factor repeated-measures design was used for each of the experiments (baseline, WD, ISL, HSL and high salt diet), with sex (female/male), treatment (control/nephrectomized), experiment (baseline, WD, ISL, HSL and high salt diet) and time (6, 12 and 18 months) as fixed effect factors and rat as random effect factor. Sex and treatment factors were crossed, experiment and month were repeated-measures factors, and rats were nested in the sex-treatment interaction. When interactions were significant, pairwise comparisons were performed using Bonferroni’s penalization. When appropriate, logarithm transformations were used. 2) The above design, but restricted to three fixed-effect factors (sex, treatment and time), was used to analyze the time course of body weight and blood pressure measurements. 3) A four-factor repeated-measures design was used to analyze variables related to salt sensitivity (BP, proteinuria, NAG and isoprostanes), with sex, treatment, time, and condition (before/after salt) as fixed-effect factors and rat as random-effect factor. Sex and treatment factors were crossed, condition and month were repeated-measures factors, and rats were nested in the sex-treatment interaction. When interactions were significant, pairwise comparisons were performed using Bonferroni’s penalization. 4) Slopes of the relationships between RPP and diuresis and natriuresis were computed for each rat, and the mean of slopes was obtained for each group. One-way ANOVA was performed to compare the means of slopes, followed by pairwise comparisons using Tukey’s method. 5) A two crossed-factor design (sex and treatment) was used to study
measures at the study endpoint. Sex-treatment interactions were analyzed and, when significance was obtained, pairwise comparisons were made using Bonferroni’s penalization because of the unbalanced sample sizes. 6) The non-parametric Kruskal-Wallis test and Mann Whitney U-test were used to analyze morphometric variables.

**Supplementary References**


**Supplementary Table**

Table S1. Morphologic variables in the experimental groups at the end of the study.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=19)</td>
<td>Uninephrectomized (n=17)</td>
</tr>
<tr>
<td>FBW (g)</td>
<td>657.35±20.3</td>
<td>577.63±17.0**</td>
</tr>
<tr>
<td>KW (mg)</td>
<td>1425.8±65.6</td>
<td>1981.4±77.1**</td>
</tr>
<tr>
<td>HW (mg)</td>
<td>1325.0±36.5</td>
<td>1258.7±31.6</td>
</tr>
<tr>
<td>LVW (mg)</td>
<td>1063.8±29.1</td>
<td>1019.5±27.2</td>
</tr>
<tr>
<td>KW/BW (mg/g)</td>
<td>2.19±0.11</td>
<td>3.47±0.16**</td>
</tr>
<tr>
<td>HW/BW (mg/g)</td>
<td>2.03±0.05</td>
<td>2.20±0.07</td>
</tr>
<tr>
<td>LVW/BW (mg/g)</td>
<td>1.63±0.04</td>
<td>1.78±0.05</td>
</tr>
<tr>
<td>LVW/HW</td>
<td>0.80±0.007</td>
<td>0.81±0.010</td>
</tr>
</tbody>
</table>

Data expressed as means ± s.e.m. FBW, final body weight; KW, final kidney weight; HW, final heart weight; LVW, final left ventricular weight; KW/BW, kidney weight versus body weight ratio; HW/BW, heart weight versus body weight ratio; LVW/BW, left ventricular weight versus body weight ratio; LVW/HW, left ventricular weight versus heart weight ratio. * P<0.05, ** P<0.01, vs their respective control group. † P<0.05, ††P<0.01, vs the control male group.
Table S2. Plasma and renal variables measured at the end of the study in the experimental groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Male Control (n=19)</th>
<th>Male Uninephrectomized (n=17)</th>
<th>Female Control (n=16)</th>
<th>Female Uninephrectomized (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mEq/L)</td>
<td>148.44±0.85</td>
<td>146.00±1.46</td>
<td>146.75±1.08</td>
<td>145.33±1.05</td>
</tr>
<tr>
<td>K (mEq/L)</td>
<td>4.08±0.08</td>
<td>4.17±0.19</td>
<td>3.87±0.14</td>
<td>4.10±0.10</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>31.59±1.42</td>
<td>40.81±3.76*</td>
<td>34.25±2.21</td>
<td>42.29±2.58*</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.31±0.01</td>
<td>0.44±0.05*</td>
<td>0.27±0.01</td>
<td>0.44±0.08*</td>
</tr>
<tr>
<td>Total Proteins (g/dL)</td>
<td>6.41±0.08</td>
<td>6.21±0.17</td>
<td>6.52±0.11</td>
<td>6.28±0.02</td>
</tr>
<tr>
<td>T. Cholesterol (mg/dL)</td>
<td>139.21±6.56</td>
<td>146.77±16.5</td>
<td>111.56±8.41</td>
<td>101.89±0.95†</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>81.80±3.28</td>
<td>79.73±3.87</td>
<td>65.78±4.29†</td>
<td>74.31±2.49</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>7.98±0.90</td>
<td>9.53±1.56</td>
<td>3.95±0.65†</td>
<td>4.98±1.33</td>
</tr>
<tr>
<td>CrC c.n (ml/min.100g)</td>
<td>0.13±0.01</td>
<td>0.07±0.02*</td>
<td>0.29±0.02†</td>
<td>0.12±0.01*</td>
</tr>
<tr>
<td>CrC post salt (ml/min.100g)</td>
<td>0.12±0.01</td>
<td>0.05±0.02</td>
<td>0.44±0.20‡</td>
<td>0.56±0.16‡</td>
</tr>
</tbody>
</table>

Data expressed as means ± s.e.m. CrC c.n, creatinine clearance in normal conditions; CrC post salt, creatinine clearance post treatment of salt. * P<0.01 vs the control group. † P<0.01 vs the male control group. ‡ vs normal conditions.

Supplementary Figures and Legends.

Figure S1: (Left panel) Time course of body weight (BW) and tail systolic blood pressure (SBP) in the groups measured by plethysmography (Right panel). (n=8, each group) Data are means ± SEM (n=8, each group). * p<0.05; ** p<0.01 versus male and female controls; + p<0.05 versus female or female uninephrectomized groups
Figure S2.- Diuresis and natriuresis in the experimental groups under baseline conditions and after several stresses. WD, water deprivation for 24 h, ISL, isotonic saline load (1% NaCl, 3 ml/100g, ip), HSL, hypertonic saline load (3% NaCl, 3 ml/100g, ip), after two weeks of increased saline intake (SALT, 1% NaCl via drinking water). All values are expressed as mean±SEM (n=8 each group) *p<0.05 versus male controls or male uninephrectomized rats.
Figure S3.- Kidney lesions in uninephrectomized 18-month-old rats. A and C) Multiple tubular casts (arrowhead), tubular dilation (asterisk) and mild inflammatory infiltrate in male uninephrectomized rat. B and D) Glomerular sclerosis (arrow) and fewer tubular casts in female uninephrectomized rat. E) Some tubular and glomerular casts in control male rat. F) Normal feature of renal cortex in control female rat (Masson’s trichrome, original magnification x10).