

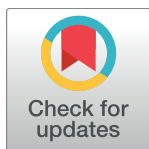
RESEARCH ARTICLE

# Treatment with enalapril and not diltiazem ameliorated progression of chronic kidney disease in rats, and normalized renal AT<sub>1</sub> receptor expression as measured with PET imaging

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## Abstract

ACE inhibitors are considered first line of treatment in patients with many forms of chronic kidney disease (CKD). Other antihypertensives such as calcium channel blockers achieve similar therapeutic effectiveness in attenuating hypertension-related renal damage progression. Our objective was to explore the value of positron emission tomography (PET) imaging of renal AT<sub>1</sub> receptor (AT<sub>1</sub>R) to guide therapy in the 5/6 subtotal-nephrectomy (Nx) rat model of CKD. Ten weeks after Nx, *Sprague-Dawley* rats were administered 10mg/kg/d enalapril (Nx<sub>E</sub>), 30mg/kg/d diltiazem (Nx<sub>D</sub>) or left untreated (Nx) for an additional 8–10 weeks. Kidney AT<sub>1</sub>R expression was assessed using *in vivo* [<sup>18</sup>F]fluoropyridine-losartan PET and *in vitro* autoradiography. Compared to shams, Nx rats exhibited higher systolic blood pressure that was reduced by both enalapril and diltiazem. At 18–20 weeks, plasma creatinine and albuminuria were significantly increased in Nx, reduced to sham levels in Nx<sub>E</sub>, but enhanced in Nx<sub>D</sub> rats. Enalapril treatment decreased kidney angiotensin II whereas diltiazem induced significant elevations in plasma and kidney levels. Reduced PET renal AT<sub>1</sub>R levels in Nx were normalized by enalapril but not diltiazem, and results were supported by autoradiography. Reduction of renal blood flow in Nx was restored by enalapril, while no difference was observed in myocardial blood flow amongst groups. Enhanced left ventricle mass in Nx was not reversed by enalapril but was augmented with diltiazem. Stroke volume was diminished in untreated Nx compared to shams and restored with both therapies. [<sup>18</sup>F]Fluoropyridine-Losartan PET allowed *in vivo* quantification of kidney AT<sub>1</sub>R changes associated with progression of CKD and with various pharmacotherapies.

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## Introduction

Chronic kidney disease (CKD) is a growing health problem worldwide with increasing annual incidence at a rate of 8%, and consumes up to 2% of the global health expenditure [1]. The prevalence of hypertension is substantial in patients suffering from kidney disease [2], and is considered a major risk factor for progression to end-stage renal disease, and for cardiovascular complications [3–6], which represent the leading cause of death in CKD [7, 8]. Most recent guidelines recommend the use of renin angiotensin (Ang) system (RAS) inhibitors in the initial antihypertensive regimen due to class-specific renoprotective mechanisms independent of their blood pressure lowering effect [9–11]. RAS blockade with Ang-converting enzyme inhibitors (ACEIs) or AT<sub>1</sub> receptor (AT<sub>1</sub>R) blockers (ARBs) have been shown in landmark clinical trials to dramatically attenuate the decline in renal function associated with CKD [12–16]. We have identified AT<sub>1</sub>R as a key molecular imaging target because of its direct involvement with the development and progression of CKD and other aspects of renal pathophysiology; and can eventually assist in prediction and monitoring of therapy.

Nevertheless, alternative antihypertensive drugs given alone or in combination were shown to be successful in reaching optimal blood pressure target and in slowing progression of CKD. In this regard, calcium channel blockers (CCBs) are similarly as efficient as RAS blockers at attenuating hypertension-related renal damage progression, when administered during the non-proteinuric stages of CKD [17–21]. The use of dihydropyridine and nondihydropyridine CCBs has been reported to be safe and effective in management of CKD, provided that a tight control of blood pressure was achieved (reviewed in [22–25]).

Our group has synthesized several positron emission tomography (PET) radioligands derived from the clinically used ARBs for imaging of the AT<sub>1</sub>R in kidney [26–30]. Preliminary PET studies with [<sup>18</sup>F]fluoropyridine (FPyr)-losartan radioligand exhibited high binding selectivity for kidney AT<sub>1</sub>R over AT<sub>2</sub>R and rapid metabolism in rats, which supported the potential of this tracer for further renal AT<sub>1</sub>R evaluation. We have successfully demonstrated an *in vivo* reduction of renal AT<sub>1</sub>R cortical expression in rats with CKD at 8–10 weeks post 5/6 nephrectomy (Nx) using [<sup>18</sup>F]FPyr-losartan with PET imaging [31]. The hypothesis of the present work was that PET imaging of AT<sub>1</sub>R would aid in identification of the kidney AT<sub>1</sub>R alterations in association with different actions of the drug treatments used in management of CKD.

## Materials and methods

### Animals

All animal experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and with approval of the University of Ottawa Animal Care Committee. Male *Sprague-Dawley* rats (200–250 g; Charles River Laboratories, Montreal, Canada) were housed in pairs on a 12h:12h light:dark cycle and fed standard rat chow and water *ad libitum*. *Sprague-Dawley* rats were subjected to either sham or Nx surgery in two steps one week apart. Ten weeks after surgery, rats (N = 34) were administered 10mg/kg/d enalapril (NxE) [32, 33], 30mg/kg/d diltiazem (NxD) [34, 35] in drinking water or left untreated (Nx) for an additional 8–10 weeks. Enalapril and diltiazem were obtained from Apotex, Inc. (Ottawa, ON, Canada). Drugs were supplied as tablets that were powdered and dissolved in water and administered to animals in the drinking bottles and doses were calculated according to water consumption for each individual rat. Animals were weighed weekly till the end of study. After sacrifice by decapitation, kidney, heart and left ventricle (LV) weights were obtained.

### 5/6 nephrectomy surgical procedure

Rats were subjected to either sham or Nx surgery in two sittings under total anesthesia with 2% isoflurane by inhalation throughout the surgery. In the first step, the right kidney was exposed through a lateral dorsal incision, then decapsulated and completely removed. One week later, the left kidney was exposed in the same way and reduced to 1/3 of its original size by resecting the superior and inferior poles to induce a total of 5/6 Nx [36, 37]. Post-operative analgesia was provided by subcutaneous administration of buprenorphine twice daily for 3 days following surgery. Sham animals underwent the same 2 surgeries one week apart to simulate Nx conditions without removing the kidneys (Fig 1 shows the study timeline).

### Systolic blood pressure measurements

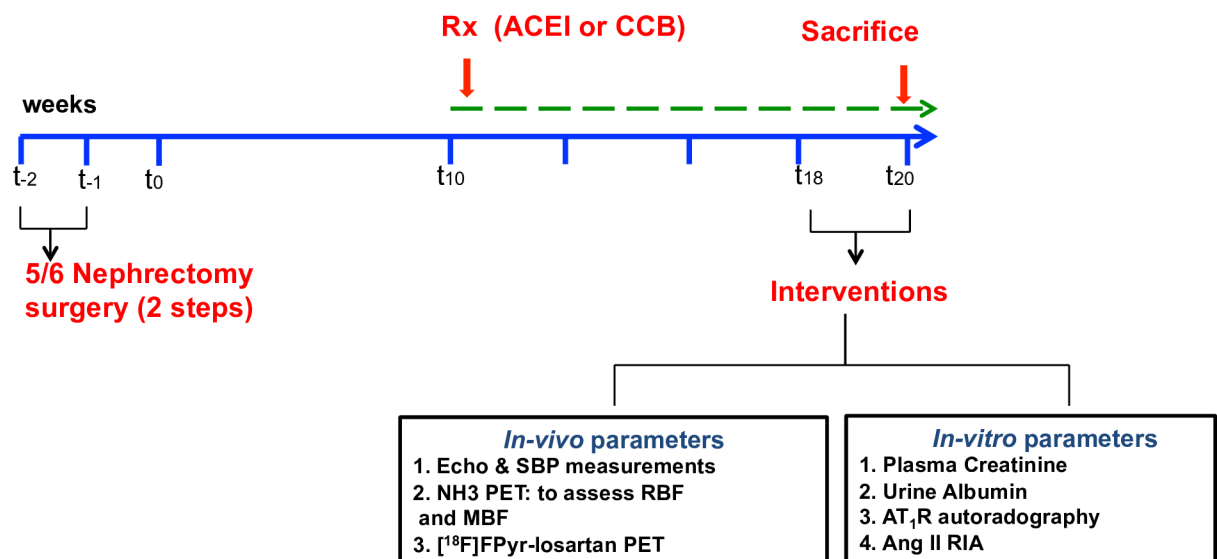
SBP was measured using indirect tail-cuff plethysmography (CODA-S2 multi-channel, Kent Scientific Corporation, Torrington, CT, USA) in conscious rats at 18–20 weeks' post-surgery. Animals were placed in rat holders and trained for 3 days to the measuring conditions. On the 4th day, 4–8 consecutive measurements were recorded from each rat per session, and an average blood pressure was calculated.

