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Sexual Dimorphism of Glomerular Capillary Morphology in Rats

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by

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Abstract

Chronic kidney disease (CKD) progresses faster in males than females; however, the underlying mechanisms remain poorly understood. Sex differences in glomerular capillary morphology has been hypothesized to contribute, in part, to the increased susceptibility to hypertension-induced renal injury and CKD progression in males, but this has not been investigated. The goal of the present study was to assess glomerular capillary morphology in male vs. female rats with intact kidneys and after uninephrectomy (UNX). We hypothesized that glomerular capillary radii (R_{CAP}) and length (L_{CAP}) would be greater in male rats.

Male (n=4) and female (n=4) with intact kidneys and UNX (n=4 males, n=4 females) provided a 0.4% NaCl diet and water *ad libitum*. Kidneys were perfusion-fixed, the left kidney was excised, and a 3 mm transverse section through the midline of the kidney was selected for further processing. Multiple 1 mm^3 cubes were randomly excised from the left, middle, and right regions of the outer cortex, embedded in EPON^{TM}, sectioned (1 μm), and stained with toluidine blue. Four glomeruli from each region were randomly selected for stereological analysis. Glomerular tuft volume (V_G), R_{CAP}, and L_{CAP} were assessed.

In rats with intact kidneys, no significant sex differences were observed in V_G, R_{CAP}, or L_{CAP}. V_G, R_{CAP}, and L_{CAP} were significant greater in both male and female rats with UNX vs. respective rats with intact kidneys. In rats with UNX, males exhibited a significantly greater V_G and L_{CAP}, but not R_{CAP}, as compared to females despite no significant differences in relative kidney weight.

These data indicate that males exhibit greater compensatory increases in L_{CAP} following UNX. The greater capillary length may lead to reduced podocyte density, a well-known mechanism that increases the susceptibility to CKD progression.

Introduction

Statement of the Problem

Chronic kidney disease (CKD) affects about 15% of the general population, significantly increases the risk of morbidity and mortality, and is associated with over $100 billion in annual Medicare expenditures (1). The mainstay of treatment for CKD is to slow the progression to end-stage renal disease (ESRD), or renal failure. Regardless of etiology, CKD tends to progress to ESRD albeit at different rates depending on well-known risk factors. The major modifiable risk factors for CKD include diabetes and hypertension, and the major non-modifiable risk factors include age, genetics, race, and sex. While the prevalence of CKD is higher in females, males exhibit an increased rate of progression of CKD to ESRD. Previous research has led to important insights into the sex disparity in CKD progression; however, the underlying mechanisms remain ill defined. A better understanding of the mechanisms contributing to sex differences in CKD progression may provide key insights that could lead to improved treatments or guidelines to slow the progression of CKD in both males and females.

Hypertension, or high blood pressure, plays a major role in the progression of most forms of CKD (2-4). Decades of research has led to the well-accepted role of hypertension in contributing to the continued loss of nephrons via barotrauma-mediated glomerular capillary injury and loss. Moreover, recent clinical studies indicate that males exhibit a greater susceptibility to hypertensive-renal injury for any given level of blood pressure as compared to females (2). Such data indicates that elevated levels of systemic blood pressure are either more readily transmitted to the glomerular capillaries in males, or that glomerular capillaries of males are more susceptible to injury for any given level of glomerular capillary pressure. The main goal of this project was to assess whether sex differences exist in the size of glomerular capillaries to

explain the discrepancy in susceptibility to hypertension-induced glomerular capillary injury between males and females.

