Kidney and Blood Pressure Research

Kidney Blood Press Res , DOI: 10.1159/000537806
Received: October 29, 2023
Accepted: February 7, 2024
Published online: February 13, 2024

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ISSN: 1420-4096 (Print), eISSN: 1423-0143 (Online)
https://www.karger.com/KBR
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Hemodynamic Renal Reserve Response in Conscious Normotensive and Hypertensive Mice

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Running title: Acute volume expansion in conscious mouse

Key words: Renal functional reserve, acute volume expansion, hypertension, sodium excretion, conscious

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ABSTRACT

Introduction Renal function may be compromised following recovery from kidney insults. Renal functional reserve (RFR) is a measure of the difference between the kidney's maximum capacity and its baseline function which helps identify any areas of the kidney with compromised function. Usually, RFR is evaluated using acute volume expansion (AVE), but this is typically done in anesthetized animals, which may not accurately represent the kidney's complete functional capacity. In this study, we have introduced a novel method that enables AVE to be conducted in conscious mice.

Methods We have implemented this innovative approach in two animal models representing either intact or impaired renal function, specifically utilizing a lower nephron hypertensive model. Mice were implanted with radio-transmitters for mean artery blood pressure (MAP) monitoring during the experiment. After recovery, half of the mice were induced hypertension by right kidney nephrectomy combined with the ligation of the upper branch of the left kidney. For the AVE, a volume equivalent to 5% of the mouse's body weight was administered via intravenous (IV) or intraperitoneal (IP) bolus injection. Subsequently, the mice were individually housed in cages covered with plastic wrap. Urine was collected every hour for a total of 3 hours for the measurement of urine and sodium excretion.

Results The MAP for all normotensive mice were consistent throughout the AVE, but it increased 5-16mmHg in the hypertensive mice upon AVE. Remarkably, conscious mice exhibited a significantly stronger response to IV-administered AVE when compared to anesthetized mice. This response was evident in the increase in urinary flow, which was approximately 170% and 145% higher in conscious normotensive and hypertensive mice, respectively, compared to their respective baselines. In contrast, anesthetized normotensive and hypertensive mice showed only around a 130% and 100% increase in urinary flow, respectively. Additionally, upon AVE, conscious normotensive mice excreted approximately 47% more sodium than conscious hypertensive mice. In contrast, anesthetized normotensive mice excreted only about 30% more sodium than their anesthetized hypertensive counterparts.
Conclusion Performing a kidney stress test with a significant solution load in conscious mice seems to be a superior method for evaluating RFR compared to conducting the test under anesthesia. Assessing kidney clearance while the mice are conscious has the potential to enhance the precision of diagnosing and predicting both acute and chronic kidney diseases.

INTRODUCTION

Glomerular filtration rate (GFR), urine and sodium excretion are considered critical indexes of kidney function [1-3]. GFR is determined by measuring clearance of certain exogenous markers or endogenous waste products. Nonetheless, GFR is not a constant value, as the kidneys do not consistently operate at their maximum filtration capacity [4-6]. They have the ability to adapt their performance in response to hemodynamic and metabolic demands [7, 8]. Renal function is often influenced by meals and diurnal cycles [9]. Thus, single-point assessments of renal function ignore varying rates of glomerular filtration. Particularly, kidney function evaluation by GFR measurement may not be able to reveal the underlying dysfunctional portion under pathological situation. This is because in these situations, the remaining healthy nephrons may become stimulated and increase their filtration rate in order to compensate for the loss of function in the damaged nephrons. The undetected alteration in GFR due to compensatory effect may mask the underlying lost function. Many investigators suspected that the functional recovery assessment might be clouded by the stimulated single-nephron GFR to compensate for nephron loss after kidney insults [10, 11]. Therefore, testing the renal functional response to stimuli in these recovered individuals could possibly unveil this undetected loss of functional units.

The concept of reserve forces of the kidney was proposed by Verney in 1930 and named renal functional reserve, which is defined as the difference between the stimulated GFR and basal GFR [11-13]. The concept of RFR was subsequently refined to encompass the renal functional response assessed by administering a substantial liquid volume/substance to the animal and measuring the resulting hemodynamic changes, denoted as acute volume expansion (AVE).

Conventionally, the AVE was carried out either in anesthetized animals by iv bolus injection or in conscious animals by intravenous (IV) infusion of a larger volume of solution [14, 15]. Both strategies require anesthesia or surgery preparation. However, anesthesia or surgical stress can directly or indirectly affect renal hemodynamics and influence renal function and body fluid regulation, possibly through lowered blood pressure and cardiac output, increased sympathetic outflow, increased release of renin, angiotensin and vasopressin, alterations in the autoregulation of renal blood flow, changes in the impact of antidiuretic hormone (ADH), and modifications to tubular transport of sodium and organic acids [16-18]. Inhalational anesthetics generally reduce GFR and urine output mainly by extra-renal effects [19, 20]. Anesthetic management frequently have a physiologically significant impact on renal physiology [21, 22]. Furthermore, IV infusion via implanted catheters and urine collection using metabolic cages may introduce potential biases or confound the results, stemming from urine evaporation and sodium loss. Additionally, AVE conducted on anesthetized animals typically serves as a terminal experiment, constraining its applicability in repeated monitoring studies.

In this context, we have presented experimental data obtained through both conventional and modified protocols. The adjustments made to these protocols have demonstrated enhancements in the accuracy and precision of experimental outcomes, improved efficiency, and addressed various limitations associated with the conventional methods.

MATERIALS and METHODS

All procedures and experiments were performed with the approval of the Institutional Animal Care and Use Committee at the University of South Florida College of Medicine and in accordance with all federal guidelines. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Animals were euthanized as needed according to the guidelines set forth by the American Veterinary Medicine Association.

Animals

Male C57BL/6J mice, aged 10-12 weeks (22–25g) were obtained from a vendor (Jackson Lab, Indianapolis, IN). The mice were randomly divided into 7 groups: normotensive anesthesia IV (NTN-A-IV), normotensive conscious IV (NTN-C-IV), normotensive conscious IP (NTN-C-IP), hypertensive anesthesia IV (HTN-A-IV), hypertensive conscious IV (HTN-C-IV), hypertensive conscious IP (HTN-C-IP), and sham control groups (normotensive and hypertensive) which followed all the procedures but without acute saline load.

Radio-telemetry transmitter implantation and MAP measurement

Radio-telemetry transmitters (PA-C10) were implanted subcutaneously in all groups of mice as we described previously [23-25]. The mice were anesthetized via inhalation of isoflurane (2% in air; flow 200 mL/min). A small incision was made in the middle of the neck, and the catheter of telemetry transmitter was inserted into the left carotid artery, which was then guided down to the aortic arch. The body of the transmitter was placed subcutaneously in the right ventral flank of the animal. The wound in the middle of the neck was closed, and the mice were given a recovery period of 10 days. Basal MAP was then measured for 5 days at an interval of 2 minutes with the measurement duration of 20 seconds.

Low nephron hypertensive model induction

After the basal MAP measurement, half of the mice were randomly selected and induced hypertension by ligating the left renal artery branch followed by right kidney nephrectomy as we described previously with modification [26]. Briefly, the mice implanted with transmitters were anesthetized with isoflurane. A midline abdominal incision was performed to expose two kidneys. The upper branch of the left renal artery was carefully dissected from the renal vein and ligated using a 7–0 braided silk suture (07C1500160; Teleflex Medical OEM, Gurnee, IL). The right kidney was removed and the ischemic portion of left kidney induced by upper branch ligation was left in place. The incision was closed and the mouse recovered in the normal cages. Plasma and urine were collected at two weeks to evaluate the kidney function and injury by plasma creatinine and Blood urea nitrogen (BUN) levels measured at Bioanalytical Core of O'Brien Center at Uiversity of Alabama at Birmingham as previously described [24, 27-30].

Cages preparation for AVE

Pure mouse urine was collected in transparent plastic-wrapped cages for qualitative analysis. Regular cages were cleaned and dry. The bottom of the cage was covered by white paper and then the whole cage was covered by Glad® cling wrap. Mouse was housed individually. To minimize stress and anxiety, three days before the experiment the mice were housed in the cages for four hours every day and 2 hours before the experiment on the same day to adapt to the new enviroment.