### Measurement of plasma creatinine

Plasma Cr was assessed at 18–20 weeks post Nx surgery. Blood samples were collected, centrifuged (4000 rpm, 4°C) for 10 minutes, then plasma was obtained and stored at -80°C till assayed. Prior to measuring of Cr in samples, plasma proteins were precipitated using deproteinizing sample preparation kit (Biovision). Cr levels were determined using a Cr assay kit (Cell Biolabs, Inc. San Diego, CA, USA) following the manufacturer's instructions.

### Measurement of albumin: creatinine ratio in urine

Urinary albumin excretion was determined using albumin:Cr ratio from spot urine collection from rats. Albumin was quantified with a commercially available rat ELISA kit (Genway



**Fig 1. Study timeline of the 5/6 nephrectomy animal model of chronic kidney disease.** Treatment (Rx): ACEI (Enalapril 10mg/kg) or CCB (Diltiazem 30mg/kg) started at 10 weeks post-surgery and all parameters assessed at 18–20 weeks post-surgery. SBP: systolic blood pressure; RBF: renal blood flow; MBF: myocardial blood flow; RIA: radioimmunoassay.

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Biotech, Inc. San Diego, CA, USA) and Cr was measured with same assay kit mentioned in previous section.

### *In vivo* PET imaging

MicroPET studies were performed using the Inveon DPET (Siemens Preclinical Imaging, Knoxville, TN) small animal scanner. Animals were anaesthetized and kept unconscious throughout the scan using 2% isoflurane. Rats were placed on the microPET scanner bed in supine position and body temperature was maintained at 37°C. Animals were positioned to include heart and both kidneys in the field of view (FOV). Body temperature, heart rate and respiratory rate were monitored throughout the scans. A <sup>57</sup>Co transmission scan was obtained for scatter and attenuation correction either immediately preceding or following the emission scan. Images were analyzed with Inveon® Research Workplace software version 1.4 (Siemens Preclinical Imaging, Knoxville, TN) unless indicated otherwise. Dynamic PET images were reconstructed using vendor-provided 3-dimensional ordered subset expectation maximization / maximum a posteriori algorithm OSEM3D/MAP ( $\beta = 1$ , OSEM3D iterations = 2, MAP iterations = 18) with all corrections enabled. Volumes of Interest (VOIs) were defined on reconstructed images to obtain time-activity-curves (TACs) in units of Bq/cc.

### [<sup>18</sup>F]FPyr-Losartan AT<sub>1</sub>R

[<sup>18</sup>F]FPyr-Losartan was synthesized as previously described and dissolved in 0.9% saline for injection [26, 38]. Rats were injected with 18–81 MBq (0.5–2.2 mCi) of [<sup>18</sup>F]FPyr-losartan (<1ml volume) via a 26 gauge catheter into the lateral tail vein. The specific activity ranged from 112–1630.52 mCi/ $\mu$ mol (4.144–60.33 GBq/ $\mu$ mol) at time of injection. A dynamic 60 min scan was acquired as 12x10 s, 3x60 s, 11x300 s frames. The arterial input function was obtained from the average blood pool activities within the left atrium (LA) or the left ventricle (LV) cavity. Briefly, a sphere was drawn inside the cavity of LA or LV at an early frame (10–40 s post-injection) and an 80% threshold was used to define the contour of the arterial blood VOI. Kidney VOIs were drawn at frame 16 or 17 (5–15 min post-injection where the highest tissue-to-background contrast was observed) by tracing a segment of the cortex at the inferolateral side of the left kidney away from liver and bowel to avoid spillover, and the final VOI was defined using a 50% threshold contour. [<sup>18</sup>F]FPyr-Losartan retention was measured using the Logan derived distribution volume (DV) values. The tracer DV provides a quantifiable parameter for repeated measurements and assessment of repeatability and reliability. Provided the tracer binds to its receptor reversibly, Logan graphical analysis [39], of the PET time-activity data can be used to calculate the DV. In essence, tracer uptake in the kidney is plotted against concentration in the plasma at equilibrium (steady-state). Plotting  $0 \int \text{TCPET}(t)dt / \text{CPET}(T)(\text{min})$  against  $0 \int \text{TCP}(t)dt / \text{CPET}(T)(\text{min})$ , where CPET(T) is the concentration of tracer in the tissue and CP(t) is the concentration of tracer in the plasma, will transform the tissue activity to a linear plot, as if the tracer was injected as a continuous infusion. The slope of this line during the steady-state phase corresponds to an estimate of the DV (ml/cm<sup>3</sup>).

### [<sup>13</sup>N]Ammonia blood flow

Heart and kidneys perfusion were assessed within the same week of [<sup>18</sup>F]FPyr-losartan PET scans. Animals were injected [<sup>13</sup>N]ammonia 55–110 MBq (1.5–3mCi) intravenously and scanned for 30 min. Myocardial blood flow (MBF) was quantified using FlowQuant software [40]. Blood and kidney TACs were generated and flow values (ml/g/min) were produced. For calculation of renal blood flow (RBF), the initial 2 min of the dynamic PET data was used to

avoid contamination by plasma metabolites of N-13 radioactivity. The images were reconstructed into 12 x 10 s frames applying the corrections for dead-time, isotope decay, detector efficiencies, and random events. Renal TACs were derived from VOI drawn over renal cortex and derived from blood pools inside the LA and LV for average arterial input function. The one-tissue-compartment kinetic model was used to calculate  $K_1$  (ml/g/min) for RBF analysis.

### *In vitro* autoradiography

*In vitro* <sup>125</sup>I-[Sar<sup>1</sup>, Ile<sup>8</sup>]Ang II binding was assessed using the method published previously [41]. [<sup>125</sup>I]-Sar<sup>1</sup>, Ile<sup>8</sup>]Ang II was purchased from Perkin Elmer (USA). The reagent was supplied as a powder that was dissolved in distilled water, each vial contained 50 μCi (1.85 MBq) with a specific activity of 2200 Ci (81.4 TBq)/mmol. Briefly, rats were sacrificed 2–3 days after PET imaging studies. Following decapitation, dissected kidneys were quickly immersed in OCT Compound (Tissue-Tek), frozen on dry ice and stored at -80 °C until subsequent autoradiography studies. Kidneys were sectioned in the longitudinal axis into 20 μm-thick slices at -18 °C with a cryostat (Leica CM3050 S). Tissue sections were thaw-mounted on glass slides (VWR) and stored at -80 °C. On the day of the experiment, slides were pre-washed in assay buffer (150 mM NaCl, 50 mM sodium phosphate dibasic, 1 mM EDTA, 0.1 mM Bacitracin, 0.1% BSA) for 15 min then incubated with 0.8 nM <sup>125</sup>I-[Sar<sup>1</sup>, Ile<sup>8</sup>]Ang II for 90 min at room temperature in the presence of AT<sub>2</sub>R antagonist, PD 123,319 (10 μM) to determine total (non-AT<sub>2</sub>R) binding or with unlabelled angiotensin II for non-specific binding. Specific binding of <sup>125</sup>I-[Sar<sup>1</sup>, Ile<sup>8</sup>]Ang II was calculated as total (non-AT<sub>2</sub>R) minus non-specific binding. After incubation, slides were washed 3 times (15 min at 4 °C) sequentially in assay buffer, deionized water, and again in assay buffer then gently air dried. Sections were then exposed to phosphor imaging plates (Kodak Screen-K, Biorad) for 48 hours in complete darkness. Phosphor plates were then read at a 100 μm resolution (BioRad Molecular Imager FX) and analyzed using Quantity One Software (BioRad, Philadelphia). Quantification was done by manually tracing the whole kidney cortex and the radioactivity density (counts/mm<sup>2</sup>) was recorded for that area.

### Measurements of angiotensin II plasma and tissue levels

Ang II analyses were performed by the Hypertension Core Laboratory at Wake Forest University Health Science Center using previously described method [42]. After decapitation of animals, trunk blood was collected in EDTA tubes containing a cocktail of protease inhibitors including 0.44 mM 1,20 ortho-phenanthroline monohydrate (Sigma, St. Louis MO.), 0.12 mM pepstatin (Peninsula Labs, Belmont CA), and 1 mM Na p-hydroxymercuribenzoate (Sigma, St. Louis MO), then centrifuged at 4000 rpm for 5 min to obtain plasma. Kidneys were rapidly collected and snap frozen on dry ice. Plasma and tissue samples were stored at -80 °C until shipped for assay. Tissues were homogenized in an acidic ethanol [80% vol/vol 0.1N HCl] solution containing peptidase inhibitors described previously [43]. Samples were Sep-Pak extracted and measured by RIA (ALPCo, Windham, NH, USA). An aliquot for protein determination was taken from the acid ethanol homogenized tissue. Protein was measured in this aliquot and results are expressed per mg protein.