In general, the kidneys of males are larger than females (5). However, data is lacking with respect to whether such differences in kidney size are accompanied by differences in glomerular capillary size. There are two important features of glomerular capillaries that can theoretically alter the susceptibility to hypertensive injury. The first is the diameter of glomerular capillaries. For any given glomerular capillary pressure, the Law of Laplace states that wall tension will be higher when the diameter is greater. Thus, it is possible that males exhibit greater capillary diameters, which would lead to increased wall tension for any given level of blood pressure. The second is the length of glomerular capillaries. Glomerular capillaries are surrounded by podocytes, which are specialized epithelial cells that provide structural support against the relatively high capillary pressures. As podocytes are terminally differentiated, increases in either the diameter or length of glomerular capillaries requires that podocytes support a greater surface area of the capillary wall. Either longer capillaries or greater capillary diameters will reduce podocyte density, which is a well-accepted mechanism that increases the risk of CKD progression (2, 6).

Overview of Renal Functional Anatomy

The kidneys function in several capacities that are essential to life including the excretion of waste products from the blood, the maintenance of water and electrolyte balance, and the regulation of blood pressure (7). Each kidney consists of approximately 1 million nephrons, the functional unit of the kidney, consisting of a glomerulus and a tubule. The glomerulus contains numerous capillaries where blood is initially filtered, and urine formation begins. The renal

tubule varies in structure and function along the entire length of the nephron and is important for the reabsorption and secretion of water and various solutes (7). At the termination of the tubule, the composition of the urine is complete and proceeds to the renal pelvis, ureter, and bladder for excretion.

Remarkably, the approximate 2 million glomeruli of both kidneys produce about 180 liters of ultrafiltrate per day. The high filtration rate through the glomerular capillaries is largely due to relatively high glomerular capillary pressures (P_{GC}). The glomerular capillaries are unique in that they lie between two resistance vessels, the afferent and efferent arteriole, to alter blood flow (7). The afferent arteriole precedes the glomerular capillaries and is especially important in regulating glomerular capillary blood flow, P_{GC}, and glomerular filtration rate (GFR), or the volume of plasma filtered through the glomerulus per unit time (3, 4, 7, 8). As the glomerular capillaries exit the glomerulus, they adjoin with the efferent arteriole, which is under hormonal control to aid regulation of P_{GC} and GFR. While this unique organization of the glomerular capillary network ensures an adequate P_{GC} to maintain a normal GFR, it also increases glomerular capillaries susceptibility to hypertension-induced injury and the development or worsening of kidney disease if these regulatory mechanisms are impaired.

Chronic Kidney Disease Definition

Chronic kidney disease is defined as a decrease in kidney function due irreversible loss of nephrons over a period of at least three consecutive months (9). Loss of kidney function is denoted by a reduction in the GFR, which is typically estimated using formulas that incorporate an individual's serum creatinine (S_{Cr}) concentration, age, weight, and sex (9). Estimated GFR (eGFR) values are normalized to body surface area and expressed as ml/min/1.73m^2. CKD is

divided into 5 stages based of the level of eGFR (10). Stage 1 consists of a normal eGFR (≥ 90 ml/min/1.73m^2) with evidence of possible kidney damage based on other tests (8). Stage 2 CKD is evidence of kidney damage with mild reductions in eGFR (60-89 ml/min1.73m^2). Stage 3 CKD is mild to moderate reduction in the eGFR ranging from 30-59 ml/min/1.73m^2, and Stage 4 CKD is severe reduction in eGFR with a concentration between 15-29 ml/min/1.73m^2. Stage 5 CKD is kidney failure, or end-stage renal disease (ESRD), and is denoted by an eGFR of less than 15 ml/min/1.73m^2 (8). Patients with ESRD require dialysis or a kidney transplant to maintain life.

In addition to eGFR, proteinuria, or the amount of protein in the urine, is commonly used to assist in the diagnosis of CKD. Proteins are not normally found in urine because healthy glomerular capillaries prevent large solutes from being filtered (7). The glomerular filtration barrier, which consists of fenestrated endothelium, glomerular basement membrane, and specialized epithelial cells called podocytes exclude large negatively charged solutes, such as proteins, from being filtered. However, when P_{GC} is elevated or the glomerular filtration barrier is altered or damaged, protein is filtered into the tubular lumen and excreted via the urine. Therefore, proteinuria can be indicative of glomerular capillary hypertension and damage. Lastly, imaging tests or a renal biopsy is sometimes performed to confirm the presence of CKD; however, renal biopsies are often a last resort due to potential risks, like infections or post-op bleeding (11).

