

**The blood pressure lowering effect of 20-HETE blockade in *Cyp4a14*(-/-) mice is
associated with natriuresis**

Varunkumar Pandey, Victor Garcia, Ankit Gilani, Priyanka Mishra, Frank Fan Zhang,
Mahesh P. Paudyal, John R. Falck, Alberto Nasjletti,
Wen-Hui Wang, Michal Laniado Schwartzman

Department of Pharmacology, New York Medical College School of Medicine, Valhalla, NY
VP, VC, AG, PM, FFZ, AN, WW, MLS

Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX
MPP, JRF

a) Running title: 20-HETE-mediated Na Retention in Cyp4a14(-/-) Mice

b) Corresponding Author: Michal Laniado Schwartzman, Department of Pharmacology,
New York Medical College, 15 Dana Road, Valhalla, NY 10595, Tel (914) 594-4153; Fax (914)
347-4956; E-mail: michal_schwartzman@nymc.edu

c) Text pages:

Tables: 0

Figures: 5

References: 39

Abstract: 216 words

Introduction: 473 words

Discussion: 1,126 words

d) **Abbreviations:** 20-HETE, 20-Hydroxy-5,8,11,14-eicosatetraenoic acid; 20-SOLA, 2,5,8,11,14,17-hexaoxonadecan-19-yl-20-hydroxyeicosa-6(Z),15(Z)-dienoate; RBF, Renal blood flow; GFR, glomerular filtration rate.

e) Cardiovascular

ABSTRACT

20-Hydroxy-5,8,11,14-eicosatetraenoic acid (20-HETE) has been linked to pro- and anti-hypertensive actions through its ability to promote vasoconstriction and inhibit Na transport in the ascending limb of the loop of Henle, respectively. In this study, we assessed the effects of 20-HETE blockade on blood pressure, renal hemodynamics and urinary sodium excretion in *Cyp4a14*(-/-) male mice, which display androgen-driven 20-HETE-dependent hypertension. Administration of 2,5,8,11,14,17-hexaoxonadecan-19-yl 20-hydroxyicosa-6(Z),15(Z)-dienoate (20-SOLA), a water-soluble 20-HETE antagonist, in the drinking water normalized blood pressure of male *Cyp4a14*(-/-) hypertensive mice ($124 \pm$ vs $153 \pm$ mmHg) while having no effect on age-matched normotensive wild-type male mice. Hypertension in *Cyp4a14*(-/-) male mice was accompanied by decreased renal perfusion and reduced glomerular filtration rates which were corrected by treatment with 20-SOLA. Interestingly, *Cyp4a14*(-/-) male mice treated with 20-SOLA displayed increased urinary sodium excretion that was paralleled by the reduction of blood pressure suggestive of an anti-natriuretic activity of endogenous 20-HETE in the hypertensive mice. This interpretation is in line with the observation that the natriuretic response to acute isotonic saline loading in hypertensive *Cyp4a14*(-/-) male mice was significantly impaired relative to that in WT mice; this impairment was corrected by 20-SOLA treatment. Hence, endogenous 20-HETE appears to promote sodium conservation in hypertensive *Cyp4a14*(-/-) male mice, presumably, as a result of associated changes in renal hemodynamics and/or direct stimulatory action on tubular sodium reabsorption.

INTRODUCTION

20-Hydroxyeicosatetraenoic acid (20-HETE) is a metabolite of arachidonic acid produced by the cytochrome P450 (CYP) ω -hydroxylases including members of the CYP4A and 4F gene families (Capdevila et al., 1992). This eicosanoid affects vascular and renal functions in a manner consistent with a potential involvement in the implementation of both pro- and anti-hypertensive mechanisms (Williams et al., 2010; Wu et al., 2014).

Previous studies documented that arterial hypertension is associated with derangement in 20-HETE production at vascular and/or renal tubular sites in various experimental models. For example, the development of hypertension in animals displaying augmentation of 20-HETE biosynthesis at vascular sites was attributed to one or more actions of this eicosanoid promoting increased reactivity to myogenic and neuro-hormonal stimuli (Miyata and Roman, 2005; Hoopes et al., 2015), endothelial dysfunction (Wang et al., 2006; Singh et al., 2007; Cheng et al., 2008), and/or upregulation of angiotensin converting enzyme (Sodhi et al., 2010; Garcia et al., 2015; Garcia et al., 2016), all actions capable of bringing about vasoconstriction and blood pressure elevation. On the other hand, the salt-sensitive hypertension featured by transgenic mice overexpressing the human 20-HETE synthase CYP4A11 was attributed to enhanced retention of sodium resulting from 20-HETE- and angiotensin II-dependent upregulation of the sodium chloride co-transporter (NCC) (Savas et al., 2016). Interestingly, salt-sensitive hypertension also was shown to develop in animals with depressed renal medullary synthesis of 20-HETE, which conditions retention of sodium in the ascending limb of the loop of Henle (Roman et al., 1993; Roman et al., 1997; Stec et al., 1997; Ito and Roman, 1999). Inasmuch as 20-HETE contributes to pro-hypertensive mechanisms via vascular and tubular actions that foster vasoconstriction and/or conservation of sodium, as well as to anti-hypertensive mechanisms via actions that facilitate sodium excretion, it is expected that the blood pressure effect of interventions that

interfere with 20-HETE actions is conditioned by the mix of the various vascular and renal blood pressure regulatory mechanisms involving this eicosanoid.

The present study was undertaken to examine this notion using *Cyp4a14*(-/-) male mice, which develop 20-HETE-dependent hypertension linked, sequentially, to upregulation of plasma androgen levels and enhanced renal expression of CYP4A12, the dominant 20-HETE synthase in mice (Holla et al., 2001; Wu et al., 2013; Gangadhariah et al., 2015). First, we contrasted male *Cyp4a14*(-/-) hypertensive mice with corresponding 129/SVE normotensive wild type controls (WT) in terms of the effects of a newly developed water-soluble 20-HETE antagonist - 2,5,8,11,14,17-hexaoxonadecan-19-yl-20-hydroxyeicosa-6(Z),15(Z)-dienoate (20-SOLA)- on systolic blood pressure, in relation to associated effects on renal blood perfusion, glomerular filtration rate, urine volume and sodium excretion. Second, we compared male *Cyp4a14*(-/-) hypertensive mice and 129/SVE normotensive WT undergoing treatment with 20-SOLA or its vehicle control only in terms of the ability to excrete sodium load. The results suggest that in addition to its effects on renal hemodynamics, 20-HETE promotes sodium conservation that also contributes to hypertension in this model.

MATERIALS AND METHODS

Materials. 2,5,8,11,14,17-hexaoxonadecan-19-yl 20-hydroxyicosa-6(Z),15(Z)-dienoate (20-SOLA) was synthesized as a PEG-conjugate of 20-6,15-hydroxyeicosadecaenoic acid (20-HEDE), an effective 20-HETE antagonist (Alonso-Galicia et al., 1999; Ding et al., 2013). The 20-HETE antagonistic activity of 20-SOLA was examined by its ability to prevent 20-HETE-mediated sensitization of the constrictor activity of phenylephrine in renal interlobar arteries as previously described (Zhang et al., 2001). Briefly, cumulative concentration-response curves to phenylephrine (1×10^{-9} - 1×10^{-4} M)-induced contraction of renal interlobar arterial rings mounted on a myograph were constructed in the presence of DDMS (30 μ M) to inhibit endogenously generated 20-HETE, with and without exposure to 20-HETE (1 μ M) alone or to 20-HETE (1 μ M) + 20-SOLA (1 μ M). Phenylephrine elicited concentration-dependent increases of isometric tension as seen in **Figure 1A**. In vessels bathed in media containing 20-HETE, the concentration-dependent increase in tension produced by phenylephrine was shifted to the left revealing a major decrease in the EC₅₀ of phenylephrine (**Fig. 1B**). Co-addition of 20-SOLA prevented 20-HETE-mediated sensitization of phenylephrine-induced vasoconstriction (**Fig. 1**). FITC-inulin was obtained from Sigma-Aldrich (St. Louis, MO).