### Echocardiography

Echocardiography was carried out at 18–20 weeks under light anesthesia (1–2% isoflurane) using the Vevo 770 system (VisualSonics, Toronto, ON, Canada) and a 23.5 MHz probe. All echocardiography studies were performed and analyzed by a single not-blinded operator. Parasternal long-axis views were recorded as sequential ECG-gated M-mode sweeps (EKV-

mode) to generate two-dimensional cines of the left ventricle. Analysis of results was completed with the VisualSonics cardiac measurements program. The LV wall mass and LV ejection fraction (LVEF), end diastolic volume (EDV) and stroke volume (SV) were assessed with the standard VisualSonics cardiac measurement formulas.

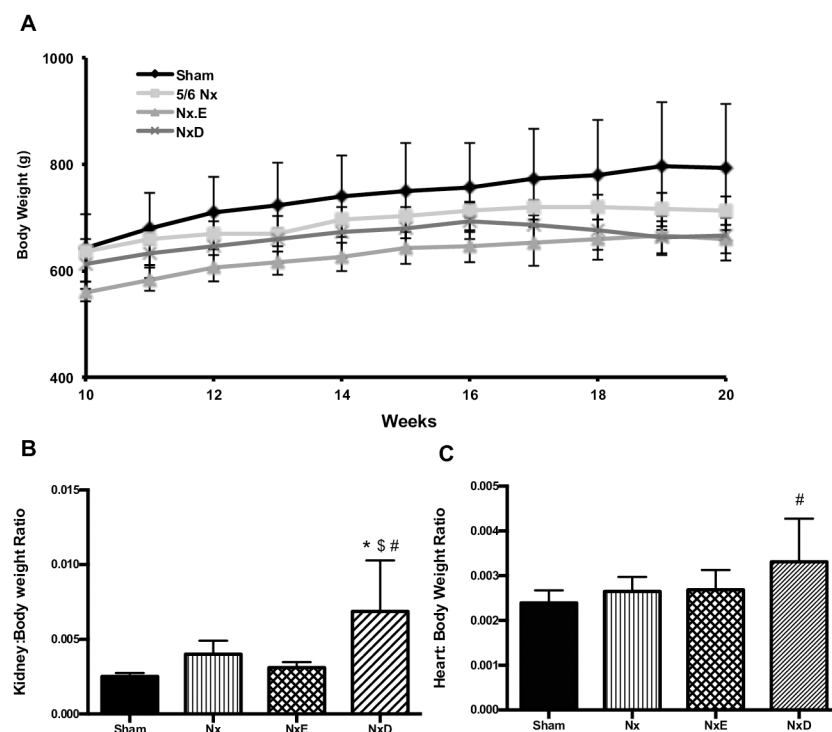
### Statistical analysis

All data are presented as mean ± standard deviation. Statistical analysis was performed with one way ANOVA using Tukey test for posthoc analysis (Prism; GraphPad Software; version 6.0h); *p*<0.05 was considered significant. N values for each comparison are given in the Figures and Tables. Data are expressed either as mean and standard deviation in the Tables, or medians and interquartiles in the boxplot Figures. Pearson correlation coefficient (*r*) was calculated to determine correlations between various parameters when required.

## Results

### Body and organ weights

At 8–10 weeks before the start of treatments, body weights were comparable among all groups of animals. Similarly, at 18–20 weeks post Nx, no significant differences in body weight or the percentage increase per week were observed between the groups (Fig 2A). No significant changes in left kidney weight/body weight were observed in Nx or NxE rats, compared to shams, although there was a tendency for kidney hypertrophy in the Nx group. Left kidney weight/body weight was significantly increased in diltiazem-treated rats, compared to all



**Fig 2. Body weight data obtained weekly over 10 weeks (A), terminal left kidney weight for sham (N = 8), Nx (N = 8), NxE (N = 6) and Nx.D (N = 8) (B) and heart weight for sham (N = 8), Nx (N = 9), NxE (N = 9) and Nx.D (N = 8) (C) normalized to body weight at 18–20 weeks post-surgery.** Data are presented as mean±SD. \* *p*<0.05 vs sham, \$ *p*<0.05 vs Nx, # *p*<0.05 vs Nx.E (*p*<0.05).

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groups (1.7–2.7-fold,  $p < 0.05$ ; Fig 2B). Cardiac hypertrophy was not observed in the sham, Nx and NxE groups. However, treatment with diltiazem induced a significant increase in heart weight/body weight compared to shams (1.4-fold,  $p < 0.05$ ; Fig 2C).

### Systolic blood pressure and renal function

Nx rats were hypertensive and had significantly higher plasma Cr levels compared to sham rat group before the start of therapy at 8–10 weeks post-surgery as we previously reported [31]. Nx rats had significantly higher SBP at 18–20 weeks post-surgery compared to shams (1.5-fold;  $p < 0.001$ ). Both enalapril and diltiazem treatments attenuated this increase (Table 1).

Plasma Cr concentration was significantly higher in Nx rats in comparison to shams at 18–20 weeks post-surgery (1.6 fold;  $p < 0.05$ ). By contrast, plasma Cr was not increased in the enalapril-treated group, compared to untreated Nx animals, while diltiazem induced a significant increase compared to all groups (1.8–3 fold;  $p < 0.05$ ) (Table 1). At the end of study, significant albuminuria (corrected to urine Cr concentration) developed in the untreated Nx animals, which was normalized by enalapril and exacerbated in diltiazem-treated rats (NxD: 4.8-fold increase versus sham, 1.8-fold increase versus Nx;  $p < 0.001$ ) (Table 1).

### Plasma and tissue angiotensin II levels

Ang II levels in plasma, kidney and LV were elevated in Nx rats compared to sham, however did not reach significance ( $p$  value = 0.15, 0.13, 0.22 respectively). Administration of enalapril did not affect Ang II levels in plasma or LV but significantly reduced levels in the kidney compared to Nx rats ( $p < 0.05$ ; Fig 3). Interestingly, treatment with diltiazem induced significant elevations in plasma and kidney Ang II levels, in comparison to the sham and NxE groups at 18–20 weeks ( $p < 0.05$  for plasma and  $p < 0.001$  for kidney; Fig 3A & 3B). Moreover diltiazem caused a marked elevation of Ang II in LV compared to all other groups (3.75-fold versus sham, 5.6-fold versus Nx, and 5-fold versus NxE; Fig 3C).

### AT<sub>1</sub>R expression

*In vivo* PET scans of the Nx rats revealed a distorted shape of the kidney after resection with less tracer uptake as compared to the normal kidney in shams (Fig 4A & 4B). While enalapril preserved the shape of the remnant left kidney and tracer retention, diltiazem-treated rats displayed further distortion of the kidney structure with minimal uptake of the tracer (Fig 4C & 4D). [<sup>18</sup>F]FPyr-Losartan distribution volume (DV, ml/cm<sup>3</sup>) determined by Logan analysis was quantified as an indication of AT<sub>1</sub>R expression in the kidney of rats (Fig 4E). DV values were significantly lower in Nx rats compared to sham animals (-28%,  $p < 0.05$ ). Treatment with enalapril normalized these values while no change was observed in the diltiazem-treated group.

