Supporting Information

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SI Methods

Hepatic Glucocorticoid Assay. Whole rodent livers were snap-frozen and stored at −80 °C, then weighed and homogenized in RIPA buffer using a rotary-blade homogenizer. The homogenate was deproteinated immediately with 6% perchloric acid (1:1), then centrifuged at 10,000 × *g* for 15 min. The supernatant was then collected separately and neutralized 1:1 with 1 M potassium hydroxide. The supernatant after centrifugation (10,000 × *g*, 10 min) was then passed through a Chromabond C18 column (Macherey-Nagel) using (*i*) two times 3 mL methanol, (*ii*) 3 mL H_2O, (*iii*) 3 mL 50 mM HCl, (*iv*) 6 mL homogenate, (*v*) 3 mL 50 mM HCl, and (*vi*) 13 mL H_2O. The steroids were collected using 6 mL 80% methanol (vol/vol), evaporated to dryness using a vacuum drier and reconstituted using 200 μL 80% methanol. Reconstituted hepatic steroids were quantified by HPLC using an apparatus consisting of a PU-2089 Quarternary Low Pressure Gradient Pump and MD-2010 Photometric Diode Array UV/Vis Detector (195–650 nm) from Jasco Instruments Ltd. The pump was connected to an AS-2055 Autosampler. Peak area was calculated by EZChrome Elite software (Agilent Technologies UK Ltd). For both systems, a reversed-phase ACE C18 column (0.46 cm × 15 cm; 5 μm-particle size) from Hichrom Ltd. was used. The column was equilibrated with a solvent mixture consisting of 70:30 water:acetonitrile (CH_3CN). After injection, the solvent mix was adjusted so that at 7 min, the mixture was 60:40 water:CH_3CN. Between 7 and 9 min the gradient was increased further so that 0:100 water:CH_3CN was reached. Between 9 and 10 min the solvent mix was maintained at 0:100 water:CH_3CN. Between 10 and 11 min the original solvent mix of 70:30 water:CH_3CN was reapplied. UV chromatograms for 220 and 240 nm were analyzed and a 240:220 ratio was obtained and compared against steroid standard data. The AUC was analyzed on Prism 5.0 software to quantify unknown steroids against calibration curves.

11β-Hydroxysteroid Dehydrogenase Type 1 Reductase Activity. Oxoreductase 11β-HSD1 "reductase" activity was measured using methods as previously described by Hu et al. (1). Snap-frozen rodent livers from the experimental groups were lysed with RIPA buffer, then treated with 1% Triton X-100 in assay buffer containing 100 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1 mM $MgCl_2$, 250 mM sucrose, and 20 mM Tris·HCl. Enzyme assays were performed using 50 mg sample protein incubated for 1 h at 37 °C in 600 μL of assay buffer containing NADPH (500 μM) and 11-dehydrocorticosterone (2,000 nM). Immediately following incubation, the samples were placed in a boiling water bath for 5 min. 11β-Hydroxysteroid dehydrogenase type 1 (11βHSD1) oxoreductase activity was then assessed by measuring corticosterone production using a corticosterone EIA kit (Cayman Chemical), and enzyme activity was expressed in units of pg of corticosterone produced per milligram of protein per minute.

1. Hu A, et al. (2009) Th2 cytokine-induced upregulation of 11beta-hydroxysteroid dehydrogenase-1 facilitates glucocorticoid suppression of proasthmatic airway smooth muscle function. *Am J Physiol Lung Cell Mol Physiol* 296(5):L790–L803.