Animals. All experimental protocols were approved by the Institutional Animal Care and Use Committee. The *Cyp4a14*(-/-) mouse model has been previously characterized (Holla et al., 2001). The male but not female *Cyp4a14*(-/-) mice show a marked induction of Cyp4a12, the sole 20-HETE synthase in the mouse, and exhibit 20-HETE-dependent hypertension (Wu et al., 2013; Gangadhariah et al., 2015). Male *Cyp4a14*(-/-) mice and age and gender-matched background wild type 129/SVE mice (WT, 8-10-week-old) were used.

Experimental Protocols.

Protocol 1: Mice were housed in metabolic cages with free access to food and drinking water containing or not containing 20-SOLA (10 mg/kg per day) for 10 days. Food and water

intake, and urinary output were monitored daily. No differences were observed in food and water intake between *Cyp4a14*(-/-) and WT mice receiving 20-SOLA (data not shown). For blood pressure measurements, mice were acclimatized to the tail-cuff measurement for seven days prior to the start of experiments. In brief, mice were placed on an infrared heating pad for 7-10 minutes. Systolic blood pressure was recorded using the CODA non-invasive blood pressure system (Kent Scientific, Torrington CT) as previously described (Wu et al., 2013). Urinary sodium concentration was determined by flame photometry and daily excretion rate was calculated. Renal blood flow (RBF) and glomerular filtration rate (GFR) were measured at the end of the 10-day experimental protocol as follows:

RBF was evaluated under anesthesia by using Laser Doppler flowmetry (Shi et al., 2007). Briefly, a midline incision was performed to access the kidneys. Laser Doppler scanner was utilized to obtain a pseudo-colored scan of renal blood perfusion in live anesthetized mice. A laparotomy was performed to expose the kidneys followed by assessment of cortical renal blood perfusion values using the laser-Doppler Perfusion Monitoring probes, image scanner, and accompanying software (PeriScan PIM II, PeriFlux system 5000, and LDPIwin version 2.6.1, respectively; PeriMed Instruments, PA). The correct positioning of laser-Doppler probes was verified after completion of each experiment.

GFR was measured by the FITC-Inulin clearance method (Rieg, 2013). Briefly, FITC-inulin (5% in 0.85% NaCl) was dialyzed against 0.85% NaCl for 24 h resulting in a ~2% solution that was used to establish the standard curve. The dialyzed FITC-inulin solution was sterile filtered and injected into the retro-orbital plexus (3.74 μ l/g/body weight) during brief isoflurane anesthesia. At 3, 7, 10, 15, 35, 55 and 75 min after injection, blood was collected from the tail into a Na⁺ heparinized 70 μ l capillary tube. After centrifugation, 10 μ l of plasma was diluted 1:5 in 500 mM HEPES (pH 7.4) and fluorescence was determined on a 96-well plate using Synergy HT, Biotek with 485 nm excitation, and 538 nm emission. GFR was calculated using a two-compartment model of two-phase exponential decay (GraphPad Prism, San Diego, CA).

Protocol 2: WT and *Cyp4a14*(-/-) mice undergoing pretreatment with either vehicle or 20-SOLA (10 mg/kg per day) for 72 hours were placed in metabolic cages for urine collection before and 4 hours after IP injection of isotonic saline (10% V/W). The amount of sodium excreted and the ratio of injected versus excreted sodium were assessed (Trott et al., 2014).

Statistical Analysis. Data are expressed as mean \pm SEM. Significance of difference in mean values was determined by using two-tailed t test for comparison between two groups or one-way ANOVA for multiple groups comparisons, followed by the Tukey-Kramer post hoc test. $P < 0.05$ was considered to be significant.

RESULTS

20-SOLA Normalizes Blood Pressure in *Cyp4a14*(-/-) Male Hypertensive Mice

The *Cyp4a14*(-/-) mouse is a model of androgen-driven male-specific 20-HETE-dependent hypertension; *Cyp4a14*(-/-) male mice are hypertensive whereas corresponding females are normotensive (Holla et al., 2001; Wu et al., 2013). As previously reported, administration of the 20-HETE antagonist 20-HEDE or 20-SOLA normalizes blood pressure in the male *Cyp4a14*(-/-) mice without affecting renal or vascular levels of 20-HETE (Wu et al., 2013; Gangadhariah et al., 2015). As seen in **Figure 2**, administration of 20-SOLA (10mg/kg/day) in the drinking water to *Cyp4a14*(-/-) male mice significantly reduced systolic blood pressure within 4 days of the onset of treatment, and normalized the blood pressure after 10 days of treatment. In contrast, the same treatment had no effect on systolic blood pressure in age-matched WT mice (**Figure 2**).

Impaired renal hemodynamic function is corrected by treatment with 20-SOLA in *Cyp4a14*(-/-) mice

We contrasted male WT and *Cyp4a14*(-/-) mice treated with vehicle or 20-SOLA in terms of renal blood perfusion and GFR. As seen in **Figure 3A**, renal blood perfusion in hypertensive *Cyp4a14*(-/-) mice was significantly lower compared to WT controls. Treatment with 20-SOLA for 10 days did not affect renal perfusion in WT controls but increased renal perfusion in the hypertensive *Cyp4a14*(-/-) mice to the level observed in the WT controls (**Figure 3A**). GFR of hypertensive *Cyp4a14*(-/-) mice was surpassed by that of WT mice (1.83 ± 0.15 vs 2.41 ± 0.12 $\mu\text{L}/\text{min}/\text{mg}$ kidney weight, $p < 0.05$ vs corresponding WT). Treatment with 20-SOLA did not affect the GFR of WT mice but increased the GFR of *Cyp4a14*(-/-) mice (2.38 ± 0.04 $\mu\text{L}/\text{min}/\text{mg}$, $p < 0.05$) to levels similar to that of WT mice (**Figure 3B**). Hence, 20-HETE appears to be a critical determinant not only of the hypertension featured by *Cyp4a14*(-/-) male mice but also of the

accompanying alterations in renal hemodynamics, viz., reduction in renal blood perfusion and GFR.

20-SOLA treatment increases urinary sodium excretion in male *Cyp4a14*(-/-) mice

Urinary volume of 7x24 h collections over 10 days was significantly lower in *Cyp4a14*(-/-) mice when compared to corresponding WT mice (55.90 ± 1.89 vs 60.29 ± 3.14 $\mu\text{l/g BW/day}$, $n=7$, $p=0.02$). Likewise, uNaV (average of 7x24 h collections over 10 days) was lower in *Cyp4a14*(-/-) as compared to WT (5.77 ± 0.25 vs 7.17 ± 0.22 $\mu\text{mol/g BW/day}$, $n=7$, $p=0.0006$). Urinary volume and sodium excretion in WT mice were unaffected by treatment with 20-SOLA (**Figure 4A and C**). On the other hand, treatment of male *Cyp4a14*(-/-) mice with 20-SOLA elicited increases in both urinary volume and sodium excretion, beginning on day 2 after the onset of treatment and lasting until day 6 (**Figure 4B-D**) at which time the blood pressure is appreciably decreased (**Figure 2**). There were no changes in body weight between treated and untreated *Cyp4a14*(-/-) mice with 20-SOLA during the course of the study (e.g., 19.3 ± 1.8 vs 20.5 ± 0.2 g in *Cyp4a14*(-/-) mice treated with vehicle and 20-SOLA for 10 days, respectively). Urinary protein was also unchanged (e.g., 2.64 ± 0.66 vs 3.38 ± 0.44 mg/g bw/day in *Cyp4a14*(-/-) mice treated with vehicle and 20-SOLA for 10 days, respectively). Likewise, no changes in these parameters were observed between WT treated and untreated with 20-SOLA (data not shown).