**Table 1. SBP, plasma creatinine (Cr) and urinary albumin:Cr in sham, untreated Nx, NxE and NxD (at 18–20 weeks post-surgery).**

Parameters	SBP (mmHg)	Plasma Cr (mg/dl)	Urine Albumin:Cr (ug/mg)
Sham	142.7±12* N = 8	0.48±0.06 N = 5	81.4±30.0 N = 4
Nx	188.8±17.3 N = 4	0.77±0.07* N = 6	211.37±39* N = 4
NxE	152±8.65 <sup>§</sup> N = 6	0.43±0.06 <sup>§</sup> N = 5	116.5±32.1 <sup>§</sup> N = 4
NxD	145.8±5.78 <sup>§</sup> N = 5	1.46±0.27* <sup>§</sup> # N = 6	385.6±53.5* <sup>§</sup> # N = 4

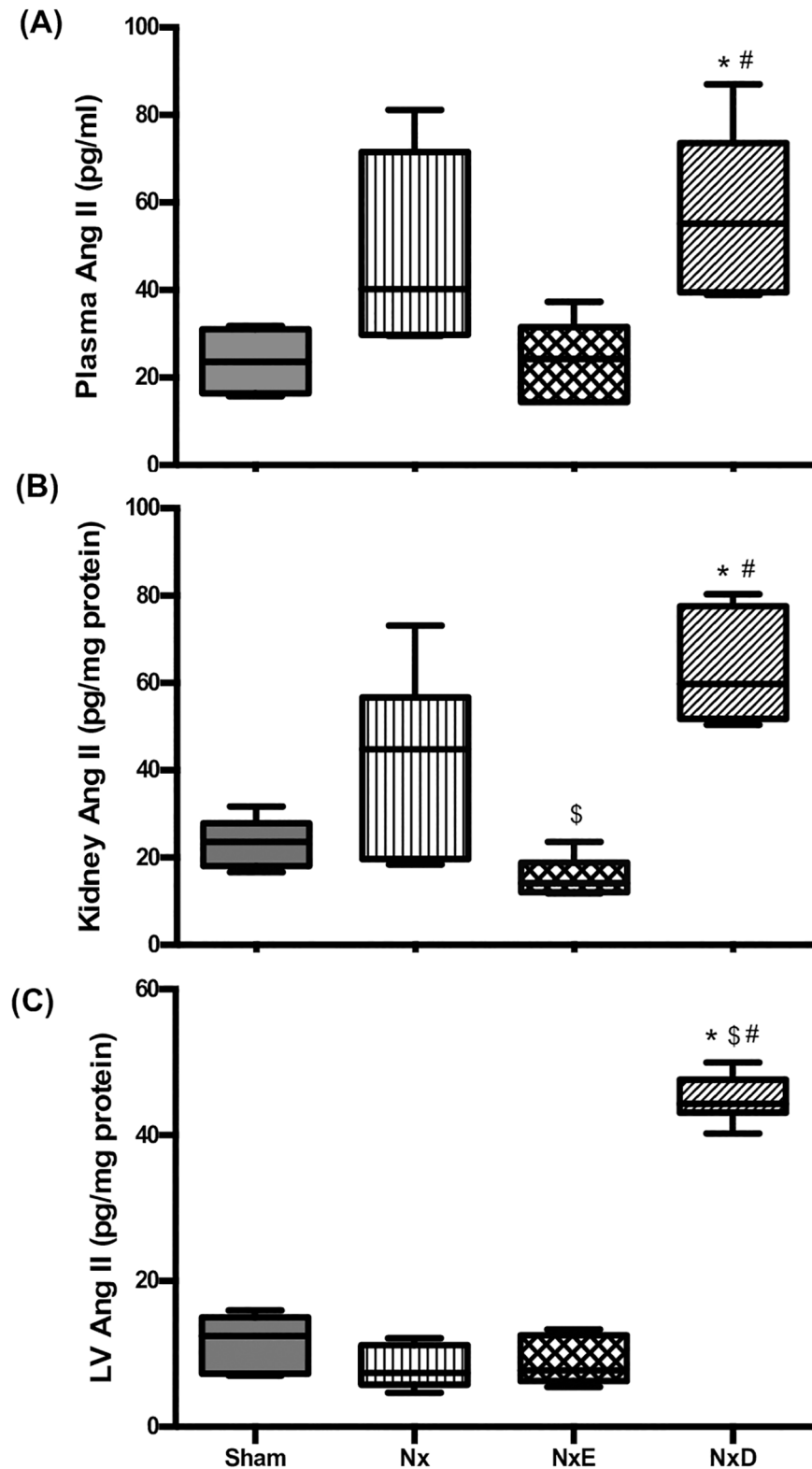
Data are presented as mean±SD.

\*  $p < 0.05$  vs sham

<sup>§</sup>  $p < 0.05$  vs Nx

#  $p < 0.05$  vs NxE ( $p < 0.05$ ).

<https://doi.org/10.1371/journal.pone.0177451.t001>



**Fig 3.** Ang II levels in plasma samples of sham (N = 4), Nx (N = 5), NxE (N = 5) and NxD (N = 6) (A), left kidney samples of sham (N = 5), Nx (N = 6), NxE (N = 5) and NxD (N = 5) (B) and left ventricle samples of sham (N = 6), Nx (N = 7), NxE (N = 5) and NxD (N = 6) (C) samples of Sham, Nx, NxE and NxD groups at 18–20 weeks post-surgery. In boxplots, horizontal lines represent median and whiskers represent



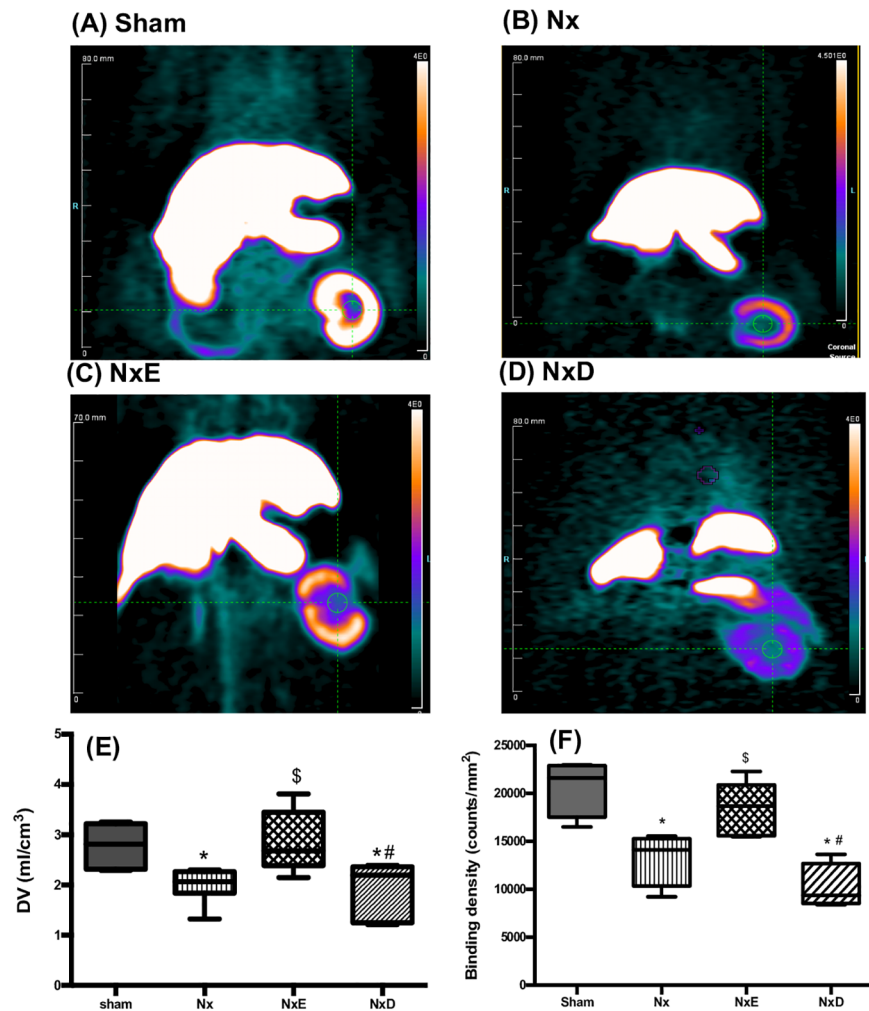
minimum and maximum values. Data are presented as mean±SD. \*  $p < 0.05$  vs sham, §  $p < 0.05$  vs Nx, #  $p < 0.05$  vs NxE ( $p < 0.05$ ).

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[<sup>125</sup>I]-[Sar<sup>1</sup>, Ile<sup>8</sup>]Ang II specific binding density confirmed reduction of AT<sub>1</sub>R in the untreated Nx group at 18–20 weeks (-36%,  $p < 0.05$ ), and normalization of the receptor expression in rats receiving enalapril but not with diltiazem therapy (Fig 4F). These results correlated well with the *in vivo* PET findings ( $r = 0.47$ ,  $p = 0.05$ ).