Causes of CKD

The two most common causes of CKD are diabetes and hypertension (1). Importantly, hypertension is thought to play a major role in the progression of CKD to ESRD, regardless of

etiology, including in the majority of patients with diabetes (2, 12-14). Roughly 85-95% of individuals with CKD have clinical hypertension as a comorbidity (12). Hypertension has increased in prevalence over the last four years affecting men more than women, older generations more than younger, and non-Hispanic Black individuals more than non-Hispanic white or Hispanic individuals (15). Other common causes of CKD include polycystic kidney disease and glomerulonephritis (1). For the overwhelming majority of the population, diabetes and hypertension are important modifiable risk factors that lead to CKD and its progression.

Prevalence of Chronic Kidney Disease in the United States

According to the Centers for Disease Control and Prevention, CKD affects 1 in 7 adults within the United States where a majority are over the age of 65 (1). CKD affects some groups more than others (e.g., non-Hispanic Blacks, Hispanics) and is slightly more common in females (1, 16). CKD is associated with several comorbidities including anemia, bone disease, hypertension, and cardiovascular disease. Regardless of the initial cause of CKD, it tends to worsen over time, albeit at different rates depending on sex, race, ethnicity, and the presence of diabetes and/or hypertension. CKD is the 9th leading cause of death in the US and associated with significant financial burden. In 2019, Medicare spent nearly $90 billion on patients with CKD, averaging about $25,000 per beneficiary over the age of 65 (1). Additionally, Medicare distributed nearly $40 billion for ESRD related incidences. High-income families may handle sustaining repetitive treatment well; however, low-income households will likely struggle to maintain financial stability.

Treatment of CKD

Once CKD is diagnosed, the main goal is to slow progression to ESRD. When nephrons are permanently damaged, the remaining uninjured nephrons undergo hypertrophy. Hypertrophy increases the functional units' productivity; however, it increases susceptibility to stress and damage, especially hypertension-induced injury (2). Unfortunately, any sustained damage from CKD is currently irreversible. The mainstay of CKD treatment involves antihypertensive therapy and the reduction of blood glucose levels if necessary (17). Similarly, many patients are instructed to maintain a low-sodium and low-protein diet to prevent acute hypertension. If the patient's condition develops into ESRD, dialysis or renal transplant are two prominent options for treatment (1).

Sex Differences in the Prevalence and Progression of CKD

Recent studies have demonstrated that the incidence of CKD is higher in females, but males exhibit a greater rate of CKD progression to ESRD (16, 18, 19). Interestingly, females are less likely to experience a decline in GFR, less likely to receive dialysis or kidney transplant, and are at a much lower risk of renal-related death (16, 19). While the prevalence of hypertension, cardiovascular disease, and diabetes are higher in males than premenopausal females, previous studies have demonstrated that the sex disparity in the rate of CKD progression is independent of systolic blood pressure levels (20-22). That is, for any given level of systemic blood pressure, males exhibit a faster rate of CKD progression as compared to females. The slower rate of CKD progression in premenopausal women has been attributed to the presence of estrogen, but the underlying mechanisms remain poorly understood (16, 23).

Pathogenesis of Hypertension-induced Renal Injury

The pattern and extent of hypertension-induced renal injury is a direct reflection of the degree to which the renal vasculature is exposed to elevated blood pressure (BP), either episodic or sustained (2). Moreover, the pathogenesis of hypertension-induced renal injury is caused by barotrauma injury to the renal microvasculature. It is important to emphasize that the spectrum of hypertension-induced CKD varies greatly in different patient populations. One of the most striking aspects of the hypertensive population in the United States is that the overwhelming majority of patients with essential hypertension will develop very modest levels of renal injury, referred to as benign nephrosclerosis (2). Injury is primarily observed at the level of the preglomerular arterioles, which are exposed to elevated BP, but the glomerular capillaries are largely spared. In contrast, injury is primarily observed at the level of the glomerular capillaries as opposed to the preglomerular vasculature in patients with CKD. Moreover, it is well-established that patients with CKD exhibit an increased susceptibility to hypertensive renal injury at any given level of systemic BP (2, 3). Importantly, elevated levels of systemic arterial BP, albeit modest, are required for the progression of CKD in experimental models (24). Thus, hypertension-induced glomerular capillary injury, even in the presence of modest elevations in BP, is thought to be a major driver of the continued loss of nephrons and progression to ESRD in patients with CKD (22).