AVE in conscious mouse

One hour before the experiment, the urine was voided from the experimental mouse's bladder by holding the tail of the mouse with thumb and index finger and pressing the root of the tail with middle and the forth finger. Press the bladder manually by application of gentle trans-abdominal pressure over the bladder to overcome normal urethral pressure with a cotton swab to encourage the mouse to micturate. The mouse was then put in the AVE cage and the timer was started. The voided urine was aspirated into micro-centrifuge tubes using a Pipette. The total amount of urine collected in the first hour was combined and taken as the a control sample. The mouse was then slightly anesthetized with isoflurane. The amount of 5% body weight sterile saline was administrated through retro-orbital or IP injections . After injection, the mouse was immediately returned to the prepared cage. The urine was collected immediately after micturate and centrifuged. All the urine samples colleted within each hour were combined and taken as a single sample. The total collection period lasted for three hours. The mice were euthanized and kidneys were collected at the end of experiment. The urine was stored under -20^{o}C for later analyses.

AVE and GFR measurement in anethetized mice

The natriuretic response to acute volume expansion was measured in anesthetized mice as previously described[23, 31]. The mice were anesthetized with ketamine (30 ug/g) and inactin (50 ug/g). Two catheters were placed in the femoral artery for the measurement of MAP, and in the femoral vein for an intravenous infusion of 2% BSA and FITC-sinistrin (2mg/ml) in a 0.9% NaCl solution at a rate of 0.5 ml/hour. Two more catheters were inserted into the ureters for the collection of urine. After surgery, urine was collected during a 30-min period after a 30-min equilibration period. Then infusion of 5% BW (body weight) saline in a bolus followed by the infusion of FITC-sinistrin at 0.5 ml/hour. Urine and blood were collected during a 60-min period for 3hours after volume expansion. The urine Na+ concentration was measured with a Flame Photometer (BWB Technologies). The urine flow rates and Na+ excretion rates were calculated based on Na+ concentration and total urine volume. FITC-sinistrin concentration in the urine and plasma samples was measured using a plate reader (Cytation3, BioTek).

The calculation for GFR was performed using the formula: [Urine FITC-sinistrin] x Urine volume / [Plasma FITC-sinistrin].

GFR measurement in conscious mice during AVE

GFR was measured in all the mice under conscious conditions using transdermal measurement of FITC-sinistrin clearance rate as previously reported [32-34]. The right dorsal hair of the mice was removed by shaving and depilatory lotion on the day before the measurement. The transdermal fluorescence detector (MB 0309 Mini, MediBeacon Inc.) was affixed directly onto the mouse's skin using medical tape. FITC- sinistrin was then administered by retro-orbital injection at 0.05 mg/g body weight. The excitation kinetics of the exogenous GFR tracer were recorded and fitted to a three-compartment model with the software provided by the vendor (MB Studio Ver. 2.1). GFR was calculated based on the half- life (t1/2) of plasma FITC-sinistrin decay.

Statistical analysis

All values are presented as means ± SEM. Comparison of three or more datasets was performed using two-way ANOVA followed by Sidak's multiple comparison test. A P-value of <0.05 was considered to be statistically significant. Sample sizes are provided in the figure legends. All statistics were performed using GraphPad Prism, version 9.0 (GraphPad Software).

RESULTS

Experimental protocols for AVE and GFR measurements, as well as sample collection from the mice under both anesthetized and conscious conditions, were outlined in Fig 1.

Part 1: AVE in normotensive mice

Blood pressure measurement during the AVE in normotensive mice

The basal MAP values were similar for all mice in conscious normotensive groups (NTN-C-IV and NTN-C-IP). AVE was carried out with the bolus injection of saline in the mice under slight anesthesia with isoflorane. The MAPs decreased about 10 mmHg due to the anesthesia which quickly returned to baseline in about 5 min. Thereafter, the MAP remained constant during the AVE for all the conscious groups (shown in Fig. 2). For the anesthetized IV group (NTN-A-IV), the basal MAP was aboout 16 mmHg lower than the conscious mice due to the anesthesia. The AVE on anesthetized mice was performed as described in the method section. The MAP maitained consistant during the whole AVE procedure.

GFR in response to AVE in normotensive mice

GFR was measured using the tranderminal measuring the sinistrin clearance rates. Basal GFR was first measured. Another dose of FITC-sinistrin was administrated again. Twenty minutes later, a 5% body weight saline solution was administered via penis vein or IP injection to induce AVE. For the group of mice underwent AVE in conscious conditions, GFR was enhanced by 62% and 53% for the IV and IP injected groups, respectively. However, the GFR only increased 31% in the mice under anesthesia condition (shown in Fig. 3).

Urine and sodium excretion in response to AVE in normotensive mice

Urine was collected from the normotensive mice under either conscious or anesthesia conditions every hour for 3 hours after injection. The basal urine flow rates were similar for both NTN-C-IP and NTN-C-IV groups with the value of about 10.6±1.7 µl/min.g KW (kidney weight). The IV bolus injection of 5%BW saline induced a dramatic increase in urine production rates with the maxium rate of 44.7±5.8 ul/min.g KW, which was about 420% of baseline. The IP injection of same amount of saline stimulated much less increase in the urine flow rates, which is about 178% of baseline, than the IV injected conscious group within the experimental period (shown in Fig. 4). Moreover, the NTN-C-IV mice displayed a significantly faster response in urine excretion to the AVE compared to the NTN-C-IP mice. In NTN-C-IV mice, the peak urinary flow rates were attained within one hour after injection, whereas it took nearly two hours for the NTN-C-IP mice to reach their peak urinary flow rate. By the end of three hours following injection, NTN-C-IV mice had excreted the entire volume of injected saline, whereas NTN-C-IP mice had only micturated approximately 52% of the total injected saline (Table 1.).

The AVE in anesthetized mice (NTN-A-IV) was performed by administration of 5% BW saline by bolus IV injection. The basal urie flow rate was 6.2±1.3 ul/min.g KW which was about 42% lower than that in conscious mice. AVE stimulated a 111% increase of urine flow rate with the maixum value of 13.1±1.8 ul/min.g KW. The peak value of the urinary excretion in response to the saline injection in NTN-C-IV mice was about 3-fold and 2-fold of NTN-A-IV and NTN-C-IP mice, respectively. Additionally, compared with conscious animals, the response of the urinary excretion rates in the anesthetized mice failed to maitain and returned to baseline within two hours after AVE. During the measurement period of 3 hours, the anesthetized mice showed the least increase in urine output and only about 4 0% of injected saline was micturated.

The sodium excretion rates showed the similar pattern as the urinary flow rates (shown in Fig. 4). The conscious mice exhibited quicker response to the stimulation of the bolus saline load and the maximal response maintained for longer period of time than the anesthetized mice.

These data indicated that anesthesia induced by isoflurane significantly decreased urine output and fractional sodium excretion during AVE. In response to the stressor of kidney overload, conscious mice demonstrated superior abilities in handling sodium excretion compared to anesthetized mice.

Part II: AVE in hypertensive mice

Hypertensive kidney injury induction

A low nepheron hypertensive model was succesfuly generated as described in the method and previously reported [35]. MAP was measured with a telemetry system. There were no significant differences between the different groups for basal conscious MAPs which were about 96 mmHg. Right kidney nephrectomy and left renal artery branch ligations induced drastic rise of blood pressure (shown in Fig. 5. A). The MAPs reached the maxium level in 2 weeks with a value of 136.4 ± 6.8 mmHg which increased about 40mmHg compared with baseline. The increased blood pressure was accompanied with impaired kidney function based on the plasma creatinine and urine ACR levels (shown in Fig. 5.B and C). The MAPs were consistant in the control mice.

Blood pressure measurement during the conscious AVE

We next tested the differences of hemodynamic response to a saline bolus chanllege among the conscious hypertensive (HTN-C-IV and HTN-C-IP) mice and anesthetized hypertensive (HTN-A-IV) mice. AVE was conducted in the hypertensive mice by IV injection under conscious or anesthetized conditions or IP injection under conscious condition as it was carried out in the normotensive mice. MAPs were monitored during the whole AVE process with telemetry system. The anesthesia by isoflurane induced about a decrease of 18 mmHg of the basal MAP (from 136 to 118mmHg) in the anesthesia group. The MAP gradually and steadily rose throughout the AVE, with the maxium increase of about 14mmHg than the baseline during the monitoring period. In the conscious group, a mild anesthesia administered for the IV injection of saline led to a temporary drop of approximately 16 mmHg in MAP during the injection phase. Subsequently, MAPs rebounded to around 10 mmHg above the baseline, and within the initial hour post AVE, they returned to the baseline level and persisted consistently throughout the remainder of the AVE experiment. In the conscious IP group, there was a delayed yet steady increase in MAP, peaking with a maximum rise of approximately 6 mmHg over the course of the AVE (shown in Fig. 6).