Natriuretic response to a salt challenge is attenuated in *Cyp4a14*(-/-) mice

Next we contrasted WT and *Cyp4a14*(-/-) male mice pretreated for 72 hours with vehicle or 20-SOLA (10 mg/kg/day) in terms of their ability to dispose of Na in response to acute isotonic salt loading (10% v/w, 0.15 mol/L NaCl). As shown in **Figure 5A**, acute salt loading caused urinary sodium excretion to increase significantly ($p<0.05$) relative to corresponding basal value, in both WT and *Cyp4a14*(-/-) male mice pretreated for 72 hours with 20-SOLA or

vehicle only. Of note, the natriuretic response to salt loading was comparable in WT male mice pretreated with 20-SOLA or vehicle only, but was depressed in vehicle pretreated *Cyp4a14*(-/-) male mice relative to corresponding values in similarly treated WT mice. Importantly, the impaired natriuretic response to salt loading noted in *Cyp4a14*(-/-) male mice pretreated with vehicle only was prevented in *Cyp4a14*(-/-) mice pretreated with 20-SOLA. Assessment of the fraction of the sodium load excreted in the urine during the first 4 hours after saline administration also showed that 20-SOLA pretreatment corrects the impaired natriuretic response observed in *Cyp4a14*(-/-) male mice which overexpress CYP4A12, a 20-HETE biosynthetic enzyme, but is without effect on the natriuretic response to the salt loading in WT mice which do not overexpress Cyp4a12 (**Figure 5B**).

DISCUSSION

This study demonstrates that systolic blood pressure and indices of renal hemodynamic function, renal blood perfusion and GFR, in hypertensive *Cyp4a14*(-/-) male mice and corresponding 129/SVE normotensive WT controls are dissimilarly impacted by treatment with 20-SOLA. That the 20-HETE antagonist is without effect on blood pressure and renal hemodynamics in normotensive WT mice, but lowers blood pressure and increases renal blood perfusion and GFR in hypertensive *Cyp4a14*(-/-) mice is attributable to differences in the status of 20-HETE synthesis among the strains. This interpretation derives support from previous reports documenting androgen-driven upregulation of CYP4A12, the main 20-HETE synthase in mice, and 20-HETE generation in vascular and renal structures of hypertensive *Cyp4a14*(-/-) mice (Holla et al., 2001; Wu et al., 2013). Since 20-HETE supports pro-hypertensive mechanisms relying on enhanced vasoconstriction (Williams et al., 2010; Hoopes et al., 2015; Fan et al., 2016), the heightened blood pressure and depressed renal perfusion featured by *Cyp4a14*(-/-) male mice are viewed as manifestations of increased 20-HETE generation (Wu et al., 2013). Conversely, the antihypertensive effect of 20-SOLA in *Cyp4a14*(-/-) hypertensive mice is attributable to its ability to antagonize 20-HETE-mediated vasoconstriction. Of note, recently we reported that a 20-HETE antagonist related to 20-SOLA, viz., 20-6,15-HEDGE, prevents the binding of 20-HETE to an orphan G-protein coupled receptor (GPCR) identified as GPR75 (Garcia et al., 2017). Since the pairing of 20-HETE and GPR75 is an essential feature of the above referenced vasoconstrictor-pressor actions of 20-HETE (Garcia et al., 2017), it is plausible that the antihypertensive response to treatment of *Cyp4a14*(-/-) mice with 20-SOLA results from interference of the latter with the binding of 20-HETE to GPR75, its putative receptor.

Our study also demonstrates that the treatment with 20-SOLA affects differently urinary sodium excretion and urine volume in hypertensive *Cyp4a14*(-/-) mice and corresponding WT

normotensive mice. The 20-HETE antagonist had no effect on sodium excretion or urine volume in normotensive mice but in hypertensive *Cyp4a14*(-/-) mice it caused slight elevation of urine volume and a robust increase of urinary sodium excretion. Since the natriuretic effect of 20-SOLA in *Cyp4a14*(-/-) hypertensive mice precedes by two days the onset of blood pressure reduction, it is conceivably that the enhanced sodium loss prompted by the 20-HETE antagonist is a primary contributor to the slightly delayed hypotensive effect, presumably via a mechanism involving reduction of extracellular fluid volume with attendant reduction of cardiac output (Williams et al., 2010). This interpretation is not necessarily incongruent with the established concept that urinary sodium excretion is negatively affected by an associated reduction of blood pressure (Reckelhoff et al., 1998). Indeed, in our study, the natriuretic effect of 20-SOLA in *Cyp4a14*(-/-) hypertensive mice had largely waned on days 8 and 10 after the onset of treatment, at which time the hypertension had nearly subsided. Based on these considerations, it would appear that the antihypertensive effect of treatment with 20-SOLA in *Cyp4a14*(-/-) mice results from antagonism of pro-hypertensive actions of 20-HETE, at vascular and perhaps at renal tubular sites promoting vasoconstriction and conservation of sodium, respectively.

The notion that over production of 20-HETE-contributes to excessive renal conservation of sodium in *Cyp4a14*(-/-) hypertensive mice, fits well with our current findings confirming that these animals are impaired in their ability to increase urinary sodium excretion after an acute saline load (Fidelis et al., 2010). That treatment with 20-SOLA results in complete normalization of the natriuretic response to acute saline loading in *Cyp4a14*(-/-) hypertensive mice is strong evidence that in these animals the over production of 20-HETE and the accompanying impairment to excrete sodium are causally related. Enhanced activity of 20-HETE-driven sodium conserving mechanisms in *Cyp4a14*(-/-) hypertensive mice may be attributed to actions of this eicosanoid targeting renal vascular structures to reduce renal perfusion and glomerular filtration and/or tubular structures to directly increase sodium reabsorption (Evans et al., 2005; Williams et al., 2007). Quigley and coworkers have documented augmentation of renal proximal

convoluted tubule transport in *Cyp4a14*(-/-) hypertensive mice, along with enhanced brush border proximal tubule expression of the sodium/hydrogen exchanger type 3 (NHE3) (Quigley et al., 2009), the major sodium transporter in this segment of the nephron (McDonough, 2010). Upregulation of renal 20-HETE synthesis, along with increased proximal tubule volume reabsorption and NHE3 protein abundance in kidney brush border membranes, also has been documented in rats treated with dihydrotestosterone (DHT) (Quigley, 2008) which increases blood pressure via mechanisms supported by 20-HETE (Wu et al., 2013) and angiotensin II (Quan et al., 2004; Yanes et al., 2009), that foster vasoconstriction and sodium conservation. However, the concept that 20-HETE-driven sodium reabsorption in animals with androgen-dependent hypertension relies on upregulation of proximal tubule NHE3 expression is missing critical confirmatory evidence. In this regard, Quigley and associates have reported that rabbit proximal tubule volume transport is enhanced following pharmacological inhibition of 20-HETE synthesis, a finding which is reversed by acute exposure to exogenous 20-HETE (Quigley et al., 2000). Hence, these observations argue strongly against the notion that endogenous 20-HETE fosters direct activation of renal NHE3. On the other hand, it would be premature to exclude from consideration a more complex mechanism for engagement of 20-HETE in promotion of sodium conservation, viz., a mechanism which includes an early step whereby 20-HETE of tubular origin stimulates the expression of components of the renin-angiotensin system intrinsic to renal tubular structures, ACE and/or angiotensinogen, with attendant elevation of angiotensin levels which, in turn, augment sodium reabsorption via activation of NHE3 and/or NCC (Dos Santos et al., 2004; Sodhi et al., 2010; Cheng et al., 2012; Savas et al., 2016). Experimental support for this concept includes reports documenting that 20-HETE-dependent induction of ACE leads to elevation of angiotensin II tissue levels (Sodhi et al., 2010; Cheng et al., 2012), that NHE3 is subject to stimulatory regulation by angiotensin II (McDonough, 2010), and that hypertension in transgenic mice overexpressing the human CYP4A11 relies on excessive sodium retention dependent on 20-HETE- and angiotensin II-dependent upregulation of NCC

(Savas et al., 2016). Further experiments are required to examine the activity of NHE3 in proximal tubule and NCC in distal convoluted tubule in *Cyp4a14*(-/-) mice.