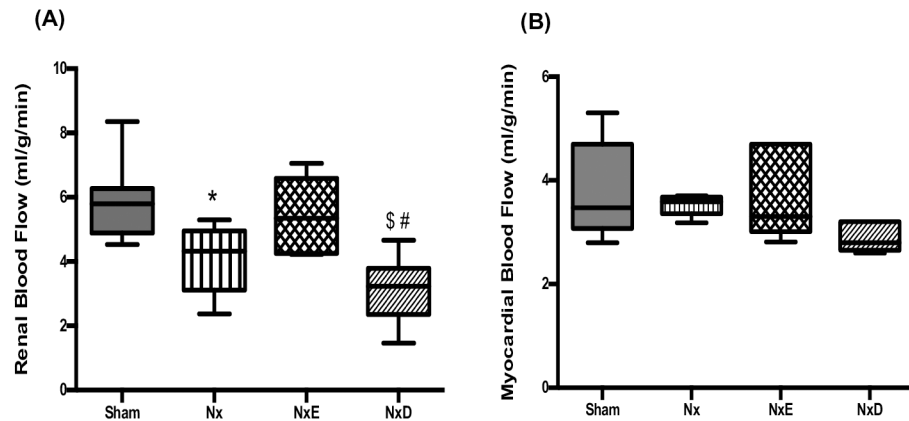
### Organ blood flow

Renal blood flow as assessed by [<sup>13</sup>N]ammonia PET was significantly reduced in Nx rats, compared to shams ( $p < 0.05$ ), and tended to normalize with enalapril treatment, although this did



**Fig 4. Representative coronal view microPET scans showing liver and kidney uptake obtained at 5–10 min post-injection of [<sup>18</sup>F]FPyr-losartan in all groups at 18–20 weeks post-surgery; Sham (A), Nx (B), NxE (C) and NxD (D). Images are displayed using same SUV scale. Kidney distribution volume (DV, ml/cm<sup>3</sup>) of [<sup>18</sup>F]FPyr-losartan obtained with PET *in vivo* in sham (N = 6), Nx (N = 7), NxE (N = 7) and NxD (N = 6) (E); and [<sup>125</sup>I]-[Sar<sup>1</sup>, Ile<sup>8</sup>]Ang II binding density (counts/mm<sup>2</sup>) obtained with *in vitro* autoradiography in sham (N = 4), Nx (N = 4), NxE (N = 6) and NxD (N = 4) (F) at 18–20 weeks post-surgery. In boxplots, horizontal lines represent median and whiskers represent minimum and maximum values. Data are presented as mean±SD. \*  $p < 0.05$  vs sham, §  $p < 0.05$  vs Nx, #  $p < 0.05$  vs NxE ( $p < 0.05$ ).**

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**Fig 5. Renal blood flow in sham (N = 7), Nx (N = 7), NxE (N = 5) and NxD (N = 7) groups (A); and myocardial blood flow values in sham (N = 6), Nx (N = 5), NxE (N = 6) and NxD (N = 5) (B) at 18–20 weeks post-surgery. Blood flow was assessed by [<sup>13</sup>N]ammonia PET imaging.** In boxplots, horizontal lines represent median and whiskers represent minimum and maximum values. Data are presented as mean ±SD. \*  $p < 0.05$  vs sham, \$  $p < 0.05$  vs Nx, #  $p < 0.05$  vs NxE ( $p < 0.05$ ).

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not achieve statistical significance. By contrast, diltiazem caused a further significant reduction in renal blood flow, compared to untreated Nx rats (Fig 5A). No significant differences in myocardial blood flow occurred amongst groups (Fig 5B).

### Heart function

Echocardiographic measures of heart functions are summarized in Table 2. There was no difference in LVEF between all groups. However, the EDV and SV were significantly diminished in untreated Nx rats as compared to shams. Treatment with enalapril and diltiazem restored only SV to normal levels (Table 2). Echocardiographic LV mass was significantly increased in the Nx group and was not reversed in rats receiving enalapril. Diltiazem treatment further enhanced the LV mass compared to all groups (3.5-fold versus sham, 1.7-fold versus Nx, and 1.7-fold versus NxE;  $p < 0.001$ ).

### Significant correlations

A strong inverse correlation was found between AT<sub>1</sub>R PET kidney DV value and albuminuria ( $r = -0.731$ ,  $p = 0.0009$ ) also between AT<sub>1</sub>R PET DV kidney value and LV mass ( $r = -0.733$ ,  $p = 0.0019$ ). Interestingly, a weak negative correlation was found between the AT<sub>1</sub>R PET DV and SBP measurements ( $r = -0.28$ ,  $p = 0.28$ ).

**Table 2. Echocardiographic parameters in sham, untreated Nx, NxE and NxD groups at 18–20 weeks post-surgery.**

Parameters	LV EF (%)	SV (ml)	EDV (ml)	LV mass (g)
Sham	73.3±5.3 N = 6	450±89 N = 6	611±91.8 N = 6	449.8±89.03 N = 6
Nx	63.6±7.9 N = 5	285±42.3* N = 5	462±74.3* N = 6	923.4±165.32* N = 6
NxE	73.3±6.5 N = 9	414±89.4 <sup>\$</sup> N = 8	542±78.2 N = 9	920.1±126.9* N = 7
NxD	72.2±5.5 N = 6	437±71.7 <sup>\$</sup> N = 5	577±112.3 N = 6	1600.8±302.3* <sup>\$</sup> # N = 6

Data is presented as mean±SD

\*  $p < 0.05$  vs sham

\$  $p < 0.05$  vs Nx

#  $p < 0.05$  vs NxE ( $p < 0.05$ ). LV: left ventricle, EF: ejection fraction, EDV: end diastolic volume, SV: stroke volume.

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## Discussion

In this study, we used 5/6 nephrectomy rat model to investigate AT<sub>1</sub>R alterations in CKD with PET. The major finding is the *in vivo* demonstration of renal AT<sub>1</sub>R reduction associated with the activated RAS due to CKD-induced hypertension in Nx rats, which was normalized following administration of the ACEI enalapril. RAS inhibition with ACEI was selected in this study instead of an ARB for management of CKD so as not to interfere with the binding of the losartan analog radiotracer used in PET imaging. The PET quantification was validated by *in vitro* <sup>125</sup>I-[Sar<sup>1</sup>, Ile<sup>8</sup>]Ang II autoradiography studies.

Subtotal Nx rats developed hypertension, renal dysfunction and albuminuria at 18–20 weeks post-surgery. In addition to being an important prognostic factor in progressive CKD, albuminuria is considered as an independent predictor of cardiovascular morbidity and mortality [44, 45]. We have recently reported that induction of Nx in rats caused elevations in SBP and plasma Cr without evidence of albuminuria at 8–10 weeks post-surgery indicating intact glomerular barrier at that stage [31]. In the current work, development of significant albuminuria was observed with sustained hypertension and renal impairment at 18–20 weeks post-surgery. Consistent with previous clinical and experimental findings [46, 47, 33], the administration of ACEI enalapril normalized SBP, plasma Cr, and attenuated albuminuria associated with Nx.

The presence of heightened systemic and local RAS activity in CKD is well established [48, 36]. It can be postulated that maladaptive changes in both the circulatory and tissue RAS activity seem to be a driving force in the pathogenesis of CKD. In this work, the low levels of kidney Ang II can be attributed to the assessment of Ang II in homogenized total kidney tissue, which does not allow assessment of the concentrations within specific compartments. Thus, an increase in Ang II in some areas of the remnant kidney may not be detected if levels are decreased in other locations [49, 50].

Chronic administration of enalapril resulted in decreasing Ang II in the kidney while unexpectedly Ang II plasma levels were not changed. Normally the kidneys have much greater tissue concentrations of Ang II that is mainly produced through local ACE-dependent pathways. Therefore the administered dose of enalapril has been sufficient to suppress the conversion of Ang I to Ang II in the kidney only whereas displayed minimal effect on other organs which may have attenuated the reduction of plasma Ang II [49]. Another possible theory could be related to a feedback mechanism process compensating for the inhibition of ACE mediated synthesis of Ang II, either by decreasing the degradation of Ang II or by increasing alternative pathways of Ang II synthesis [51, 52]. Nevertheless, the effect of enalapril was sufficient to increase AT<sub>1</sub>R densities to normal levels in the remnant kidney by attenuating processes of Ang II-induced AT<sub>1</sub>R downregulation, confirming an inverse correlation between Ang II levels and AT<sub>1</sub>R expression.

Previous experimental studies investigating the renal RAS (mainly AT<sub>1</sub>R expression) in various animal models of CKD have yielded conflicting results that may reflect differences in the methodology and/or the time points of measurements [36, 53, 37]. Joly et al. reported a decrease (30–40%) in AT<sub>1</sub>R mRNA levels in cortex and outer medulla at 4 weeks post-surgery [53]; however, another study found no change in AT<sub>1</sub>R protein expression at the same time point [36]. Whereas, an increase (>70%) in AT<sub>1</sub>Rs was obtained using Western blotting at 8 weeks post-surgery by Kujal et al (2010) [37]. A non-invasive molecular imaging probe will provide relevant information regarding the dynamic changes in tissue AT<sub>1</sub>R levels.