GFR in response to AVE in hypertensive mice

Initially, basal GFR was determined. Subsequently, another dose of FITC-sinistrin was administered. Twenty minutes later, a 5% body weight saline solution was introduced via penile vein or intraperitoneal (IP) injection to induce AVE. In the group of mice that underwent AVE under conscious conditions, GFR showed a respective enhancement of 25-28% for the IV and IP injected groups. However, under anesthesia conditions, the GFR only increased by 12% (shown in Fig. 7).

Urine and sodium excretion in response to AVE in hypertensive mice

The basal urine flow rate were similar for both HTN-C-IV and HTN-C-IP groups with the value of 16.2±2.8 ul/min.g KW (kidney weight). The IV bolus injection of 5% BW saline in the conscious mice induced a dramatic increase in urine production rates. The urinary flow rate reached the maxium value of 38.7±2.8 ul/min.g KW, which was about 238% of baseline, in two hours. The urine excretion maitained the highest rate until three hours after injection and decreased gradually after that. The IP injection of same amount of saline also induced the similar levels of increase as the IV group, however the increase of the urinary flow rate was significantly delayed (shown in Fig. 8). HTN-C-IV group showed much quicker response to the stimulation of massive saline load in the urine excretion than HTN-C-IP injection.

Basal urinery flow for the anesthetized mice was suppressed compared with the conscious mice with a value of 10.42±1.38 ul/min.g KW. The bolus injection of saline induced an increase of urinary flow rate upto 20.12±1.86 ul/min.g KW for the HTN-A-IV mice. The peak value of the urinary excretion in response to the saline injection in conscious mice HTN-C-IV was three times of anesthetized mice HTN-A-IV. It is very interesting that the conscious animal showed more prolonged response to the stimulation of saline bolus load than the anesthetized mice based on the sustained peak urinary flow rates for upto three hours for the HTN-C-IV verse one hour for the HTN-A-IV. HTN-C-IP mice showed drastically delayed response to the saline loading challenge. Within three hours after injection, HTN-C-IV goup excreted 59% of the injected saline, however, IP injected hypertensive mice and anesthetized hypertensive mice only excreted about 44% and 28% of the total injected saline, respectively.

The sodium excretion rates showed the similar pattern as the urinary flow rates (shown in Fig. 8). The IV injection induced quicker response of urineary sodium excretion to the volume epansion than IP injection. Additionally, conscious mice exhibited faster and longer sustained stimulation in the excretion of sodium compared to the anethitized mice.

Part III: conscious AVE is more likely to reveal underlying renal sodium handling

Both the conscious and anesthetized mice that received IV injections showed higher urinary flow rates per kidney weight in hypertensive mice compared to normotensive mice. This increase in flow rates may be attributed to hyperfiltration induced by kidney mass ablation. However, the delta of total urinary excretion in responding to AVE between hypertensive and normotensive mice is greater in conscious mice than the that in anesthetized mice. In addition, normotensive mice excreted 47% more urine than hypertensive mice during the three hours of conscious AVE period, whereas there was only a 30% difference in urine output between normotensive and hypertensive mice when the mice were subjected to AVE under anesthetic conditions (Table 1).

DISCUSSION

Our research group has developed a new methodology to evaluate the hemodynamic response to AVE in unencumbered mice. This is accomplished through the administration of a bolus intravenous injection of a saline solution, with a volume equating to 5% of the animal's body weight.

This method has been validated in health normotensive and hypertensive animal models and compared with conventional method under anesthesia conditions in this study. Our findings indicated that conscious animals, regardless of their health or hypertensive status, display a notably stronger renal hemodynamic response to a large volume infusion of saline compared to anesthetized animals. Therefore, we concluded that evaluating the full kidney functional capacity may be more accurate and informative in conscious animals compared to animals under anesthesia. This is because the state of consciousness appears to impact urinary excretion patterns in response to AVE, with anesthesia potentially concealing or reducing these distinctions.

This method demonstrated that using conscious animals in the evaluation of RFR may provide a better strategy compared to using anesthetized mice, and that this approach may lead to improved diagnosis and prognostication of both acute and chronic kidney disease.

GFR and urine flow rates are common standards used in the evaluation of kidney function, and they can be used together to provide a more comprehensive assessment of kidney health [4-6]. However, the GFR is not a fixed parameter, and it can vary in response to various factors such as changes in blood pressure, medications, and other physiological stimuli [36, 37]. Thus, baseline GFR does not describe the entire functional capacity of the kidneys and a stress test is required to quantify the functional reserve of the kidney, which has been described as RFR. Especially under the pathphysiological conditions, like acute kidney injury and hypertension induced by less nephrons, even if a normal basal kidney function is observed after a recovery, a reduction in the functional reserve of the kidney due to the loss of nephrons and damage to the remaining nephrons may have occurred. Therefore, measurement of kidney function response to a stimuli can provide important information about the functional reserve of the kidney and can help identify early stages of kidney disease or dysfunction before significant damage occurs.

In clinical situations characterized by hyperfiltration, like during pregnancy, hypertension, or diabetic nephropathy, the baseline GFR can often stay within normal ranges or even exceed normal levels. This can persist until around 50% of nephrons are lost, or in cases involving patients with just one remaining kidney. This can obscure any underlying reduction in kidney functional reserve. The RFR test can be used to evaluate the ability of the kidney to increase its filtration rate in response to a stressor, such as an infusion of amino acids or glucose. This may represent a sensitive and early way to assess the functional decline in the kidney.

In animal studies, the RFR is usually determined by measuring the renal responses to ECVE with IV bolus injection under anesthesia condition or perfusion of isotonic saline in conscious animals [38]. As we and others did previously, IV bolus injection of 3-5% body weight saline induced 40-60% increase in GFR and Urine flow rate [29, 39]. In the present study, we first confirmed that IV bolus injection of 5% body weight saline did not significantly affect systemic blood pressure in conscious mice, which is similar to anesthetized mice. We then repeated the AVE experiments in anesthetized mice as we or other labs did before [29, 39]. The AVE in conscious mice showed greater and more sustained response to the saline load than the anesthetized mice with greater urinary excretion. The lower blood pressure due to anesthesia, the surgical stress and nociceptive input followed by loss of fluid during the operation can affect the renal function to different extents. It has been reported that most barbiturates and inhalational anesthetics tend to decrease renal blood flow (RBF) and GFR. The effects of

anesthetics on the kidney go beyond a simple change in basal hemodynamics and include, for some drugs, an alteration in the ability for the kidney to autoregulate its blood flow and GFR [40, 41].

We also tested the AVE in conscious mice of a hypertensive kidney disease model which was generated by uninephrectomy combined with 2/3 renal infarction via a ligation of upper renal artery branch on the contralateral kidney as reported previously [35]. We first applied the AVE with the traditional method under anesthetic conditions. The anesthetic AVE showed that the normotensive mice excreted about 30% more sodium than hypertensive mice which is consistent with the results of previous studies of the GFR differences between the hypertensive and uninephrectomized mice [42-44]. However, conscious AVE showed about 47% more sodium output in normotensive mice than hypertensive mice. This may be because the suppressed renal sodium excretion was further decreased due to the application of anesthesia. Although the sodium output was much less in the hypertensive mice than the normotensive mice, we did not test the increase of the blood pressure during the AVE, which may be due to the short time of monitoring after AVE.

In summary, this study introduced and validated a novel approach for assessing renal functional capacities in conscious animals. This innovative method holds the potential to enhance diagnostic and prognostic capabilities in both acute and chronic kidney disease. Furthermore, the new AVE procedure in conscious animals offers the advantage of repeated use in monitoring kidney functional capacities, in contrast to the AVE conducted in anesthetized animals, which is typically a terminal experiment.

Statement of Ethics

All animal use and welfare adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. This study followed the protocol approved by the Institutional Animal Care and Use Committee (IACUC, IS000011615R) at the University of South Florida.

Conflict of Interest Statement

The authors declare that they have no competing financial interests or personal relationships that could have potentially influenced the findings presented in this paper.