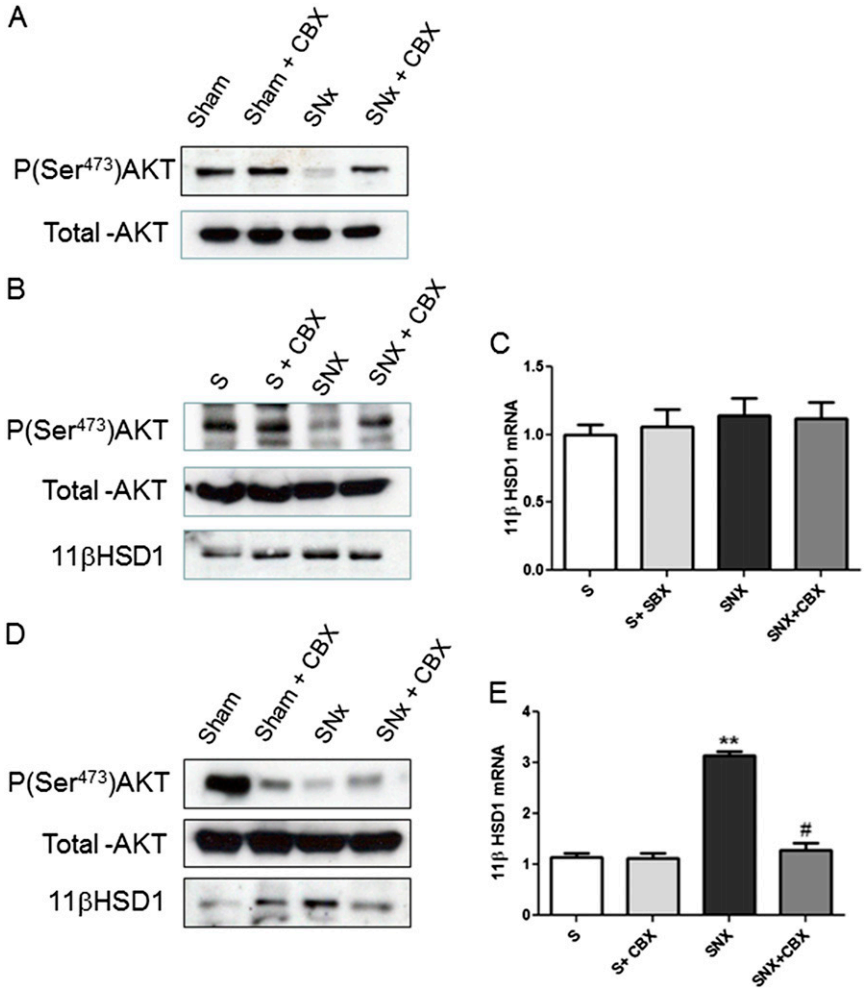

Fig. S1. Uremia-induced changes in peripheral insulin signaling and 11βHSD1 levels. Experimental uremia was induced in rats by SNx ($n = 8$ per group). CBX (50 mg·kg^{-1}·d^{-1}) or vehicle was administered by oral gavage for 2 wk. (*A*) Hepatic phospho(Ser^{473})-AKT and total-AKT protein; (*B*) skeletal muscle protein levels of phospho(Ser^{473})-AKT, total-AKT, and 11βHSD1; (*C*) skeletal muscle 11βHSD1 mRNA; (*D*) epididymal white adipose tissue protein levels of phospho(Ser^{473})-AKT, total-AKT, and 11βHSD1; and (*E*) epididymal white adipose tissue 11βHSD1 mRNA. Western blots are representative ($n = 4$). Data are expressed as mean ± SEM. Statistically significant differences between sham and SNx are indicated by $^{**}P < 0.01$. Statistically significant effects of CBX treatment are indicated by $^{\#}P < 0.05$.