In summary, this study underscores the significance of excessive 20-HETE production to the hypertensive phenotype displayed by *Cyp4a14*(-/-) male mice, which is associated with diminished renal blood perfusion and GFR and impaired ability to excrete an acute sodium load. That this phenotype is corrected by treatment with 20-SOLA, an antagonist of 20-HETE, implicates endogenous 20-HETE in the mechanisms underlying development of hypertension, renal hypoperfusion, reduced glomerular filtration, and excessive sodium conservation in *Cyp4a14*(-/-) male mice. There is a wealth of information on the mechanisms underlying the vascular mechanisms mediating the actions of 20-HETE which implement elevation of blood pressure, and decrease renal blood flow and glomerular filtration (Williams et al., 2010). On the other hand, information is limited on 20-HETE-mediated tubular actions that promote sodium conservation.

Authorship contributions

Participated in research design: Schwartzman, Nasjletti, Falck, Wang.

Conducted experiments: Pandey, Gilani, Garcia, Mishra, Zhang, Paudyal

Performed data analysis: Pandey, Gilani, Schwartzman.

Wrote or contributed to the writing of the manuscript: Schwartzman, Nasjletti, Falck, Pandey,
Garcia, Wang

REFERENCES

- Alonso-Galicia M, Falck JR, Reddy KM and Roman RJ (1999) 20-HETE agonists and antagonists in the renal circulation. *Am J Physiol* **277**:F790-796.
- Capdevila JH, Falck JR and Estabrook RW (1992) Cytochrome P450 and the arachidonate cascade. *FASEB J* **6**:731-736.
- Cheng J, Garcia V, Ding Y, Wu CC, Thakar K, Falck JR, Ramu E and Schwartzman ML (2012) Induction of Angiotensin-Converting Enzyme and Activation of the Renin-Angiotensin System Contribute to 20-Hydroxyeicosatetraenoic Acid-Mediated Endothelial Dysfunction. *Arterioscler Thromb Vasc Biol* **32**:1917-1924.
- Cheng J, Ou JS, Singh H, Falck JR, Narsimhaswamy D, Pritchard KA, Jr. and Schwartzman ML (2008) 20-Hydroxyeicosatetraenoic acid causes endothelial dysfunction via eNOS uncoupling. *Am J Physiol Heart Circ Physiol* **294**:H1018-1026.
- Ding Y, Wu CC, Garcia V, Dimitrova I, Weidenhammer A, Joseph G, Zhang F, Manthathi VL, Falck JR, Capdevila JH and Schwartzman ML (2013) 20-HETE induces remodeling of renal resistance arteries independent of blood pressure elevation in hypertension. *Am J Physiol Renal Physiol* **305**:F753-763.
- Dos Santos EA, Dahly-Vernon AJ, Hoagland KM and Roman RJ (2004) Inhibition of the formation of EETs and 20-HETE with 1-aminobenzotriazole attenuates pressure natriuresis. *Am J Physiol Regul Integr Comp Physiol* **287**:R58-68.
- Evans RG, Majid DS and Eppel GA (2005) Mechanisms mediating pressure natriuresis: what we know and what we need to find out. *Clin Exp Pharmacol Physiol* **32**:400-409.
- Fan F, Ge Y, Lv W, Elliott MR, Muroya Y, Hirata T, Booz GW and Roman RJ (2016) Molecular mechanisms and cell signaling of 20-hydroxyeicosatetraenoic acid in vascular pathophysiology. *Frontiers in bioscience (Landmark edition)* **21**:1427-1463.

- Fidelis P, Wilson L, Thomas K, Villalobos M and Oyekan AO (2010) Renal function and vasomotor activity in mice lacking the Cyp4a14 gene. *Experimental biology and medicine* (Maywood, NJ **235**:1365-1374.
- Gangadhariah MH, Luther JM, Garcia V, Pauksakon P, Zhang MZ, Hayward SW, Love HD, Falck JR, Manthathi VL, Imig JD, Schwartzman ML, Zent R, Capdevila JH and Pozzi A (2015) Hypertension is a major contributor to 20-hydroxyeicosatetraenoic acid-mediated kidney injury in diabetic nephropathy. *J Am Soc Nephrol* **26**:597-610.
- Garcia V, Gilani A, Shkolnik B, Pandey V, Zhang FF, Dakarapu R, Gandham SK, Reddy NR, Graves JP, Gruzdev A, Zeldin DC, Capdevila JH, Falck JR and Schwartzman ML (2017) 20-HETE Signals Through G Protein-Coupled Receptor GPR75 (Gq) to Affect Vascular Function and Trigger Hypertension. *Circ Res*.
- Garcia V, Joseph G, Shkolnik B, Ding Y, Zhang FF, Gotlinger K, Falck JR, Dakarapu R, Capdevila JH, Bernstein KE and Schwartzman ML (2015) Angiotensin II receptor blockade or deletion of vascular endothelial ACE does not prevent vascular dysfunction and remodeling in 20-HETE-dependent hypertension. *Am J Physiol Regul Integr Comp Physiol* **309**:R71-78.
- Garcia V, Shkolnik B, Milhau L, Falck JR and Schwartzman ML (2016) 20-HETE Activates the Transcription of Angiotensin-Converting Enzyme via Nuclear Factor-kappaB Translocation and Promoter Binding. *J Pharmacol Exp Ther* **356**:525-533.
- Holla VR, Adas F, Imig JD, Zhao X, Price E, Jr., Olsen N, Kovacs WJ, Magnuson MA, Keeney DS, Breyer MD, Falck JR, Waterman MR and Capdevila JH (2001) Alterations in the regulation of androgen-sensitive Cyp 4a monooxygenases cause hypertension. *Proc Natl Acad Sci U S A* **98**:5211-5216.
- Hoopes SL, Garcia V, Edin ML, Schwartzman ML and Zeldin DC (2015) Vascular actions of 20-HETE. *Prostaglandins Other Lipid Mediat* **120**:9-16.