Funding Sources

This work was supported by American Heart Associate Career Development Grant (18CDA34110441) and National Institutes of Health Grant (R01DK122050)

Author contributions

MT, CL and LW designed the research study and generated the draft of the manuscript. MT generated the hypertensive model, performed the acute volume expansion experiment, performed data analysis and prepared figures. CL conducted radio-transmitter implantation, measured the blood pressure and performed data analysis. MN performed GFR and sodium excretion measurement. CP measured plasma creatinine and urine ACR. LW participated in the design of the study and contributed to the interpretation of results and revision of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Availability of Data and Materials

All the data and materials supporting the findings of this study are available within the article. Further enquiries can be directed to the corresponding author.

References

1. Smith, H.W., W. Goldring, and H. Chasis, *The Measurement of the Tubular Excretory Mass, Effective Blood Flow and Filtration Rate in the Normal Human Kidney.* J Clin Invest, 1938. **17**(3): p. 263-78.
2. Rahn, K.H., S. Heidenreich, and D. Bruckner, *How to assess glomerular function and damage in humans.* J Hypertens, 1999. **17**(3): p. 309-17.
3. Stevens, L.A., et al., *Assessing kidney function--measured and estimated glomerular filtration rate.* N Engl J Med, 2006. **354**(23): p. 2473-83.
4. Asmar, A., et al., *Extracellular Fluid Volume Expansion Uncovers a Natriuretic Action of GLP-1: A Functional GLP-1-Renal Axis in Man.* J Clin Endocrinol Metab, 2019. **104**(7): p. 2509-2519.
5. Fattah, H., A. Layton, and V. Vallon, *How Do Kidneys Adapt to a Deficit or Loss in Nephron Number?* Physiology (Bethesda), 2019. **34**(3): p. 189-197.
6. Kaufman, D.P., H. Basit, and S.J. Knohl, *Physiology, Glomerular Filtration Rate*, in *StatPearls*. 2023: Treasure Island (FL) ineligible companies. Disclosure: Hajira Basit declares no relevant financial relationships with

ineligible companies. Disclosure: Stephen Knohl declares no relevant financial relationships with ineligible companies.
7. Ronco, C., R. Bellomo, and J. Kellum, *Understanding renal functional reserve.* Intensive Care Med, 2017. **43**(6): p. 917-920.
8. King, A.J. and A.S. Levey, *Dietary protein and renal function.* J Am Soc Nephrol, 1993. **3**(11): p. 1723-37.
9. Koopman, M.G., et al., *Circadian rhythm of glomerular filtration rate in normal individuals.* Clin Sci (Lond), 1989. **77**(1): p. 105-11.
10. Molitoris, B.A., *Rethinking CKD Evaluation: Should We Be Quantifying Basal or Stimulated GFR to Maximize Precision and Sensitivity?* Am J Kidney Dis, 2017. **69**(5): p. 675-683.
11. Sharma, A., M.J. Mucino, and C. Ronco, *Renal functional reserve and renal recovery after acute kidney injury.* Nephron Clin Pract, 2014. **127**(1-4): p. 94-100.
12. Ronco, C. and L.S. Chawla, *Glomerular and Tubular Kidney Stress Test: New Tools for a Deeper Evaluation of Kidney Function.* Nephron, 2016. **134**(3): p. 191-194.
13. Chawla, L.S. and C. Ronco, *Renal Stress Testing in the Assessment of Kidney Disease.* Kidney Int Rep, 2016. **1**(1): p. 57-63.
14. LaGrange, L.P., G.M. Toney, and V.S. Bishop, *Chronic angiotensin II infusion attenuates the renal sympathoinhibitory response to acute volume expansion.* Am J Physiol Regul Integr Comp Physiol, 2003. **284**(4): p. R1098-107.
15. Cervenka, L., K.D. Mitchell, and L.G. Navar, *Renal function in mice: effects of volume expansion and angiotensin II.* J Am Soc Nephrol, 1999. **10**(12): p. 2631-6.
16. Zuurbier, C.J., V.M. Emons, and C. Ince, *Hemodynamics of anesthetized ventilated mouse models: aspects of anesthetics, fluid support, and strain.* Am J Physiol Heart Circ Physiol, 2002. **282**(6): p. H2099-105.
17. Janssen, B.J., et al., *Effects of anesthetics on systemic hemodynamics in mice.* Am J Physiol Heart Circ Physiol, 2004. **287**(4): p. H1618-24.
18. Mercatello, A., *[Changes in renal function induced by anesthesia].* Ann Fr Anesth Reanim, 1990. **9**(6): p. 507-24.
19. Sladen, R.N., *Effect of anesthesia and surgery on renal function.* Crit Care Clin, 1987. **3**(2): p. 373-93.
20. Burchardi, H. and G. Kaczmarczyk, *The effect of anaesthesia on renal function.* Eur J Anaesthesiol, 1994. **11**(3): p. 163-8.
21. Deutsch, S., *Effects of anesthetics on the kidney.* Surg Clin North Am, 1975. **55**(4): p. 775-86.
22. Motayagheni, N., et al., *A Review of Anesthetic Effects on Renal Function: Potential Organ Protection.* Am J Nephrol, 2017. **46**(5): p. 380-389.
23. Lu, Y., et al., *Macula densa nitric oxide synthase 1 beta protects against salt-sensitive hypertension.* J Am Soc Nephrol, 2016. **27**(8): p. 2346-2356.
24. Wang, L., et al., *The role of kidneys in sex differences in Ang II-induced hypertension.* Hypertension, 2017. **70**(6): p. 1219-1227.
25. Zhang, J., et al., *Enhanced Hemodynamic Responses to Angiotensin II in Diabetes are Associated with Increased Expression and Activity of AT1 Receptors in the Afferent Arteriole.* Physiol Genomics, 2017: p. hysiolgenomics.
26. Hille, B., *Ion channels of excitable membranes*. 3rd ed. 2001, Sunderland, Mass.: Sinauer. xviii, 814 p.
27. Wang, L., et al., *A new mouse model of hemorrhagic shock-induced acute kidney injury.* Am J Physiol Renal Physiol, 2017. **312**(1): p. F134-F142.
28. Wang, L., et al., *Cross-sex transplantation alters gene expression and enhances inflammatory response in the transplanted kidneys.* American Journal of Physiology - Renal Physiology, 2017. **313**(2): p. F326-F338.
29. Wang, L., et al., *Graft function assessment in mouse models of single- and dual-kidney transplantation.* Am J Physiol Renal Physiol, 2018. **315**(3): p. F628-F636.
30. Wei, J., et al., *A new mouse model of CKD transitioned from ischemic AKI.* Am J Physiol Renal Physiol, 2019.
31. Wang, L., et al., *Effects of different storage solutions on renal ischemia tolerance after kidney transplantation in mice.* Am J Physiol Renal Physiol, 2018. **314**(3): p. F381-F387.
32. Chaudhari, S., et al., *Renal TLR-7/TNF-alpha pathway as a potential female-specific mechanism in the pathogenesis of autoimmune-induced hypertension.* Am J Physiol Heart Circ Physiol, 2022. **323**(6): p. H1331-H1342.
33. Schreiber, A., et al., *Transcutaneous measurement of renal function in conscious mice.* Am J Physiol Renal Physiol, 2012. **303**(5): p. F783-8.

34. Scarfe, L., et al., *Transdermal Measurement of Glomerular Filtration Rate in Mice.* J Vis Exp, 2018(140).
35. Wei, J., et al., *A new low-nephron CKD model with hypertension, progressive decline of renal function, and enhanced inflammation in C57BL/6 mice.* Am J Physiol Renal Physiol, 2018. **314**(5): p. F1008-F1019.
36. Kellum, J.A., et al., *Acute kidney injury.* Nat Rev Dis Primers, 2021. **7**(1): p. 52.
37. Bhaskar, A. and V. Oommen, *A simple model for demonstrating the factors affecting glomerular filtration rate.* Adv Physiol Educ, 2018. **42**(2): p. 380-382.
38. Fuhrman, D.Y., *The Role of Renal Functional Reserve in Predicting Acute Kidney Injury.* Crit Care Clin, 2021. **37**(2): p. 399-407.
39. Wang, X., et al., *Inhibition of Nitric Oxide Synthase 1 Induces Salt-Sensitive Hypertension in Nitric Oxide Synthase 1alpha Knockout and Wild-Type Mice.* Hypertension, 2016. **67**(4): p. 792-9.
40. Cupples, W.A., et al., *Spontaneous blood pressure fluctuations and renal blood flow dynamics.* Am J Physiol, 1996. **270**(1 Pt 2): p. F82-9.
41. Loutzenhiser, R., et al., *Renal autoregulation: new perspectives regarding the protective and regulatory roles of the underlying mechanisms.* Am J Physiol Regul Integr Comp Physiol, 2006. **290**(5): p. R1153-67.
42. Gava, A.L., et al., *Effects of 5/6 nephrectomy on renal function and blood pressure in mice.* Int J Physiol Pathophysiol Pharmacol, 2012. **4**(3): p. 167-73.
43. Cowley, A.W., Jr., et al., *Progression of glomerular filtration rate reduction determined in conscious Dahl salt-sensitive hypertensive rats.* Hypertension, 2013. **62**(1): p. 85-90.
44. Dupont, V., et al., *Impaired renal reserve contributes to preeclampsia via the kynurenine and soluble fms-like tyrosine kinase 1 pathway.* J Clin Invest, 2022. **132**(20).