There are three main ways hypertension can propagate continuous degradation of the renal system (23). The first is the level of systematic blood pressure. Simply put, the higher the systemic arterial blood pressure, the greater risk of glomerular capillary injury and CKD. The second is the degree to which systemic BP is transmitted to the glomerular capillary bed. The preglomerular vasculature exhibits autoregulation, which maintains a relatively constant RBF

and P_{GC} via proportionate vascular constriction or dilation in response to increases or decreases in systemic BP, respectively (23). There are two well-known mechanisms that contribute to renal autoregulation: myogenic response and tubuloglomerular feedback (TGF) (2, 4). The myogenic response is the more effective and faster of the two and thus is the most critical for protecting glomerular capillaries against hypertensive injury. The renal myogenic response is triggered by the myocytes of the arterioles contracting and dilating in response to increases or decreases in BP to maintain a constant rate of RBF. TGF is mediated by signals originating from the distal tubule that cause preglomerular arteriole constriction or relaxation via changes in sodium and water delivery to the distal portion of the nephron. While TGF may play a critical role in maintaining GFR when BP falls to dangerously low levels, it is not thought to have a major role in protecting the glomerular capillaries against hypertension-induced injury because of its much slower response time as compared to the myogenic response (4). Ultimately, if renal myogenic autoregulatory ability is impaired, even modest increases in systemic BP are transmitted to the glomerular capillaries, which leads to barotrauma-induced capillary injury (2-4). To date, there are a paucity of studies that have evaluated sex differences in renal autoregulatory capacity. This subject is beyond the scope of the present studies, which are instead focused on sex differences in the structural factors of glomerular capillaries that have the potential to alter the susceptibility to hypertensive renal injury. The third major modulator of susceptibility to hypertensive glomerular capillary injury is glomerular capillary morphology. For example, variations in glomerular capillary diameter and length can alter the susceptibility to capillary injury for any given level of P_{GC}. Capillaries with greater diameters will experience a higher wall tension for any given level of renal BP transmission (2), which increases the risk of barotrauma-mediated injury. Increases in either capillary diameter or length can also increase the

susceptibility to barotrauma-mediated injury by decreasing podocyte density (2). Podocytes are specialized epithelial cells that surround glomerular capillaries and provide structural support against the relatively high capillary pressures (2, 25-27). In addition, podocytes are terminally differentiated and cannot be replaced via proliferation (6, 28). During compensatory hypertrophy and the associated increase in glomerular capillary diameter or length, individual podocytes are obligated to support a greater area of the capillary wall. This increase in mechanical stress experienced by individual podocytes in settings of reduced podocyte density increases the risk of barotrauma-mediated injury and additional loss of nephrons, which creates a viscous cycle leading to progression of CKD to ESRD (6, 28). .

Previous studies have demonstrated that, on average, d males have larger kidneys and glomeruli than females. However, whether such sex differences in kidney and glomerular size are observed at the level of the glomerular capillaries has not been investigated. It is possible that, on average, glomerular capillary diameter or length are greater in males, which could contribute to the observed sex difference in the rate of CKD progression (19).

Hypothesis/Aims

We tested the hypothesis that male would exhibit larger glomerular capillary radii and length as compared to females in rats with intact kidneys and in rats with compensatory hypertrophy following a 50% reduction in renal mass via uninephrectomy (UNX).