- Ito O and Roman RJ (1999) Role of 20-HETE in elevating chloride transport in the thick ascending limb of Dahl SS/Jr rats. *Hypertension* **33**:419-423.
- McDonough AA (2010) Mechanisms of proximal tubule sodium transport regulation that link extracellular fluid volume and blood pressure. *Am J Physiol Regul Integr Comp Physiol* **298**:R851-861.
- Miyata N and Roman RJ (2005) Role of 20-hydroxyeicosatetraenoic acid (20-HETE) in vascular system. *J Smooth Muscle Res* **41**:175-193.
- Quan A, Chakravarty S, Chen JK, Chen JC, Loleh S, Saini N, Harris RC, Capdevila J and Quigley R (2004) Androgens augment proximal tubule transport. *Am J Physiol Renal Physiol* **287**:F452-459.
- Quigley R (2008) Androgens stimulate proximal tubule transport. *Gend Med* **5 Suppl A**:S114-120.
- Quigley R, Baum M, Reddy KM, Griener JC and Falck JR (2000) Effects of 20-HETE and 19(S)-HETE on rabbit proximal straight tubule volume transport. *Am J Physiol Renal Physiol* **278**:F949-953.
- Quigley R, Chakravarty S, Zhao X, Imig JD and Capdevila JH (2009) Increased renal proximal convoluted tubule transport contributes to hypertension in Cyp4a14 knockout mice. *Nephron Physiol* **113**:p23-28.
- Reckelhoff JF, Zhang H and Granger JP (1998) Testosterone exacerbates hypertension and reduces pressure-natriuresis in male spontaneously hypertensive rats. *Hypertension* **31**:435-439.
- Rieg T (2013) A High-throughput method for measurement of glomerular filtration rate in conscious mice. *Journal of visualized experiments : JoVE*:e50330.
- Roman RJ, Alonso-Galicia M and Wilson TW (1997) Renal P450 metabolites of arachidonic acid and the development of hypertension in Dahl salt-sensitive rats. *Am J Hypertens* **10**:63s-67s.

- Roman RJ, Ma YH, Frohlich B and Markham B (1993) Clofibrate prevents the development of hypertension in Dahl salt-sensitive rats. *Hypertension* **21**:985-988.
- Savas U, Wei S, Hsu MH, Falck JR, Guengerich FP, Capdevila JH and Johnson EF (2016) 20-Hydroxyeicosatetraenoic Acid (HETE)-dependent Hypertension in Human Cytochrome P450 (CYP) 4A11 Transgenic Mice: NORMALIZATION OF BLOOD PRESSURE BY SODIUM RESTRICTION, HYDROCHLOROTHIAZIDE, OR BLOCKADE OF THE TYPE 1 ANGIOTENSIN II RECEPTOR. *J Biol Chem* **291**:16904-16919.
- Shi H, Patschan D, Epstein T, Goligorsky MS and Winaver J (2007) Delayed recovery of renal regional blood flow in diabetic mice subjected to acute ischemic kidney injury. *Am J Physiol Renal Physiol* **293**:F1512-1517.
- Singh H, Cheng J, Deng H, Kemp R, Ishizuka T, Nasjletti A and Schwartzman ML (2007) Vascular cytochrome P450 4A expression and 20-hydroxyeicosatetraenoic acid synthesis contribute to endothelial dysfunction in androgen-induced hypertension. *Hypertension* **50**:123-129.
- Sodhi K, Wu CC, Cheng J, Gotlinger K, Inoue K, Goli M, Falck JR, Abraham NG and Schwartzman ML (2010) CYP4A2-induced hypertension is 20-hydroxyeicosatetraenoic acid- and angiotensin II-dependent. *Hypertension* **56**:871-878.
- Stec DE, Mattson DL and Roman RJ (1997) Inhibition of renal outer medullary 20-HETE production produces hypertension in Lewis rats. *Hypertension* **29**:315-319.
- Trott DW, Thabet SR, Kirabo A, Saleh MA, Itani H, Norlander AE, Wu J, Goldstein A, Arendshorst WJ, Madhur MS, Chen W, Li CI, Shyr Y and Harrison DG (2014) Oligoclonal CD8+ T cells play a critical role in the development of hypertension. *Hypertension* **64**:1108-1115.
- Wang JS, Singh H, Zhang F, Ishizuka T, Deng H, Kemp R, Wolin MS, Hintze TH, Abraham NG, Nasjletti A and Laniado-Schwartzman M (2006) Endothelial dysfunction and hypertension in rats transduced with CYP4A2 adenovirus. *Circ Res* **98**:962-969.

- Williams JM, Murphy S, Burke M and Roman RJ (2010) 20-hydroxyeicosatetraenoic acid: a new target for the treatment of hypertension. *Journal of cardiovascular pharmacology* **56**:336-344.
- Williams JM, Sarkis A, Lopez B, Ryan RP, Flasch AK and Roman RJ (2007) Elevations in renal interstitial hydrostatic pressure and 20-hydroxyeicosatetraenoic acid contribute to pressure natriuresis. *Hypertension* **49**:687-694.
- Wu CC, Gupta T, Garcia V, Ding Y and Schwartzman ML (2014) 20-HETE and blood pressure regulation: clinical implications. *Cardiol Rev* **22**:1-12.
- Wu CC, Mei S, Cheng J, Ding Y, Weidenhammer A, Garcia V, Zhang F, Gotlinger K, Manthati VL, Falck JR, Capdevila JH and Schwartzman ML (2013) Androgen-sensitive hypertension associates with upregulated vascular CYP4A12-20-HETE synthase. *J Am Soc Nephrol* **24**:1288-1296.
- Yanes LL, Sartori-Valinotti JC, Iliescu R, Romero DG, Racusen LC, Zhang H and Reckelhoff JF (2009) Testosterone-dependent hypertension and upregulation of intrarenal angiotensinogen in Dahl salt-sensitive rats. *Am J Physiol Renal Physiol* **296**:F771-779.
- Zhang F, Wang MH, Krishna UM, Falck JR, Laniado-Schwartzman M and Nasjletti A (2001) Modulation by 20-HETE of phenylephrine-induced mesenteric artery contraction in spontaneously hypertensive and Wistar-Kyoto rats. *Hypertension* **38**:1311-1315.

Footnotes

This study was supported by National Institute of Health grants HL034300 (M.L. Schwartzman), DK54983 (W.H. Wang) and National Heart, Lung, and Blood Institute Diversity Supplement Award HL34300-26A1S1 (V. Garcia), by the Cornelius H. Borman Research Funds (M.L. Schwartzman) and by the Robert A. Welch Foundation (I-011, J. R. Falck).

FIGURE LEGENDS

Figure 1: A) Effect of 20-SOLA on the 20-HETE-increased sensitivity to phenylephrine constrictor responses in renal interlobar arteries from WT mice. The constrictor response to phenylephrine was measured in the presence of DDMS (30 $\mu\text{mol/L}$) with and without 20-HETE (1 $\mu\text{mol/L}$) alone or together with 20-SOLA (1 $\mu\text{mol/L}$) (mean \pm SEM, n=6). **B)** Calculated EC₅₀ to phenylephrine in the presence of DDMS with and without 20-HETE and 20-SOLA (mean \pm SEM, n=6).

Figure 2: Time course of blood pressure response to 20-SOLA in WT (n=4) and Cyp4a14(-/-) (n=5) male mice. Systolic blood pressure was measured by the tail cuff method. Results are mean \pm SEM, *p<0.05 vs vehicle-treated Cyp4a14(-/-) mice.

Figure 3: Effect of 20-SOLA on **(A)** renal blood flow (RBF) and **(B)** glomerular filtration rate (GFR) in WT and Cyp4a14(-/-) male mice. Laser Doppler perfusion monitoring probes were used to quantify renal blood perfusion in LDFU units from the renal cortices of Cyp4a14(-/-) and WT mice treated for 10 days with either vehicle or 20-SOLA. GFR was measured by the FITC-inulin method. Values are mean \pm SEM from 4-6 mice in each group.

Figure 4: Urinary volume and sodium excretion (UNaV) in WT **(A and C)** and Cyp4a14(-/-) **(B and D)** mice treated with vehicle or 20-SOLA for 10 days (n=6/group, *p<0.05 vs vehicle-treated Cyp4a14(-/-) mice).

