SUPPLEMENTAL MATERIAL
Supplemental Methods

**Metabolic cages.** Animals were placed into metabolic cages 48 h before daily quantitative urine collections and measurements of food and water intake were performed. After basal 24-h urine collection, a nutrient-matched HS diet was introduced, and 24-h urine samples were collected daily for 5 consecutive days. Urine was centrifuged at 1000 × g for 5 min, aliquoted, and stored at −80°C until further analysis. At the end of the fifth day on the HS diet, rats (16-18 weeks old) were euthanized, and the blood, skin, and kidney tissues were harvested. Separate sets of animals were euthanized while maintained on a NS diet or on the first day of the HS diet to harvest blood, skin, gastrocnemius muscles, and kidney tissues. The kidney cortex, outer and inner medulla, whole body skin and gastrocnemius muscles were frozen in liquid nitrogen and stored at −80°C until molecular assays were performed.

**Telemetry.** Blood pressure measurements were made in a different cohort of animals using DSI PA-C40 transmitters (Data Sciences International, Duluth, MN) as previously detailed. At the beginning of the 2-week NS diet, rats were anesthetized with 2% isoflurane, and telemetry transmitters were implanted with catheters inserted into the abdominal aorta. The catheter was secured in place with VetBond tissue glue (3M corporation, St. Paul, MN), the transmitter body was secured in place along the incision line, and the muscle layer was closed with sutures. Staples were used to close the skin. Rats were allowed to recover for at least 10 days after surgery before the basal 24-h urine collection and subsequent HS challenge. Blood pressure was recorded for 10 sec once every 10 min throughout the study.

**Body water measurement.** In vivo body composition of rats was determined using an 2MHz Whole Body Composition Analyzer quantitative magnetic resonance (QMR) machine (Echo Medical Systems, Houston, TX), as previously validated. Rats were weighed and then placed into a clear Perspex tube, allowing constant airflow, and room for the rat to turn around. Total body water measurements were obtained at different time points in the same animals. Data were calculated relative to body weight and presented as percentage.
Ashing. As previously described, whole body skin and gastrocnemius muscles were frozen at −80°C until processed. Samples were then weighed and placed in a drying oven at 80°C for 96 h. Water content was determined by subtracting the dry weight from the wet weight. Samples were ashed by heating to 150°C, 250°C, and 350°C in 6-h increments, followed by 24 h at 450°C. Finally, samples were held at 600°C for 20 h, and the ash was then dissolved in 5% nitric acid.

Measurement of electrolytes. Urinary and serum electrolytes were measured using an EasyLyte Na⁺/K⁺/Cl⁻ analyzer (Medica, Bedford, MA). Skin and muscle electrolytes were measured using atomic absorption spectrometry (ICE 3000; Thermo Fisher Scientific, Waltham, MA).

Creatinine measurement. Plasma and urine creatinine levels were determined by non-derivitized, stable isotope dilution LC-MS/MS as previously described. An Agilent (Wilmington, DE) Infinity 1260 LC and Infinity 1290 autosampler with a 6460 Triple Quad mass spectrometer were used.

Aldosterone (ALD) measurement. Urinary ALD was measured by immunoassay as previously described.

Norepinephrine (NE) measurement. Urinary NE concentrations were measured using a Norepinephrine ELISA Fast Track kit (BA E-6200; Rocky Mountain Diagnostics, Colorado Springs, CO) according to the manufacturer’s directions.

Nitrite and nitrate (NOx) measurement. Urinary NOx concentrations were analyzed using the E-NO 30 HPLC system (Eicom, Kyoto, Japan) as previously described.

ET-1 measurement. ET-1 concentrations were measured using the Quantiglo ET-1 ELISA kit (QET00B; R&D Systems, Minneapolis, MN) according to the manufacturer’s directions.

Atrial natriuretic peptide (ANP) measurement. Serum ANP concentrations were measured using the Rat ANP ELISA Kit (ab108797, Abcam, Cambridge, MA) according to the manufacturer’s directions.