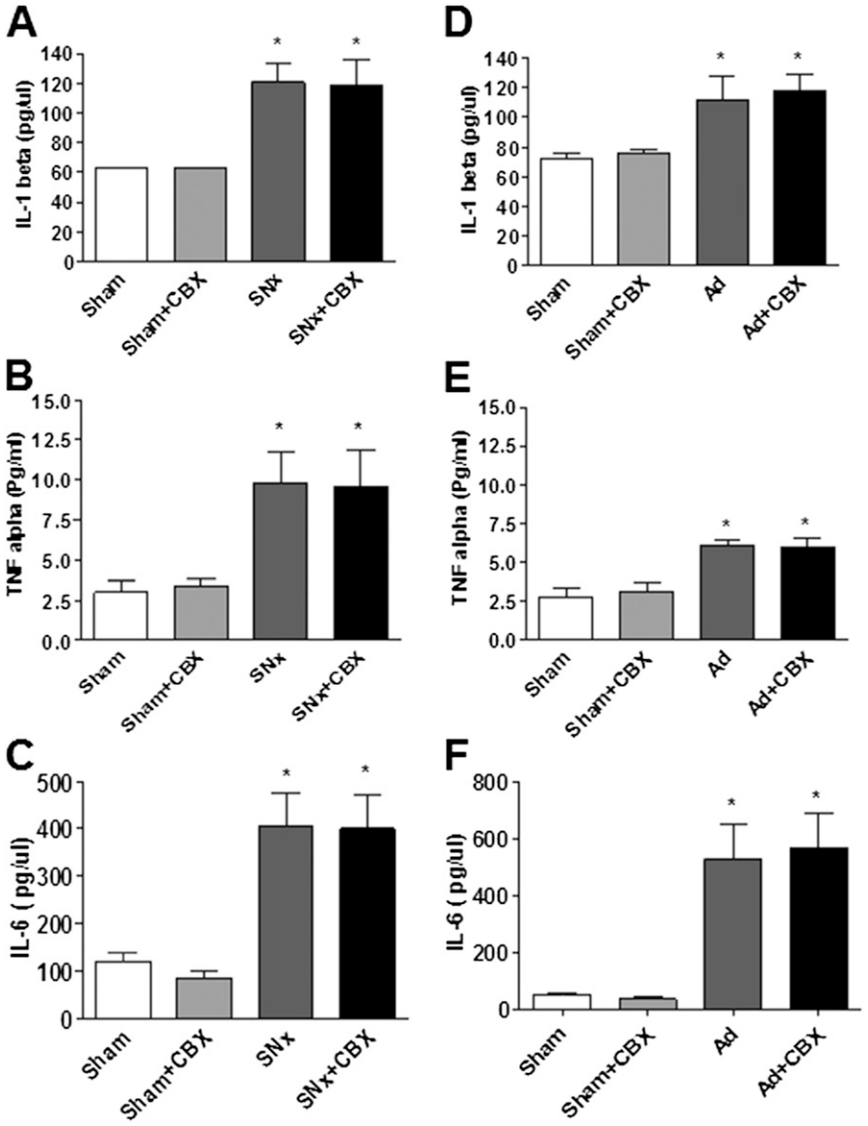

Fig. S2. Uremia induces elevated plasma cytokine levels. Experimental uremia was induced in rats by Subtotal nephrectomy (SNx) or adenine-feeding (Ad) ($n = 8$ per group). Carbenoxolone (CBX) (50 mg·kg^{-1}·d^{-1}) or vehicle was administered by oral gavage for 2 wk. Plasma levels of IL-1β (*A* and *D*), TNFα (*B* and *E*), and IL-6 (*C* and *F*) were measured in SNx and Ad models, respectively. Data are expressed as mean ± SEM $^{*}P < 0.05$ vs. sham or sham with CBX.

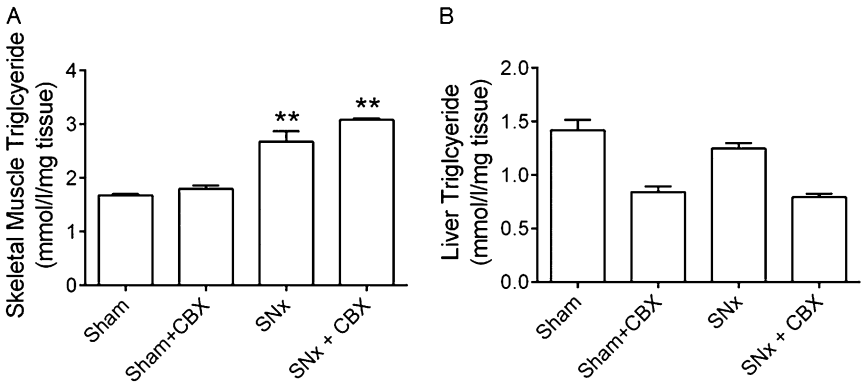

Fig. S3. Uremia induced changes in liver and skeletal muscle triglyceride levels. Experimental uremia was induced in rats by SNx ($n = 8$ per group). CBX (50 mg·kg^{-1}·d^{-1}) or vehicle was administered by oral gavage for 2 wk. (*A*) Hepatic triglyceride levels, (*B*) Skeletal muscle triglyceride levels. Data are expressed as mean ± SEM. Statistically significant differences between sham and SNx are indicated by $^{**}P < 0.01$. Statistically significant effects of CBX treatment are indicated by $^{\#}P < 0.05$.