Figure 5: Sodium excretion in response to salt loading in Cyp4a14(-/-) and WT male mice treated with vehicle or 20-SOLA. Mice were treated with vehicle or 20-SOLA for 72 h prior to administration of isotonic saline (i.p., 10% v/w) and urine collected for 4 h thereafter. The

natriuretic response was calculated as **(A)** $\mu\text{mol Na/hr/g}$ body weight and **(B)** as percentage of sodium load (mean \pm SEM, n=3/group).

Figure 1

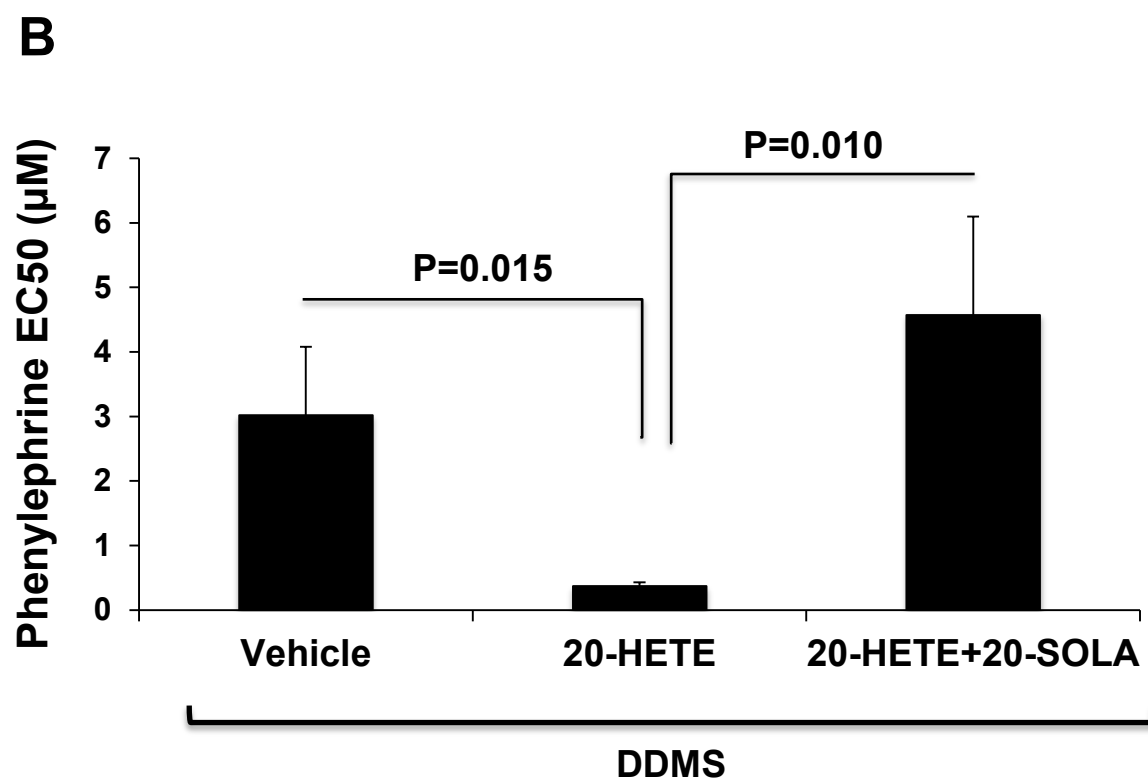
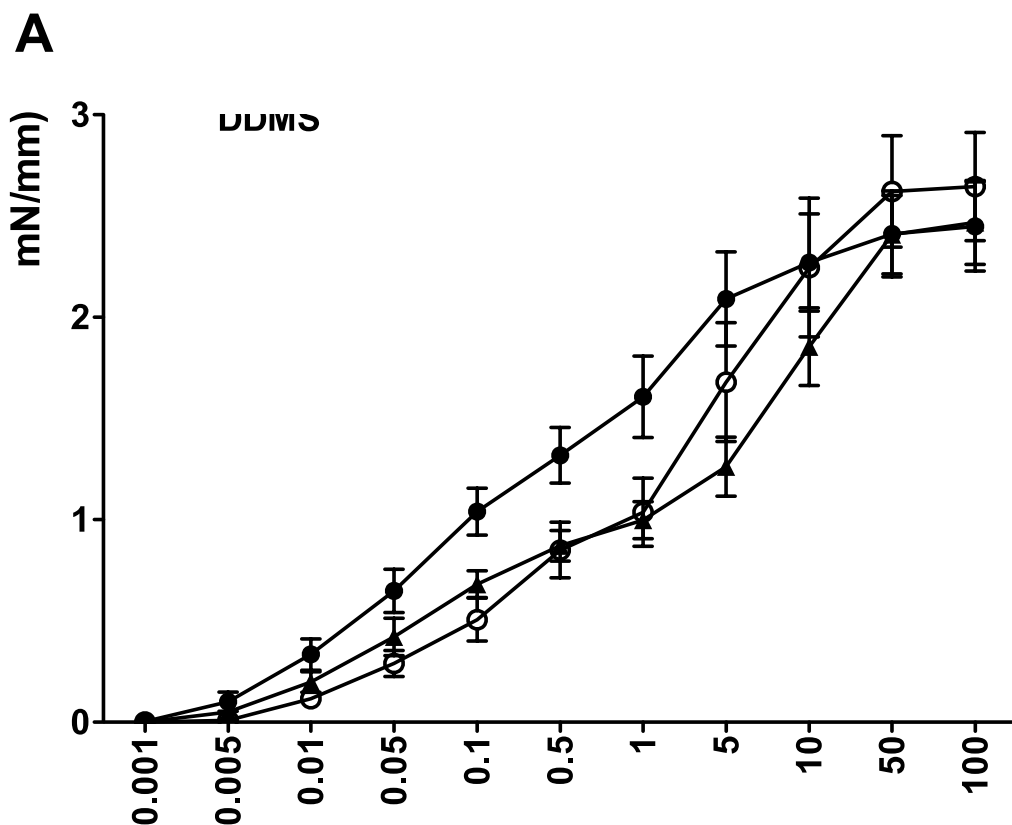