In prior work, we have shown reduced [<sup>18</sup>F]FPyr-losartan retention (-23%) in the hypertrophied remnant kidney of Nx rats at 8–10 weeks compared to shams [31]. In this study, the -28% reduction obtained at 18–20 weeks with the same tracer was comparable to the early timepoint, indicating sustained reduced expression of renal AT<sub>1</sub>Rs in the untreated Nx rat.

These reduced PET renal AT<sub>1</sub>R levels in Nx were normalized by enalapril, perhaps due to effective ACE inhibition that was sufficient to attenuate Ang II-induced AT<sub>1</sub>R downregulation. On the other hand, the deleterious effect of diltiazem on the plasma and kidney components of Ang II was associated with persistent reduction of AT<sub>1</sub>R kidney expression (similar to Nx rats) which could also provide an explanation for worsening of renal and cardiac functions in this group. PET imaging findings were supported by *in vitro* autoradiographic binding studies; thus, demonstrating reliable and accurate quantification of the dynamic changes of renal AT<sub>1</sub>Rs. This outcome emphasizes the benefits of using small animal PET in experimental work as it allows the usage of the same animal for longitudinal *in vivo* assessment of AT<sub>1</sub>R in health and disease states, and monitor effect of therapy.

The renal blood flow was reduced displaying a similar trend to [<sup>18</sup>F]FPyr-losartan decreased retention in the untreated Nx rats when compared with shams. However, this reduction in blood flow is not assumed to be the cause for diminished [<sup>18</sup>F]FPyr-losartan uptake, since the AT<sub>1</sub>R reduction with PET was confirmed by [<sup>125</sup>I]-[Sar<sup>1</sup>, Ile<sup>8</sup>]Ang II autoradiography. This assumption is further validated by the fact that using the same model of CKD, Nx rats exhibited no change in the renal blood flow associated with the PET AT<sub>1</sub>R reduction at 8–10 weeks post-surgery [31]. Indeed, local factors as Ang II, tumor growth factor-beta (TGF-βa) [24] and endothelin-1 [54], or reduction of nitric oxide production [55] among others, can be implicated in the changes in the renal hemodynamics.

Cardiac hypertrophy is a typical complication of CKD in response to the persistent hypertension and activation of the systemic RAS [8, 56]. Compared to shams, untreated Nx rats displayed significant increase in LV mass and diminished EDV with consequent decrease in SV presumably as a result of reduction of ventricular compliance due to the developed concentric hypertrophy [56]. The Ang II levels were not increased in the LV, hence the cardiac hypertrophy cannot be attributed to local activation of RAS in this study. However, other research groups suggested a role for cardiac RAS in LV hypertrophy associated with CKD [57, 7]. Nonetheless, systemic factors like anemia, dyslipidemia, and endothelial dysfunction or activated hormonal pathways such as the sympathetic nervous system that are known to be associated with CKD patients [58, 59] may have contributed to the promoting of LV hypertrophy [56, 60]. This result may also indicate the establishment of diastolic dysfunction [58], however such possibility was not further analyzed with echocardiography in this work. Interestingly, treatment with enalapril had no effect on LV levels of Ang II, however it prevented the progression of cardiac hypertrophy and partially improved cardiac efficiency.

The use of non-dihydropyridine CCB diltiazem was equally effective as antihypertensive therapy but failed to correct plasma Cr or albuminuria, and further increased their levels compared to untreated Nx rats. PET and autoradiographic renal AT<sub>1</sub>R expression results were not normalized in the diltiazem group, due to persistent elevation in renal Ang II levels. Moreover, diltiazem significantly increased Ang II levels in plasma and heart, although the mechanisms were not investigated. Cardiac hypertrophy was exacerbated in Nx rats compared to untreated Nx. However, SV was improved possibly due to normalization of SBP by diltiazem, despite high levels of Ang II in the heart. Consistent with previous research [61, 62, 34, 63], we demonstrated that late treatment with CCBs may further impair renal function and proteinuria in CKD. An interpretation for this effect can be due to their potent vasodilatory effect on the afferent arteriole dependent on pressure-induced depolarization and calcium entry through voltage-gated calcium channels [64, 65] which can have additive harmful effect induced by reduced renal mass [66, 62], and completely abolish renal autoregulatory capacity in the remnant kidneys of Nx rats [67, 62].

In summary, the Nx rat model of CKD exhibited renal impairment, proteinuria, sustained hypertension and cardiac hypertrophy. This was associated with elevated Ang II levels in

kidney and compensatory downregulation of renal AT<sub>1</sub>Rs as measured *in vivo* by PET imaging and *in vitro* autoradiography. As expected, delayed administration of ACEI enalapril attenuated renal impairment, hypertension and prevented progression of cardiac hypertrophy. This was successfully accomplished through reduction of kidney Ang II levels and consequent normalization of renal AT<sub>1</sub>R. On the other hand, use of the non-dihydropyridine CCB diltiazem was equally effective in reducing SBP but did not normalize renal AT<sub>1</sub>R expression. Diltiazem induced increases in Ang II levels in plasma, kidney and heart, associated with exacerbation of renal and cardiac dysfunction. Hence, PET can provide insights about drug responses at AT<sub>1</sub>R level, which may help identifying patients likely to respond, thereby optimizing outcome and reducing adverse effects.

In conclusion, strong *in vivo* evidence is provided in this experimental study on the beneficial effects of ACEI enalapril in reducing Ang II combined with a normalization of renal AT<sub>1</sub>R levels and hypertension. Additionally, our data reinforces the need for early intervention to prevent progression of CKD and subsequent reduction in the risk for cardiovascular morbidities and mortalities. This outcome adds value to the feasibility of non-invasive [<sup>18</sup>F]FPyr-losartan PET for determination of receptor abnormalities with progression of the disease and monitoring of therapy.

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## References

1. Levin A, Hemmelgarn B, Culeton B, Tobe S, McFarlane P, Ruzicka M et al.: Guidelines for the management of chronic kidney disease. CMAJ. 2008; 179:1154–62. <https://doi.org/10.1503/cmaj.080351> PMID: 19015566

2. Arora P, Vasa P, Brenner D, Iglar K, McFarlane P, Morrison H et al.: Prevalence estimates of chronic kidney disease in Canada: results of a nationally representative survey. *CMAJ*. 2013; 185:E417–23. <https://doi.org/10.1503/cmaj.120833> PMID: 23649413
3. Appel LJ, Wright JT Jr., Greene T, Agodoa LY, Astor BC, Bakris GL et al.: Intensive blood-pressure control in hypertensive chronic kidney disease. *N Engl J Med*. 2010; 363:918–29. <https://doi.org/10.1056/NEJMoa0910975> PMID: 20818902
4. Pohl MA, Blumenthal S, Cordonnier DJ, De Alvaro F, Deferrari G, Eisner G et al.: Independent and additive impact of blood pressure control and angiotensin II receptor blockade on renal outcomes in the irbesartan diabetic nephropathy trial: clinical implications and limitations. *J Am Soc Nephrol*. 2005; 16:3027–37. <https://doi.org/10.1681/ASN.2004110919> PMID: 16120823
5. Asselbergs FW, Diercks GF, Hillege HL, van Boven AJ, Janssen WM, Voors AA et al.: Effects of fosinopril and pravastatin on cardiovascular events in subjects with microalbuminuria. *Circulation*. 2004; 110:2809–16. <https://doi.org/10.1161/01.CIR.0000146378.65439.7A> PMID: 15492322
6. Toto RD: Management of hypertensive chronic kidney disease: role of calcium channel blockers. *J Clin Hypertens (Greenwich)*. 2005; 7:15–20.
7. Sukumaran V, Watanabe K, Veeraveedu PT, Thandavarayan RA, Gurusamy N, Ma M et al.: Telmisartan, an angiotensin-II receptor blocker ameliorates cardiac remodeling in rats with dilated cardiomyopathy. *Hypertens Res*. 2010; 33:695–702. <https://doi.org/10.1038/hr.2010.67> PMID: 20535115
8. Svirglerova J, Kuncova J, Nalos L, Tonar Z, Rajdl D, Stengl M: Cardiovascular parameters in rat model of chronic renal failure induced by subtotal nephrectomy. *Physiol Res / Academia Scientiarum Bohemoslovaca*. 2010; 59 Suppl 1:S81–8.
9. Griffin KA, Bidani AK: Potential risks of calcium channel blockers in chronic kidney disease. *Curr Cardiol Rep*. 2008; 10:448–55. PMID: 18950553
10. Jafar TH, Stark PC, Schmid CH, Landa M, Maschio G, de Jong PE et al.: Progression of chronic kidney disease: the role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition: a patient-level meta-analysis. *Ann Intern Med*. 2003; 139:244–52. PMID: 12965979
11. Macconi D: Targeting the renin angiotensin system for remission/regression of chronic kidney disease. *Histol Histopathol*. 2010; 25:655–68. <https://doi.org/10.14670/HH-25.655> PMID: 20238303
12. Brenner B, Cooper M, de Zeeuw D, Keane W, Mitch W, Parving H et al.: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Eng J Med*. 2001; 345:861–9.
13. Lewis E, Hunsicker L, al e: Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. *N Eng J Med*. 2001; 345:851–60.
14. Parving HH, Hommel E, Jensen BR, Hansen HP: Long-term beneficial effect of ACE inhibition on diabetic nephropathy in normotensive type 1 diabetic patients. *Kidney int*. 2001; 60:228–34. <https://doi.org/10.1046/j.1523-1755.2001.00790.x> PMID: 11422755
15. Parving HH, Lehnert H, Brochner-Mortensen J, Gomis R, Andersen S, Arner P et al.: The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. *N Engl J Med*. 2001; 345:870–8. <https://doi.org/10.1056/NEJMoa011489> PMID: 11565519
16. Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). *Lancet*. 1997; 349:1857–63. PMID: 9217756
17. Brown MJ, Palmer CR, Castaigne A, de Leeuw PW, Mancia G, Rosenthal T et al.: Morbidity and mortality in patients randomised to double-blind treatment with a long-acting calcium-channel blocker or diuretic in the International Nifedipine GITS study: Intervention as a Goal in Hypertension Treatment (INSIGHT). *Lancet*. 2000; 356:366–72. [https://doi.org/10.1016/S0140-6736\(00\)02527-7](https://doi.org/10.1016/S0140-6736(00)02527-7) PMID: 10972368
18. Officers A, Coordinators for the ACRGTA, Lipid-Lowering Treatment to Prevent Heart Attack T: Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA*. 2002; 288:2981–97. PMID: 12479763
19. Wright JT Jr., Bakris G, Greene T, Agodoa LY, Appel LJ, Charleston J et al.: Effect of blood pressure lowering and antihypertensive drug class on progression of hypertensive kidney disease: results from the AASK trial. *JAMA*. 2002; 288:2421–31. PMID: 12435255
20. Black HR, Elliott WJ, Neaton JD, Grandits G, Grambsch P, Grimm RH Jr. et al.: Rationale and design for the Controlled ONset Verapamil INvestigation of Cardiovascular Endpoints (CONVINCE) Trial. *Control Clin Trials*. 1998; 19:370–90. PMID: 9683312
21. Hansson L, Hedner T, Lund-Johansen P, Kjeldsen SE, Lindholm LH, Syvertsen JO et al.: Randomised trial of effects of calcium antagonists compared with diuretics and beta-blockers on cardiovascular