Table S1. Serum markers of renal failure in SNx rats

Laboratory parameters	Sham	SNx	Sham + CBX	SNx + CBX
Serum sodium, mmol/L	135.9 ± 5.3	136.1 ± 3.2	135.1 ± 2.6	138.4 ± 2.2
Serum potassium, mmol/L	4.9 ± 0.9	4.8 ± 0.5	4.4 ± 0.7	4.6 ± 0.2
Serum urea, mmol/L	5.5 ± 0.3	30.7 ± 3.7*	8.7 ± 0.4	28.5 ± 1.4*
Serum creatinine, μmol/L	38.1 ± 1.0	139.3 ± 10.9*	41.9 ± 2.0	133 ± 6.5*
Proteinuria, g/24 h	0.27 ± 0.04	3.6 ± 0.3*	0.21 ± 0.4	3.3 ± 0.5*

Renal dysfunction was determined in subtotal SNx rats ($n = 8$ per group) by measurements of serum levels of creatinine, urea, sodium, and potassium and urine levels of protein. Data are expressed as mean ± SEM. *$P <$ 0.05 vs. sham.

Table S2. Serum markers of renal failure in adenine-fed rats

Laboratory parameters	CON	Ad	CON + CBX	Ad + CBX
Serum sodium, mmol/L	136.5 ± 4.5	135.7 ± 0.2	139.5 ± 1.4	141 ± 1.2
Serum potassium, mmol/L	4.46 ± 0.2	5.24 ± 0.1*	4.75 ± 0.2	5.7 ± 0.1*
Serum urea, mmol/L	7.3 ± 0.4	86.4 ± 8.5*	6.31 ± 0.7	92.1 ± 4.0*
Serum creatinine, μmol/L	39.6 ± 2.1	325 ± 20.4*	37.48 ± 0.7	314.2 ± 18.3*
Proteinuria, g/24 h	0.1 ± 0.03	3.2 ± 0.3*	0.024 ± 0.1	3.7 ± 0.7*

Renal dysfunction was determined in adenine-fed rats ($n = 8$ per group) by measurements of serum levels of creatinine, urea, sodium, and potassium and urine levels of protein. Ad, adenine; CON, control. Data are expressed as mean ± SEM. *$P < 0.05$ vs. sham (Table S1).

Table S3. Serum markers of renal failure in adenine-fed 11βHSD$^{-/-}$ mice

Laboratory parameters	WT-CON	WT-Ad	11βHSD1$^{-/-}$-CON	11βHSD1$^{-/-}$-Ad
Serum sodium, mmol/L	148.1 ± 3.2	147.2 ± 3.6	145.1 ± 4.1	142.8 ± 2.6
Serum potassium, mmol/L	5.6 ± 0.7	5.4 ± 1.2	5.6 ± 0.9	5.3 ± 0.09
Serum urea, mmol/L	11.4 ± 2.5	43.7 ± 1.5*	10.6 ± 3.3	44.9 ± 2.6*
Serum creatinine, μmol/L	22.2 ± 2.2	68.6 ± 4.3*	23.0 ± 3.1	67.7 ± 3.7*

Renal dysfunction was determined in adenine-fed mice ($n = 8$ per group) by measurements of serum levels of creatinine, urea, sodium, and potassium. WT, wild type. Data are expressed as mean ± SEM. *$P < 0.05$ vs. control diet.