Figure 2

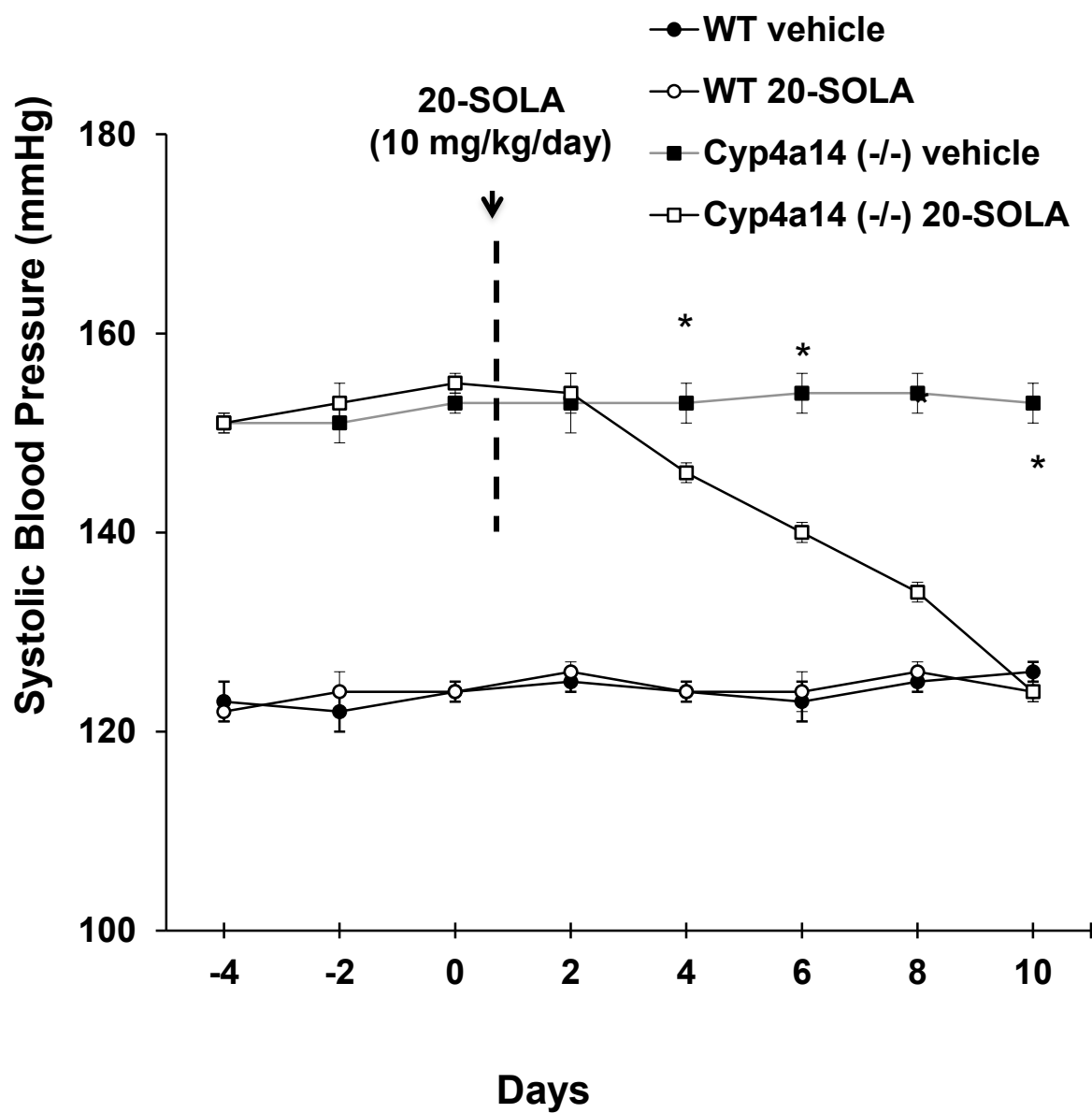


Figure 3

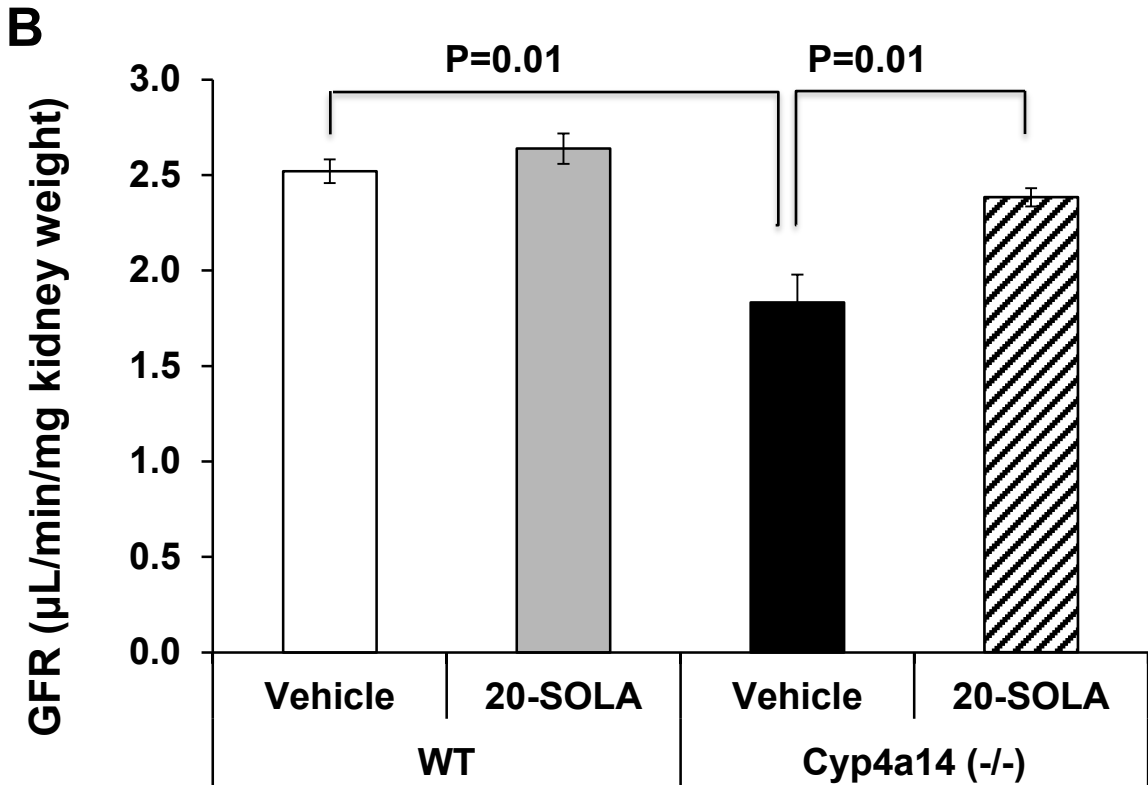
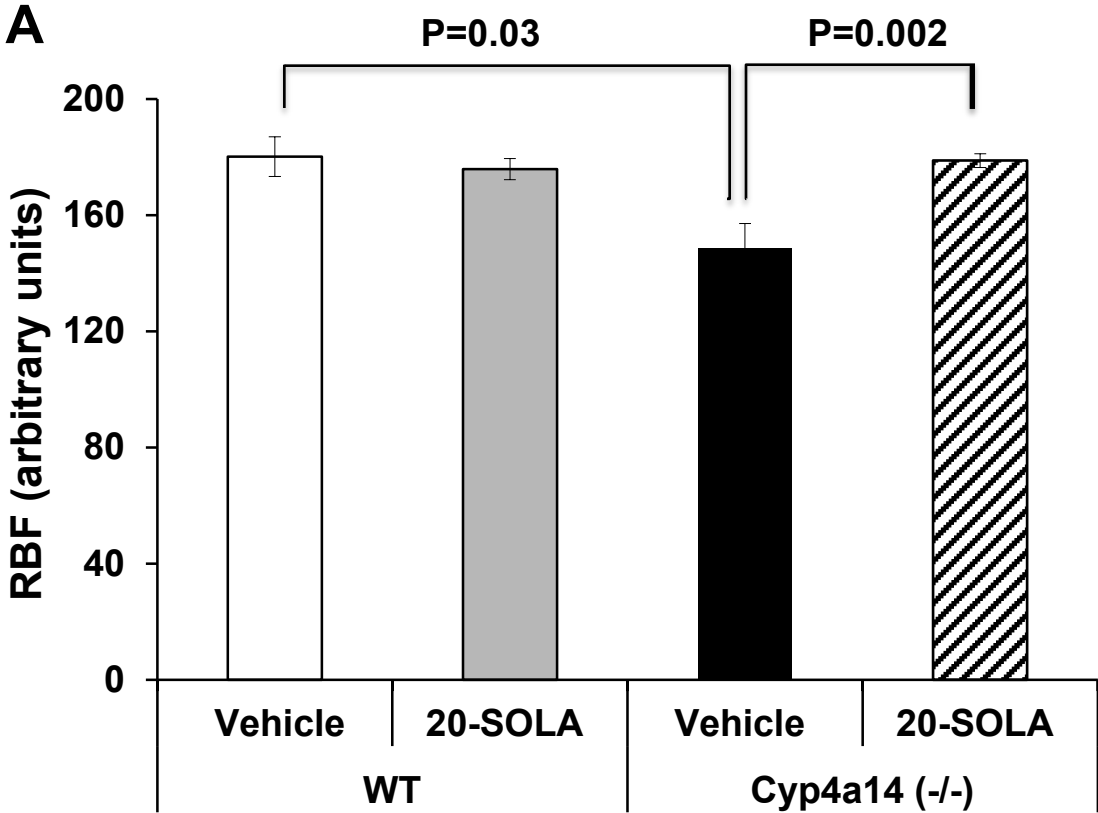