- morbidity and mortality in hypertension: the Nordic Diltiazem (NORDIL) study. *Lancet*. 2000; 356:359–65. PMID: [10972367](https://pubmed.ncbi.nlm.nih.gov/10972367/)
22. Nathan S, Pepine CJ, Bakris GL: Calcium antagonists: effects on cardio-renal risk in hypertensive patients. *Hypertension*. 2005; 46:637–42. <https://doi.org/10.1161/01.HYP.0000184541.24700.c7> PMID: [16172420](https://pubmed.ncbi.nlm.nih.gov/16172420/)
  23. Segura J, Campo C, Ruilope LM: Influence of chronic kidney disease development and renin-angiotensin system inhibition on cardiovascular prognosis. *Curr Med Chem Cardiovasc Hematol Agents*. 2005; 3:55–60. PMID: [15638744](https://pubmed.ncbi.nlm.nih.gov/15638744/)
  24. Lopez-Novoa JM, Martinez-Salgado C, Rodriguez-Pena AB, Lopez-Hernandez FJ: Common pathophysiological mechanisms of chronic kidney disease: therapeutic perspectives. *Pharmacol Ther*. 2010; 128:61–81. <https://doi.org/10.1016/j.pharmthera.2010.05.006> PMID: [20600306](https://pubmed.ncbi.nlm.nih.gov/20600306/)
  25. Bidani AK, Griffin KA: The benefits of renin-angiotensin blockade in hypertension are dependent on blood-pressure lowering. *Nat Clin Pract Nephrol*. 2006; 2:542–3. <https://doi.org/10.1038/ncpneph0299> PMID: [17003827](https://pubmed.ncbi.nlm.nih.gov/17003827/)
  26. Arksey N, Hadizad T, Ismail B, Hachem M, Valdivia AC, Beanlands RS et al.: Synthesis and evaluation of the novel 2-[(1)(8)F]fluoro-3-propoxy-triazole-pyridine-substituted losartan for imaging AT(1) receptors. *Bioorg Med Chem*. 2014; 22:3931–7. <https://doi.org/10.1016/j.bmc.2014.06.011> PMID: [25023539](https://pubmed.ncbi.nlm.nih.gov/25023539/)
  27. Hadizad T, Collins J, Antoun RE, Beanlands R, Dasilva J: [11C]Methyl-losartan as a potential ligand for PET imaging angiotensin II AT1 receptors. *J Label Compd Radiopharm*. 2011; 54:754–7.
  28. Hadizad T, Kirkpatrick SA, Mason S, Burns K, Beanlands RS, Dasilva JN: Novel O-[(11C)methylated derivatives of candesartan as angiotensin II AT(1) receptor imaging ligands: radiosynthesis and ex vivo evaluation in rats. *Bioorg Med Chem*. 2009; 17:7971–7. <https://doi.org/10.1016/j.bmc.2009.10.016> PMID: [19879152](https://pubmed.ncbi.nlm.nih.gov/19879152/)
  29. Ismail B, Hadizad T, Antoun R, Lortie M, deKemp RA, Beanlands RS et al.: Evaluation of [(11C)methyl-losartan and [(11C)methyl-EXP3174 for PET imaging of renal AT1 receptor in rats. *Nucl Med Biol*. 2015; 42:850–7. <https://doi.org/10.1016/j.nucmedbio.2015.06.012> PMID: [26300209](https://pubmed.ncbi.nlm.nih.gov/26300209/)
  30. Lortie M, DaSilva JN, Kirkpatrick SA, Hadizad T, Ismail BA, Beanlands RS et al.: Analysis of [11C] methyl-candesartan kinetics in the rat kidney for the assessment of angiotensin II type 1 receptor density in vivo with PET. *Nucl Med Biol*. 2013; 40:252–61. <https://doi.org/10.1016/j.nucmedbio.2012.10.013> PMID: [23352346](https://pubmed.ncbi.nlm.nih.gov/23352346/)
  31. Ismail B, deKemp RA, Hadizad T, Mackasey K, Beanlands RS, DaSilva JN: Decreased renal AT1 receptor binding in rats after subtotal nephrectomy: PET study with [(18)F]FPyKYNE-losartan. *EJNMMI Res*. 2016; 6:55. <https://doi.org/10.1186/s13550-016-0209-4> PMID: [27339045](https://pubmed.ncbi.nlm.nih.gov/27339045/)
  32. Ghosh SS, Massey HD, Krieg R, Fazelbhoj ZA, Ghosh S, Sica DA et al.: Curcumin ameliorates renal failure in 5/6 nephrectomized rats: role of inflammation. *Am J Physiol Renal Physiol*. 2009; 296:F1146–57. <https://doi.org/10.1152/ajprenal.90732.2008> PMID: [19225048](https://pubmed.ncbi.nlm.nih.gov/19225048/)
  33. Windt WA, Tahara A, Kluppel AC, de Zeeuw D, Henning RH, van Dokkum RP: Early, but not late therapy with a vasopressin V1a-antagonist ameliorates the development of renal damage after 5/6 nephrectomy. *J Renin Angiotensin Aldosterone Syst*. 2006; 7:217–24. <https://doi.org/10.3317/jraas.2006.041> PMID: [17347933](https://pubmed.ncbi.nlm.nih.gov/17347933/)
  34. Podjarny E, Bernheim JL, Pomeranz A, Rathaus M, Pomeranz M, Green J et al.: Effect of timing of anti-hypertensive therapy on glomerular injury: comparison between captopril and diltiazem. *Nephrol Dial Transplant*. 1993; 8:501–6. PMID: [8394529](https://pubmed.ncbi.nlm.nih.gov/8394529/)
  35. Podjarny E, Hasdan G, Bernheim J, Rashid G, Green J, Korzets Z et al.: Effect of chronic tetrahydrobiopterin supplementation on blood pressure and proteinuria in 5/6 nephrectomized rats. *Nephrol Dial Transplant*. 2004; 19:2223–7. <https://doi.org/10.1093/ndt/gfh383> PMID: [15252157](https://pubmed.ncbi.nlm.nih.gov/15252157/)
  36. Dilauro M, Zimpelmann J, Robertson SJ, Genest D, Burns KD: Effect of ACE2 and angiotensin-(1–7) in a mouse model of early chronic kidney disease. *Am J Physiol Renal Physiol*. 2010; 298:F1523–32. <https://doi.org/10.1152/ajprenal.00426.2009> PMID: [20357030](https://pubmed.ncbi.nlm.nih.gov/20357030/)
  37. Vaziri ND, Bai Y, Ni Z, Quiroz Y, Pandian R, Rodriguez-Iturbe B: Intra-renal angiotensin II/AT1 receptor, oxidative stress, inflammation, and progressive injury in renal mass reduction. *J of Pharmacol Exp Ther*. 2007; 323:85–93.
  38. Hachem M, Tiberi M, Ismail B, Hunter CR, Arksey N, Hadizad T et al.: Characterization of [18F]FPyKYNE-losartan for imaging AT1 Receptors *J Nucl Med* (In press). 2015.
  39. Logan J, Wolf AP, Shiue CY, Fowler JS: Kinetic modeling of receptor-ligand binding applied to positron emission tomographic studies with neuroleptic tracers. *J Neurochem*. 1987; 48:73–83. PMID: [2432176](https://pubmed.ncbi.nlm.nih.gov/2432176/)
  40. Klein R, Beanlands RS, deKemp RA: Quantification of myocardial blood flow and flow reserve: Technical aspects. *J Nucl Cardiol*. 2010; 17:555–70. <https://doi.org/10.1007/s12350-010-9256-9> PMID: [20596841](https://pubmed.ncbi.nlm.nih.gov/20596841/)