Tissue RNA isolation, cDNA synthesis, and real time PCR. RNA was isolated from kidney tissue using the Purelink Mini RNA Extraction kit (12183018A, Thermo Fisher Scientific) according to the
manufacturer’s instructions. RNA concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Then, the isolated RNA was reverse transcribed using the QuantiTect Reverse Transcription kit (Qiagen). Finally, the resulting cDNA was used to quantify mRNA by RT-PCR (CFX96 Real-Time System, Bio-Rad, Hercules, CA) using TaqMan primer gene expression assays with ET-1 (Rn00561129_m1), ET_{A} receptor (Rn00561137_m1), ET_{B} receptor (Rn00569139_m1) and β-Actin (Rn00667869_m1) primers. Gene expression was quantified relative to β-actin as a housekeeping gene using the \(2^{\Delta\Delta Ct}\) method.

**RNA sequencing and IPA.** Total RNA was extracted from inner medullary tissues collected from NS-fed male and female rats (n=8/sex) following the Trizol method (Ambion), pooled (n=4 rats/pool) by sex and quantified using a NanoDrop2000 spectrometer (Thermo Fisher Scientific). Thus, we sequenced two pools of four samples per sex. RNA library construction for sequencing was performed using the Illumina TruSeq Stranded Total RNA Kit. Samples were multiplexed 8 per lane on a 400 Gb flow cell, pair-end sequenced with an Illumina HiSeq 2500. Before the analysis, adapter sequences were removed from the output sequence reads using cutadapt (http://code.google.com/p/cutadapt/). Reads with low base quality (<13) were further trimmed and, if less than 25 base pairs, removed by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The trimmed sequence reads were then aligned to the rat reference genome and gene annotation database (rn5) using TopHat2 (v2.1.1) with mammalian default parameters. Transcripts in all of the sequencing libraries were assembled by Stringtie. Differential expression analysis was performed using Cufflinks (v2.2.1). The Benjamini-Hochberg procedure was used for controlling false discovery rates in multiple comparisons. A total of 26,085 unique transcripts were identified through sequencing. A list of transcripts that met the following criteria were uploaded to IPA (Qiagen) for core analysis: 1) detected in both male and female samples, and 2) identified to be differentially expressed by Cufflinks or at least a 1.5-fold different between the
sexes. Transcripts meeting these criteria are represented by color-coded points in Supplemental Figure 3. A total of 2345 transcripts mapped to genes represented in the IPA for subsequent pathway analysis.

**Interleukin-1β (IL-1β) measurement.** Cortical and inner medullary regions of the kidney were homogenized, sonicated and centrifuged as previously described. Protein concentration in the supernatant extract was determined using Quick Start Bradford 1× Dye Reagent (RLB00, Bio-Rad). IL-1β protein level in the supernatant extract was measured using rat-specific IL-1β Quantikine ELISA kits (RLB00, R&D Systems) according to the manufacturer’s instructions. Measurements were normalized to total protein measured by Bradford assay.

**Immunohistochemical staining.** Kidneys were fixed and immunohistochemically stained as previously described. Primary antibodies against IL-1β (ab9787, Abcam, Cambridge, MA) were used at 1:3000 dilution. DAB substrate (DAKO) was used to visualize the primary antibody.

**Western blotting.** Kidney cortical lysates were separated by SDS-PAGE and processed for Western blot analysis as previously described. Primary antibodies against the sodium transporters and channels have previously been validated. Supplemental Table 1 lists all primary antibodies used for Western blotting in the present study. Alexa Fluor 680 (A21109, Invitrogen, Waltham, MA) and Alexa Fluor 800 (A32730, Invitrogen) secondary antibodies were used at 1:1000 dilution except for those used to detect β-actin (1:10000). To quantify protein bands, densitometry was performed with a LI-COR Odyssey Image Studio (v5.2.5), and all values were normalized to the corresponding values in the male NS group.
Table S1. Western blotting antibody details.

| Antibody Target | Source                                                                 | Host     | Clonality    | Dilution factor | Expected molecular weight (kDa) | Validated and/or citation |
|-----------------|------------------------------------------------------------------------|----------|--------------|-----------------|-------------------------------|--------------------------|
| ENaCα           | AB3530P, Chemicon, Temecula, California                                | Rabbit   | Polyclonal   | 1:1000          | ~100                          | 71                       |
| NKAα            | Developmental Studies Hybridoma Bank at the University of Iowa         | Rouse    | Monoclonal   | 1:1000          | 112                           | 66                       |
| NCC             | a kind gift from Dr. McDonough, University of Southern California     | Rabbit   | Polyclonal   | 1:1000          | 150                           | 67                       |
| pNCCT53         | a kind gift from Dr. Loffing, University of Zurich                     | Rabbit   | Polyclonal   | 1:1000          | 150                           | 68                       |
| NHE3            | a kind gift from Dr. McDonough, University of Southern California     | Rabbit   | Polyclonal   | 1:1000          | 83                            | 69                       |
| pNHE3S552       | SC-53962, Santa Cruz Biotechnology, Dallas, TX                         | Mouse    | Monoclonal   | 0.2 µg/ml       | 83                            | 70                       |
| Actin           | A1978, Sigma, St. Louis, MO                                           | Mouse    | Monoclonal   | 1:10000         | 42                            |                          |