Figure 4

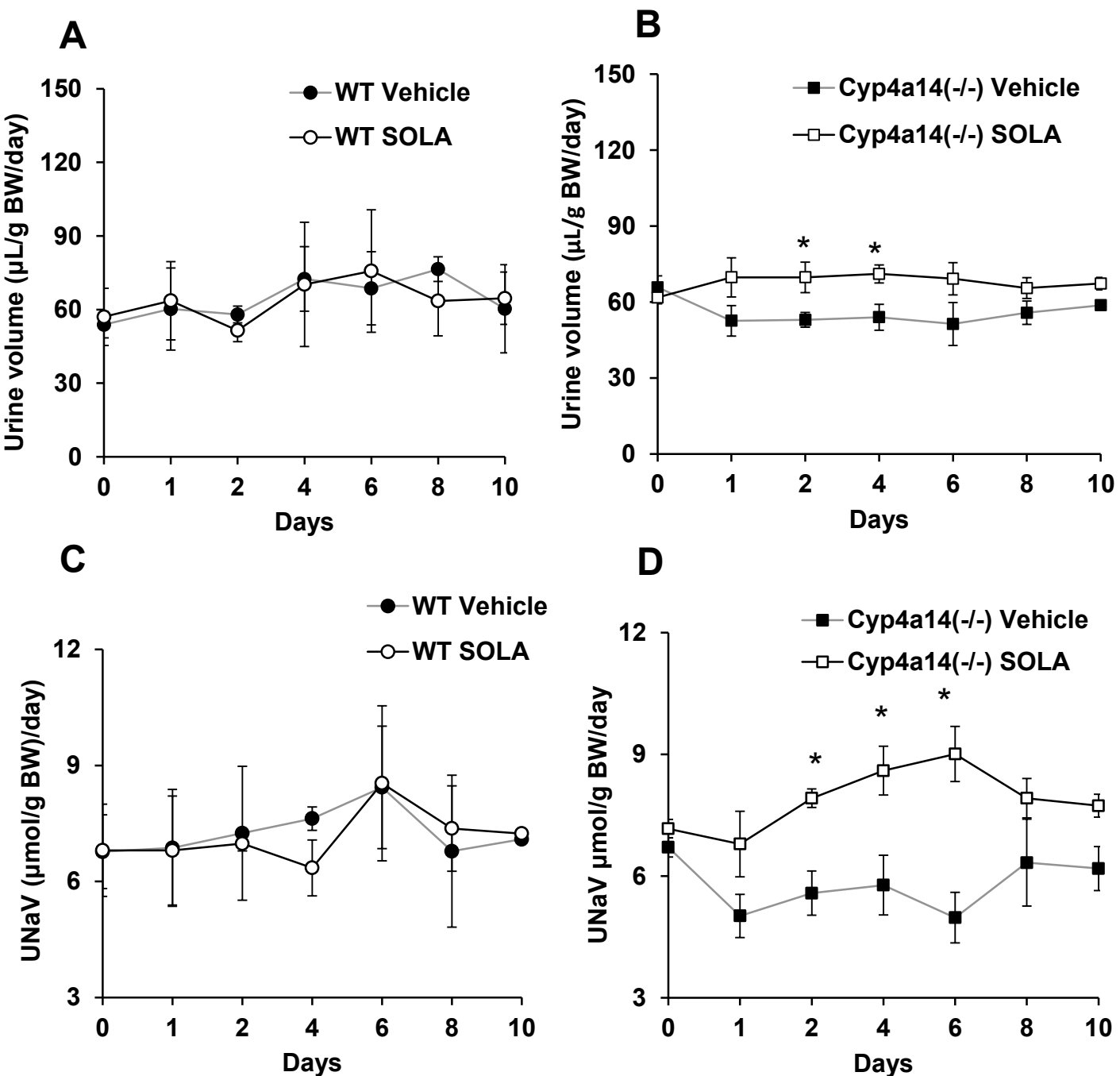
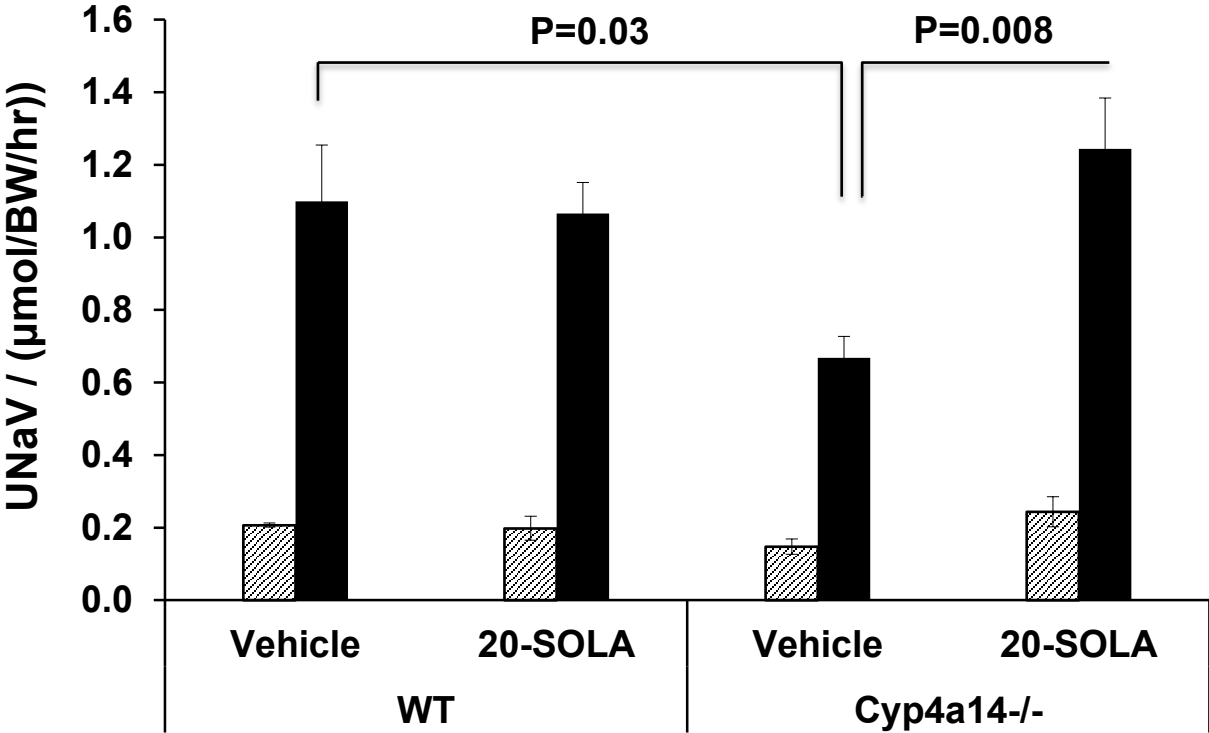


Figure 5

A

▨ Before salt loading
■ After salt loading



B