Figure Legends

Fig 1. Experimental protocols for the AVE in anesthetized and conscious mice.

A. Post surgery, the infusion of saline containing FITC-sinistrin commenced, continuing throughout the entire AVE experiment. Basal urine was collected during a 30-minute period following a 30-minute equilibration period. Subsequently, AVE was initiated, and urine and blood were collected over a 60-minute period for the next 3 hours. Urine flow rates, Na+ excretion rates, and GFR were calculated using the described methods.
B. Basal GFR was measured using the transdermal method. AVE was then initiated by bolus injection of 5% body weight of saline, and FITC-sinistrin was injected for GFR measurement during AVE. Urine was collected during a 60-minute period for the subsequent 3 hours after volume expansion. Urine flow rates and Na+ excretion rates were calculated following the outlined methodology.

Fig.2. MAP in normotensive mice in response to acute volume expansion (AVE).

The basal MAP values were similar for all mice in conscious normotensive mice. The MAPs decreased about 10 mmHg due to the anesthesia for IV injection, which quickly returned to baseline in about 5 min. Thereafter, the MAP remained constant during the AVE for all the conscious groups (NTN-C-IV and NTN-C-IP). For the anesthetized IV group (NTN-A-IV), the basal MAP was aboout 16 mmHg lower than the conscious mice due to the anesthesia. The MAP maitained consistant during the whole AVE procedure.

Fig. 3. GFR in normotensive mice in response to AVE.

For the group of mice underwent AVE in conscious conditions, GFR was enhanced by 62% and 53% for the NTN-C-IV and NTN-C-IP injected groups, respectively. However, the GFR only increased 31% in the mice under anesthesia condition. *Two-way* ANOVA followed by Sidak's multiple comparison test was performed. (*p<0.05, ****p<0.0001, n=5)

Fig. 4. Urine and sodium excretion in normotensive mice in response to AVE stimulation.

The conscious mice exhibited quicker response in urine and sodium excretion to the stimulation of the bolus saline load and the maximal response maintained for longer period of time than the anesthetized mice. Two-way ANOVA followed by Sidak's multiple comparison test was performed. (***p<0.001, ****p<0.0001, n=5)

Fig. 5. Hypertensive kidney disease induction.

A low nepheron hypertensive model was succesfuly generated as described in the method. The increased blood pressure (**A**) was accompanied with impaired kidney function based on the plasma creatinine (**B**) and urine ACR levels (C). Two-way ANOVA followed by Sidak's multiple comparison test was performed. (****p<0.0001, n=5)

Fig. 6. MAP in hypertensive mice in response to AVE stimulation.

MAPs were monitored during the whole AVE process with telemetry system. The anesthesia by isoflurane induced about a decrease of 18 mmHg of the basal MAP (from 136 to 118mmHg) in the anesthesia group. The MAP gradually and steadily rose throughout the AVE, with the maxium increase of about 14mmHg. In the conscious group, a mild anesthesia administered for the IV injection of saline led to a temporary drop of approximately 16 mmHg in MAP, which subsequently rebounded to around 10 mmHg above the baseline. In the conscious IP group, there was a delayed yet steady increase in MAP of approximately 6 mmHg over the course of the AVE.

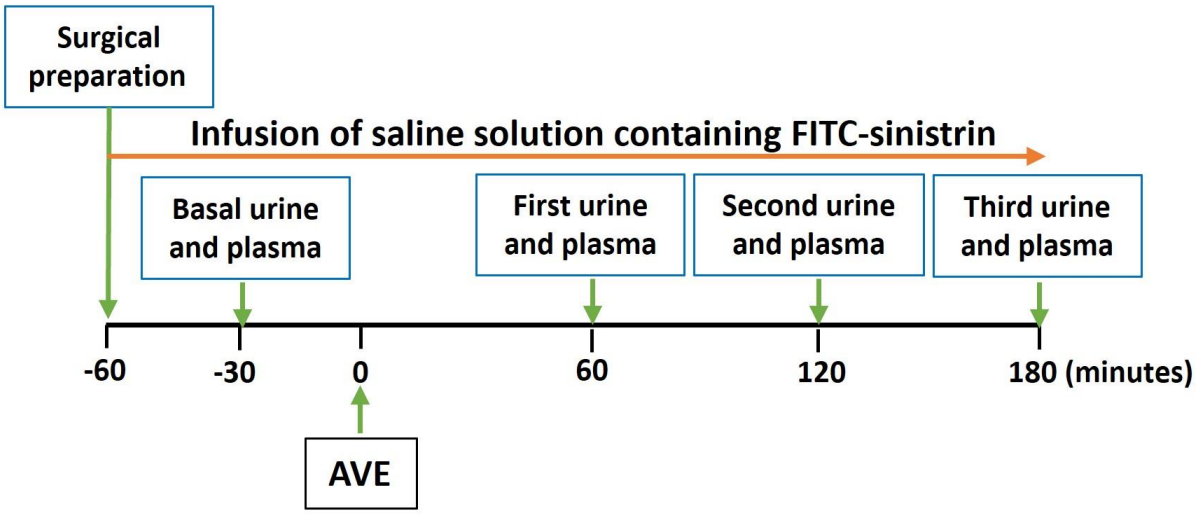
Fig. 7. GFR response to AVE for hypertensive mice.

For the group of mice underwent AVE in conscious conditions, GFR was enhanced by 29% and 24% for the NTN-C-IV and NTN-C-IP injected groups, respectively. However, the GFR only increased 12% in the mice under anesthesia condition. Two-way ANOVA followed by Sidak's multiple comparison test was performed. (**p<0.01, ***p<0.001, n=5)

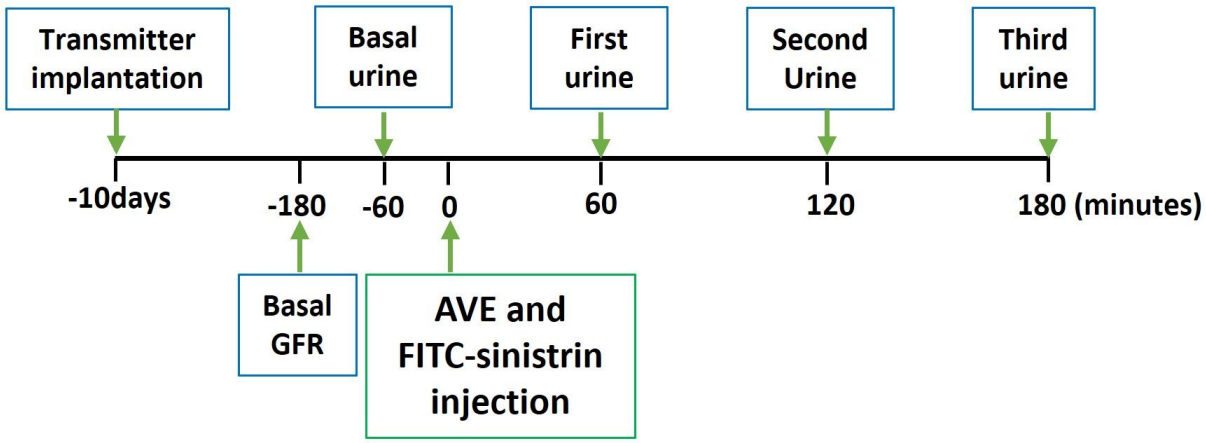
Fig. 8. Urine and sodium excretion in hypertensive mice in response to AVE.