Table S4. Baseline characteristics of SNx group

Measurements	Sham	SNx	Sham + CBX	SNx + CBX
Body weight, g	307.1 ± 92.6	303.7 ± 48.3	313.1 ± 19.6	302.1 ± 8.4
Food intake, g/24 h	27.1 ± 1.6	24.7 ± 2.2	26.8 ± 1.4	24.6 ± 2.6
Mean HR, bpm	403.8 ± 23.2	416.3 ± 36.1	415.0 ± 26.3	423.8 ± 38.2
MBP, mmHg	112.0 ± 38.1	135.5 ± 12.0	119.7 ± 26.4	152.0 ± 13.9

Measurements in experimental models of uremia ($n = 8$ per group). Data are expressed as mean ± SEM. bpm, beats per minute; HR, heart rate; MBP, mean blood pressure.

Table S5. Baseline characteristics of Ad group

Measurements	CON	Ad	CON + CBX	Ad + CBX
Body weight, g	325.4 ± 41.0	308.6 ± 41.0	312.6 ± 10.7	299.7 ± 8.3
Food intake, g/24 h	25.0 ± 2.1	21.5 ± 3.1	23.5 ± 2.5	20.5 ± 4.5
Mean HR, bpm	436.5 ± 14.3	445.3 ± 36.1	434.4 ± 16.9	440.6 ± 21.4
MBP, mmHg	122.0 ± 27.9	147.9 ± 25.5	129.5 ± 28.9	151.2 ± 15.9

Table S6. Serum markers of renal failure in adenine-fed rats treated with specific 11βHSD1 inhibitor UE2316

Laboratory parameters	CON + vehicle	Ad + vehicle	Ad + UE2316
Serum sodium, mmol/L	145.2 ± 3.0	144.8 ± 3.4	142.2 ± 5.5
Serum potassium, mmol/L	5.3 ± 0.3	4.9 ± 0.7	4.9 ± 0.6
Serum urea, mmol/L	8 ± 2.4	73.6 ± 12.8***	72.6 ± 14.1***
Serum creatinine, μmol/L	47.3 ± 11.6	301.7 ± 15.9***	291.1 ± 15.8***
Serum albumin, g/L	25.8 ± 2.8	24.6 ± 2.6	25.1 ± 2.1

Renal dysfunction was determined in adenine-fed mice ($n = 8$ per group) by measurements of serum levels of creatinine, urea, sodium, potassium, and albumin. Data are expressed as mean ± SEM. ***$P < 0.05$ vs. control diet.

Table S7. Quantitative PCR primer and probe sequences

Gene	Gene accession no.	Dir	Primer sequence	nM	Nanograms of cDNA per well
Cytosolic PEPCK	XM_342593	Fwd	GTCATCCGCAAGCTGAAGAA	300	10
		Rev	GCTTTCGATCCTGGCCACA	300	
		Probe	6-FAM-CAACTGTTGGCTGGCTCTCACTG ACC-TAMRA	100	
PPARγ coactivator 1alpha	NM_031347	Fwd	TCTGGAACTGCAGGCCTAACTC	300	10
		Rev	AGCTTTGGCGAAGCCTTGA	300	
		Probe	6-FAM-ACGACTCCTCCTCATAAAGCCAACC AAGATAA-TAMRA	100	
3-hydroxy-3-methylglutaryl CoA reductase	NM_03134	Fwd	TCAGCTGTACCATGCCGTCTAT	300	10
		Rev	CAGGCTTGCTGAGGTAGAAGGT	300	
		Probe	6-FAM-ATCGGAACCGTGGGTGGTGGG-TAMRA	100	
Fatty acid synthase	NM_017332	Fwd	AATACAAAATGGAGCCACTGCAT	300	10
		Rev	AACTTGAGGCCGGGATGTT	300	
		Probe	6-FAM-CGGTGCATCCGCCCCTCACT-TAMRA	100	
Acetyl CoA carboxylase	NM_022193	Fwd	CCGGTGCCCTCAACAAAGT	300	10
		Rev	AACTCCGTTGTGCATTATCTG	300	
		Probe	6-FAM-TCGGGAAGTATACACCTCCAACAA TCAGCTT-TAMRA	100	
11βHSD1	NM_017080.2	Fwd	GCTTTTGCAGAGCGATTTGTT	300	10
		Rev	CAGTCCACCCAAGAGCTTTCC	300	
		Probe	Sybr green	50	

Accession numbers refer to data deposited in NCBI Reference Sequence database. Dir, direction.