NHE3, Na⁺/H⁺ exchanger isoform 3; pNHE3, phosphorylated Na⁺/H⁺ exchanger isoform 3; αENaC, epithelial Na⁺ channel α subunit; NKAα, Na⁺, K⁺-ATPase α.
Table S2. Pathways identified by Ingenuity Pathway Analysis to be enriched in transcripts with differential expression between males and females.

See Excel file.
Table S3. Top 10 highly differentially expressed genes between sexes in the renal inner medullary transcriptome.

| Gene  | Fold change (female over male) | P values |
|-------|-------------------------------|----------|
| Adh1  | 53.3                          | 5.00E-05 |
| Slc22a7 | 39.6                        | 5.00E-05 |
| Mmp13 | 15.1                          | 0.00045  |
| Acsm1 | 12.8                          | 0.00095  |
| Car15 | 9.9                           | 5.00E-05 |
| Slc5a10 | 9.6                        | 5.00E-05 |
| Slc7a12 | 9.6                        | 5.00E-05 |
| Tmigd1 | 8.7                           | 5.00E-05 |
| Akr1c1 | 8.2                           | 5.00E-05 |
| Knu   | 7.7                           | 5.00E-05 |
Figure S1. Urine and serum electrolytes during the HS diet challenge. Food intake (A), urinary excretion of K⁺ (Uₖᵥ) (B) and Cl⁻ (U₉ᵥ) (C), and serum K⁺ (D, E) and Cl⁻ (F, G) in male and female SD rats during the NS diet phase or the HS challenge (n= 4-9 rats in each group). Statistical comparisons were performed by repeated measures two-way ANOVA with Sidak’s post-hoc test for multiple comparisons (A-C) or two-way ANOVA with Sidak’s post-hoc test for multiple comparisons (D-G). *P < 0.05 vs. corresponding NS values. ANOVA results: food intake: P_interaction = 0.8, P_time = 0.9, P_sex = 0.8; Uₖᵥ: P_interaction = 0.03, P_time = 0.007, P_sex = 0.4; U₉ᵥ: P_interaction = 0.6, P_time < 0.0001, P_sex = 0.3; serum K⁺: P_interaction = 0.9, P_time = 0.0003, P_sex = 0.09; serum Cl⁻: P_interaction = 0.02, P_time = 0.7, P_sex = 0.006. NS, normal salt; HS, high salt; SD, Sprague Dawley.
Figure S2. Blood pressure during the HS diet challenge. 24-h measurements of mean arterial pressure (MAP) in male and female SD rats during the NS diet phase or the HS diet challenge (n=4-6 rats in each group). Statistical comparisons were performed by repeated measures two-way ANOVA with Sidak’s post-hoc test for multiple comparisons. *$P < 0.05$ vs. corresponding NS values. **$P < 0.05$ vs. corresponding male values. ANOVA results: $P_{\text{interaction}} = 0.1$, $P_{\text{time}} = 0.008$, $P_{\text{sex}} = 0.01$. NS, normal salt; HS, high salt; SD, Sprague Dawley.
Figure S3. RNA-sequencing results plotted as A) Scatter plot comparing the mean FPKM from male and female pools and B) Volcano plot of log fold difference in gene expression (females over males) against q-value of the comparisons. Criteria for transcripts inclusion in pathway analysis were >+/− 1.5-fold difference in expression and/or q < 0.05. Transcripts meeting these criteria are represented by color-coded points, with all other transcripts in black.

Transcripts Included in Pathway Analysis
Fold difference > +/− 1.5x
q-value <0.05; fold difference > +/− 1.5x
q-value <0.05; fold difference <+/− 1.5x