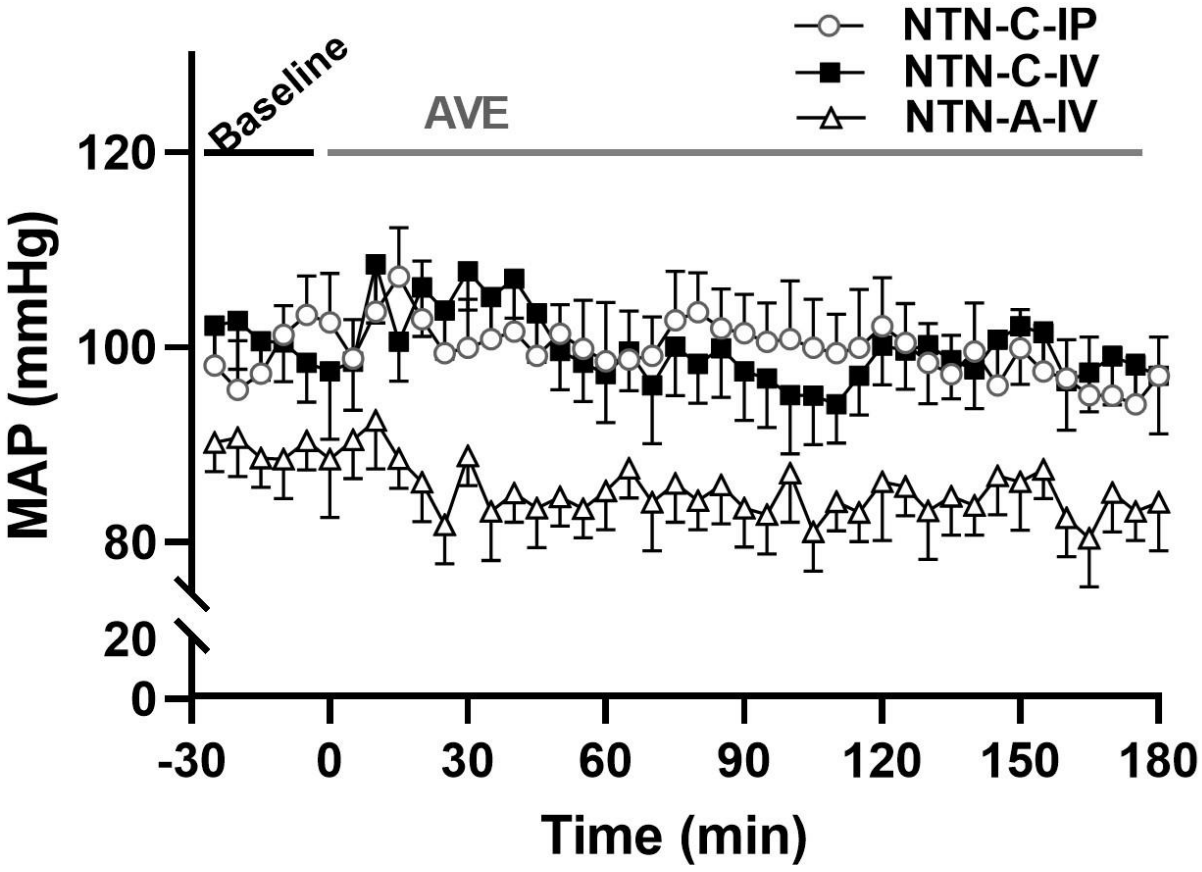
The conscious mice demonstrated a swifter response to the bolus saline stimulation, with the maximal effect sustained for a prolonged duration compared to the anesthetized mice. Both the urine and sodium excretion in anesthetized mice were suppressed compared with the conscious mice. Two-way ANOVA followed by Sidak's multiple comparison test was performed. (*p<0.05, **p<0.01, ***p<0.001, n=5)