41. Kenk M, Greene M, Thackeray J, Dekemp RA, Lortie M, Thorn S et al.: In vivo selective binding of (R)-[(11)C]rolipram to phosphodiesterase-4 provides the basis for studying intracellular cAMP signaling in the myocardium and other peripheral tissues. *Nucl Med Biol.* 2007; 34:71–7. <https://doi.org/10.1016/j.nucmedbio.2006.10.002> PMID: 17210463
42. Nakamoto H, Ferrario CM, Fuller SB, Robaczewski DL, Winicov E, Dean RH: Angiotensin-(1–7) and nitric oxide interaction in renovascular hypertension. *Hypertension.* 1995; 25:796–802. PMID: 7536715
43. Allred AJ, Chappell MC, Ferrario CM, Diz DI: Differential actions of renal ischemic injury on the intrarenal angiotensin system. *Am J Physiol Renal Physiol.* 2000; 279:F636–45. PMID: 10997913
44. Chronic Kidney Disease Prognosis C, Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS et al.: Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet.* 2010; 375:2073–81. [https://doi.org/10.1016/S0140-6736\(10\)60674-5](https://doi.org/10.1016/S0140-6736(10)60674-5) PMID: 20483451
45. van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey A et al.: Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. *Kidney Int.* 2011; 79:1341–52. <https://doi.org/10.1038/ki.2010.536> PMID: 21307840
46. Rahn KH: The role of calcium antagonists in patients with chronic renal failure. *Pediatr Nephrol.* 2005; 20:1208–13. <https://doi.org/10.1007/s00467-005-1982-x> PMID: 15971071
47. Noda M, Matsuo T, Fukuda R, Ohta M, Nagano H, Shibouta Y et al.: Effect of candesartan cilexetil (TCV-116) in rats with chronic renal failure. *Kidney Int.* 1999; 56:898–909. <https://doi.org/10.1046/j.1523-1755.1999.00614.x> PMID: 10469358
48. Kujal P, Chabova VC, Vermerova Z, Walkowska A, Kompanowska-Jeziarska E, Sadowski J et al.: Similar renoprotection after renin-angiotensin-dependent and -independent antihypertensive therapy in 5/6-nephrectomized Ren-2 transgenic rats: are there blood pressure-independent effects? *Clin Exp Pharmacol Physiol.* 2010; 37:1159–69. <https://doi.org/10.1111/j.1440-1681.2010.05453.x> PMID: 20880190
49. Kobori H, Nangaku M, Navar LG, Nishiyama A: The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacol Rev.* 2007; 59:251–87. <https://doi.org/10.1124/pr.59.3.3> PMID: 17878513
50. Mehta PK, Griendling KK: Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol.* 2007; 292:82–97.
51. Bader M: Tissue renin-angiotensin-aldosterone systems: Targets for pharmacological therapy. *Ann Rev Pharmacology Toxicol.* 2010; 50:439–65.
52. Bader M, Peters J, Baltatu O, Muller DN, Luft FC, Ganten D: Tissue renin-angiotensin systems: new insights from experimental animal models in hypertension research. *J Mol Med (Berl).* 2001; 79:76–7102.
53. Joly E, Nonclercq D, Caron N, Mertens J, Flamion B, Toubeau G et al.: Differential regulation of angiotensin II receptors during renal injury and compensatory hypertrophy in the rat. *Clin Exp Pharmacol Physiol.* 2005; 32:241–8. <https://doi.org/10.1111/j.1440-1681.2005.04181.x> PMID: 15810986
54. Bruzzi I, Remuzzi G, Benigni A: Endothelin: a mediator of renal disease progression. *J Nephrol.* 1997; 10:179–83. PMID: 9377723
55. Aiello S, Remuzzi G, Noris M: Nitric oxide/endothelin balance after nephron reduction. *Kidney Int Suppl.* 1998; 65:S63–7. PMID: 9551434
56. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, Jafar TH, Heerspink HJ, Mann JF et al.: Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet.* 2013; 382:339–52. [https://doi.org/10.1016/S0140-6736\(13\)60595-4](https://doi.org/10.1016/S0140-6736(13)60595-4) PMID: 23727170
57. Le Corvoisier P, Adamy C, Sambin L, Crozatier B, Berdeaux A, Michel JB et al.: The cardiac renin-angiotensin system is responsible for high-salt diet-induced left ventricular hypertrophy in mice. *Eur J Heart Fail.* 2010; 12:1171–8. <https://doi.org/10.1093/eurjhf/hfq146> PMID: 20870672
58. Herzog CA, Asinger RW, Berger AK, Charytan DM, Diez J, Hart RG et al.: Cardiovascular disease in chronic kidney disease. A clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int.* 2011; 80:572–86. <https://doi.org/10.1038/ki.2011.223> PMID: 21750584
59. Shiba N, Shimokawa H: Chronic kidney disease and heart failure—Bidirectional close link and common therapeutic goal. *J Cardiol.* 2011; 57:8–17. <https://doi.org/10.1016/j.jjcc.2010.09.004> PMID: 21030212
60. Moriguchi Y, Yogo K, Aizawa K, Serizawa K, Tashiro Y, Yorozu K et al.: Left ventricular hypertrophy is associated with inflammation in sodium loaded subtotal nephrectomized rats. *Biomed Res.* 32:83–90. PMID: 21551943
61. Griffin KA, Abu-Amarah I, Picken M, Bidani AK: Renoprotection by ACE inhibition or aldosterone blockade is blood pressure-dependent. *J Hypertens.* 2003; 41:201–6.



62. Griffin KA, Picken MM, Bidani AK: Deleterious effects of calcium channel blockade on pressure transmission and glomerular injury in rat remnant kidneys. *J Clin Invest.* 1995; 96:793–800. <https://doi.org/10.1172/JCI118125> PMID: 7635974
63. Brenner BM: Hemodynamically mediated glomerular injury and the progressive nature of kidney disease. *Kidney Int.* 1983; 23:647–55. PMID: 6336299
64. Davis MJ, Hill MA: Signaling mechanisms underlying the vascular myogenic response. *Physiol Rev.* 1999; 79:387–423. PMID: 10221985
65. Griffin KA, Bidani AK: Progression of renal disease: renoprotective specificity of renin-angiotensin system blockade. *Clin J Am Soc Nephrol* 2006; 1:1054–65. <https://doi.org/10.2215/CJN.02231205> PMID: 17699327
66. Griffin KA, Picken MM, Bakris GL, Bidani AK: Class differences in the effects of calcium channel blockers in the rat remnant kidney model. *Kidney Int.* 1999; 55:1849–60. <https://doi.org/10.1046/j.1523-1755.1999.00434.x> PMID: 10231447
67. Griffin KA, Hacıoglu R, Abu-Amarah I, Loutzenhiser R, Williamson GA, Bidani AK: Effects of calcium channel blockers on "dynamic" and "steady-state step" renal autoregulation. *Am J Physiol Renal Physiol.* 2004; 286:F1136–43. <https://doi.org/10.1152/ajprenal.00401.2003> PMID: 14996672