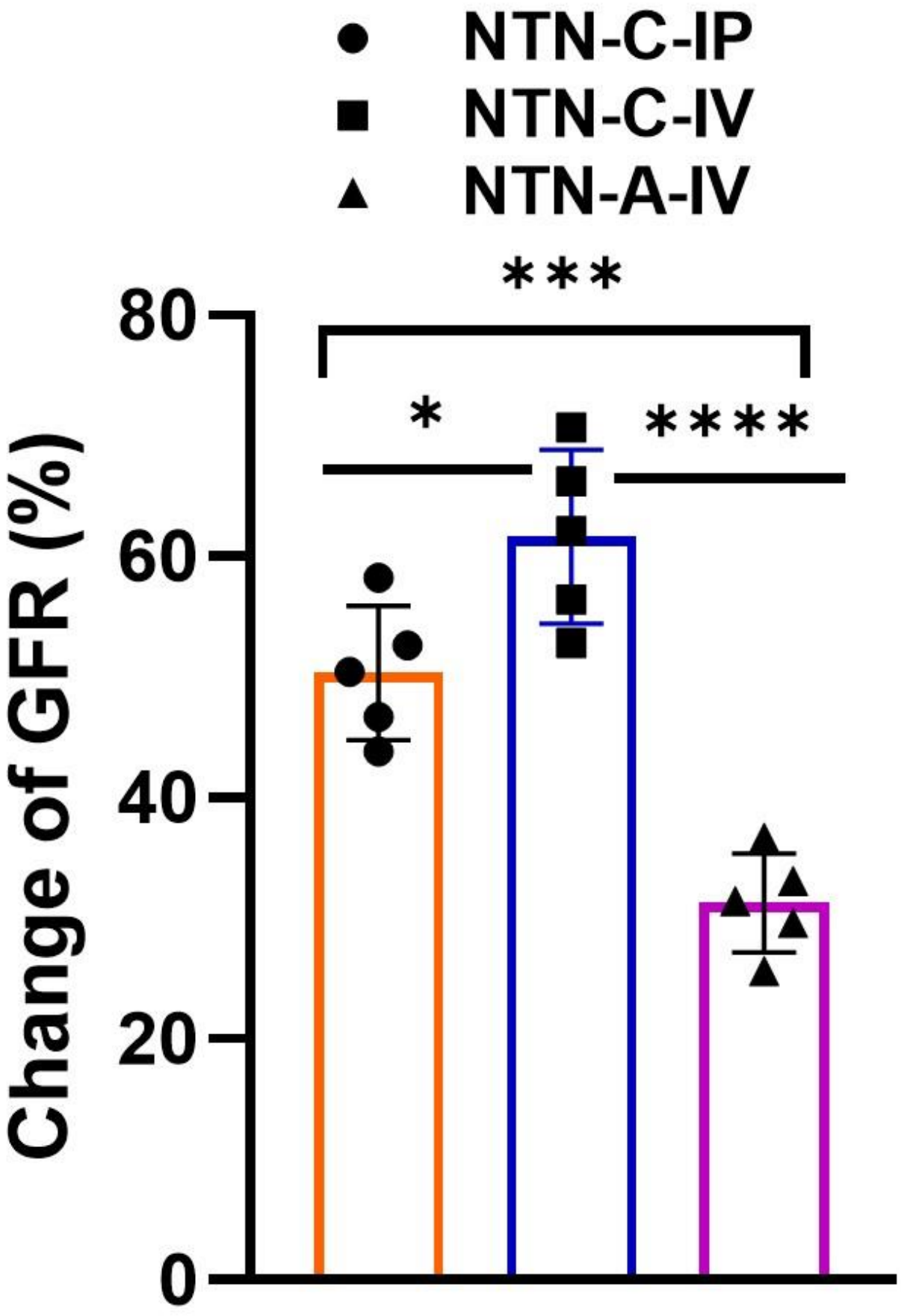
A. Anesthetized AVE

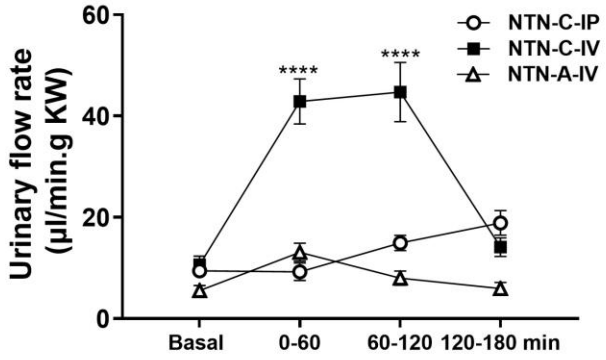


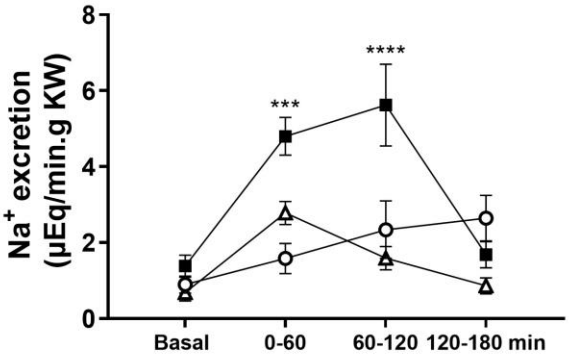
B. Conscious AVE

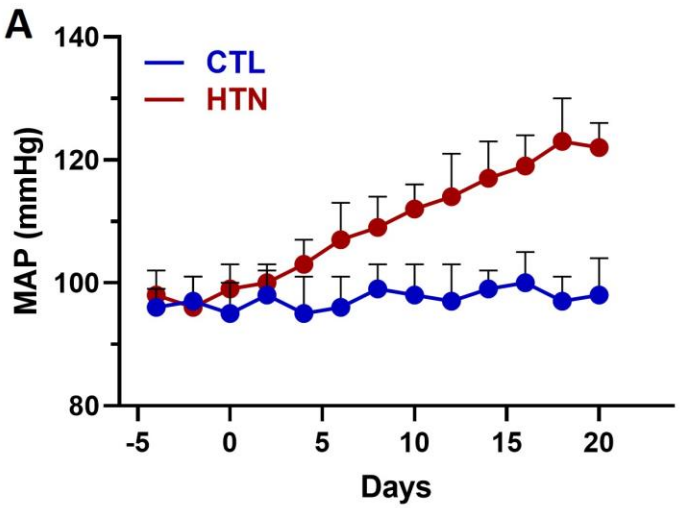


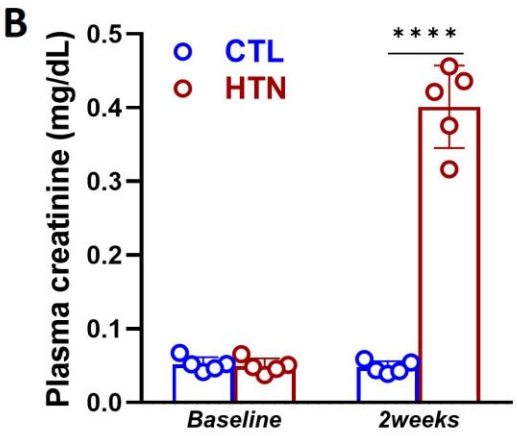


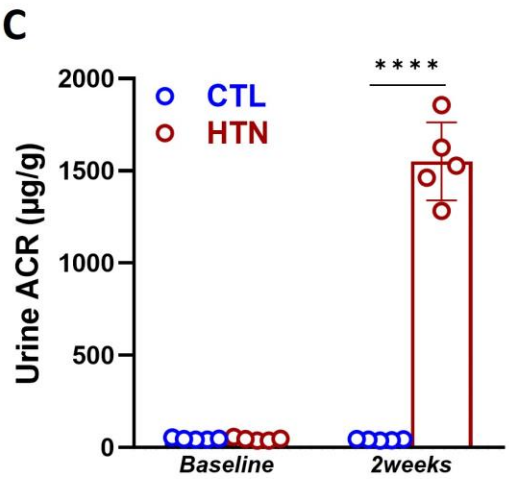


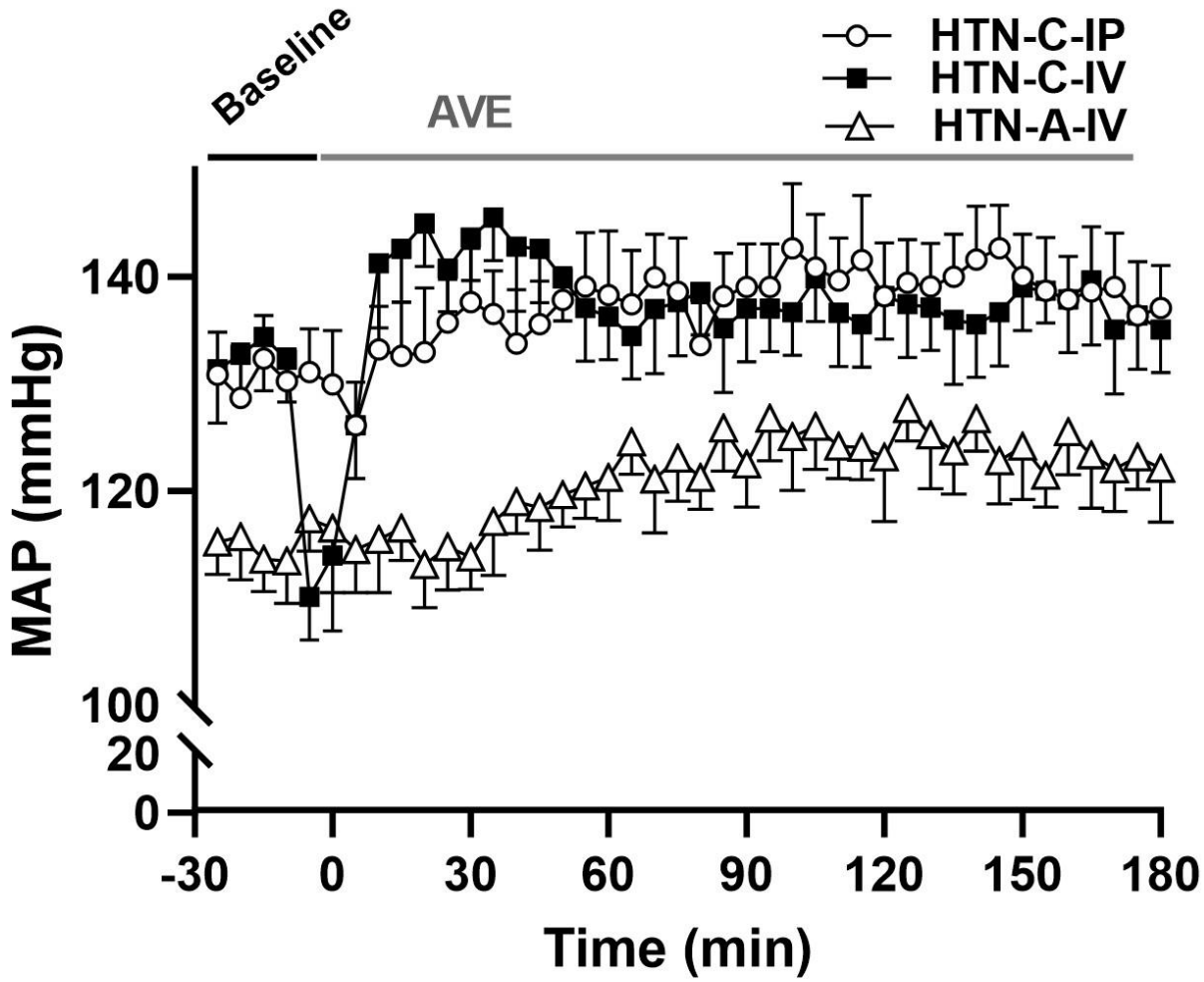


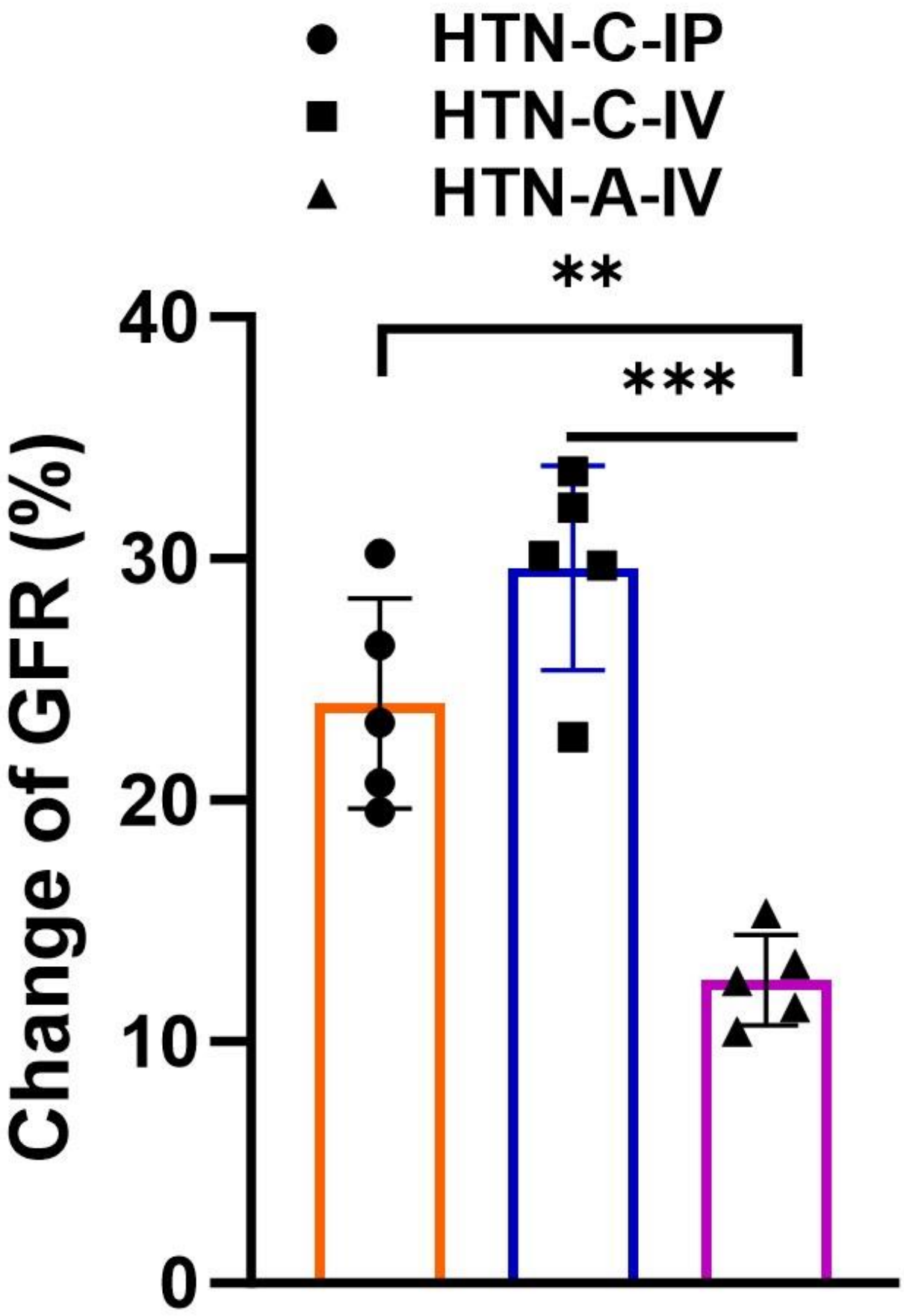


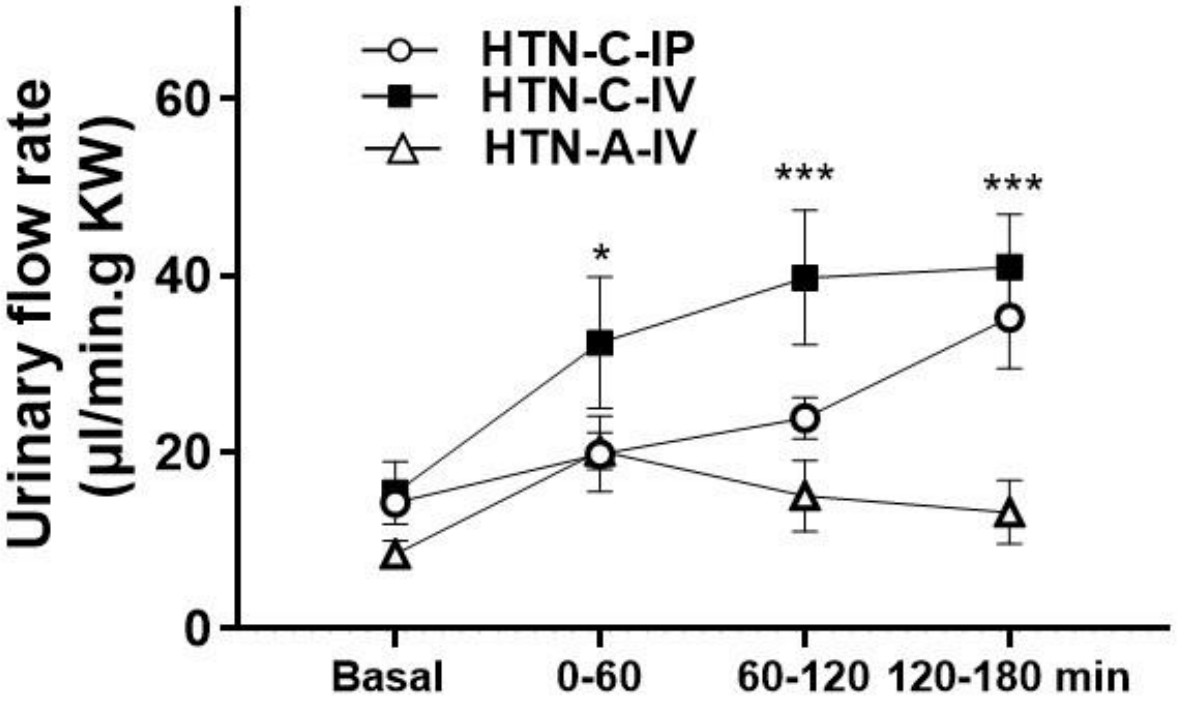












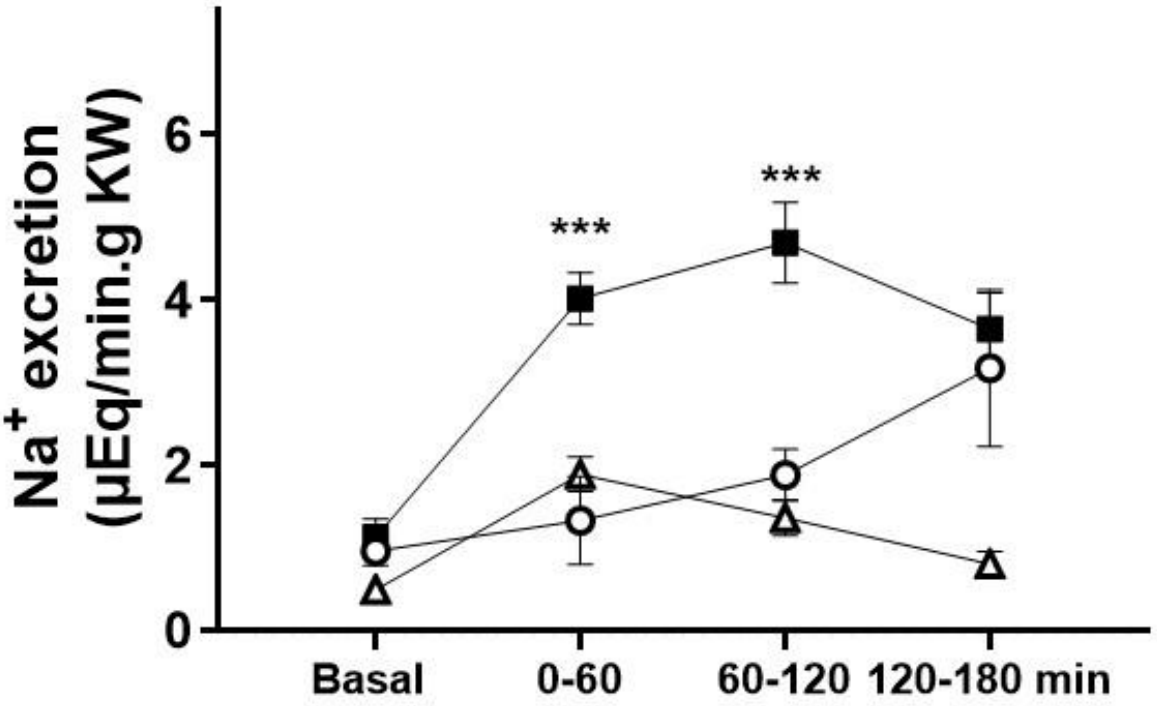


Table 1. Total urinary excretion during AVE.

			$V_{injected}$ (ml)	$V_{excretion}$ (ml)	Excretion fraction
Normotensive	conscious	IP	1.43±0.10	0.79±0.05	52%
	conscious	IV	1.44±0.12	1.61±0.04	112%
	anesthetized	IV	1.46±0.10	0.58±0.05	40%
Hypertensive	conscious	IP	1.37±0.11	0.60±0.07	44%
	conscious	IV	1.40±0.12	0.83±0.19	59%
	anesthetized	IV	1.39±0.13	0.39±0.12	28%