Methods

Rationale for using Rats

Sprague-Dawley (SD) rats were used in these studies because they exhibit a similar pathogenesis of CKD progression as humans. Upon renal mass reduction and the loss of nephrons, the remaining kidney and its nephrons undergo hypertrophy in both rats and humans (24, 29). This is a compensatory response to account for the decrease in nephrons. Importantly, rats with CKD exhibit impaired RBF autoregulation, reduced podocyte density, and an increased susceptibility to hypertensive renal injury (2, 3, 28, 31); thus, providing a clinically relevant model for CKD progression to ESRD. Thus, rats are an excellent model for determining whether there is a sexual dimorphism in glomerular capillary morphology.

Experimental Design

Male (n=8) and female (n=8) SD rats were obtained from Charles River Laboratory (Raleigh, North Carolina) at 7 and 10 weeks of age, respectively, to allow for body mass matching. All rats were provided a standard sodium and protein diet and water *ad libitum* throughout the duration of the protocol.

After one week acclimatization to the animal facility, male (n=4) and female (n=4) rats were euthanized and their kidneys perfusion-fixed with ringer's lactate followed by 2% paraformaldehyde and 2.5% glutaraldehyde in phosphate-buffered saline (PBS) with a pH of 7.4 and temperature of 37°C. At the same time, a separate group of male (n=4) and female (n=4) underwent a right uninephrectomy (UNX) (29). Briefly, a midline incision was made, the right kidney was isolated, and the renal artery and vein were ligated with 3-0 silk suture. The kidney was then removed, and the abdominal incision was closed with 4-0 Vicryl suture. Bupivacaine

was applied to the incision site and all rats were administered Tylenol via drinking water (~200 mg/kg/day) for three days following surgery. Rats with UNX were followed for six additional weeks to allow for compensatory increases in renal size and function. At the end of the protocol, the left kidney of rats with UNX was perfusion-fixed in a similar manner to rats with intact kidneys. In rats with intact kidneys and UX, the left kidney was transversely divided into 3 mm sections and kept in the perfusate solution overnight at 4°C.

Tissue Sectioning and Selection of Glomeruli

The 3 mm midline section was cut into 72 cubes of 1 mm^3 selected in a randomized fashion from the left, center, and right regions of the superficial cortex. The 1 mm^3 cubes were then embedded in EPON^{TM}, cut into 1 μm sections and placed on slides, and stained with toluidine blue. Four glomeruli from each section were selected for stereological analysis using uniform random sampling (32). An image of each glomerulus was obtained using a 40x objective lens using an Olympus BX41 microscope equipped with a motorized stage and camera (DP74, Olympus). The selected glomeruli included the hilar area or vascular pole as they were felt to represent a complete cross section close to the center of the glomerulus.

Stereological Analysis

Morphometric analysis of all glomeruli were assessed in a blinded fashion via point-counting using STEPanizer, a freely-available online stereology software program (33). Validated morphometric equations (24, 32, 34, 35) were used to assess the cross-sectional area (CSA) and volume of both the glomerular capillary lumina and the glomerular tuft, the number of capillaries per glomerular section, as well as the total length and average radius of all

glomerular capillaries. First, the surface area of the capillary lumen and tuft was determined by dividing the number of points overlaying the respective structures by the total number of counting tiles and then multiplying that quotient by the total counting area. Individually calculating the capillary lumen and tuft volumes both utilized the equation $V_G = \beta/K\ (SA_G^{3/2})$ where β equals 1.38, the size distribution coefficient, K equals 1.1, the shape coefficient for glomeruli idealized as spheres, and SA_G equals the surface area of glomerulus (35).

To determine average capillary length, glomerular capillary length density (μm/μm^3) was first calculated by multiplying the number of capillary profiles per section by two and dividing by the surface area of the glomerular tuft (24, 32-34). The, average glomerular capillary length (μm) was calculated from the product of the length density of glomerular capillaries and glomerular tuft volume (34). Next, the CSA of glomerular capillaries was calculated by dividing glomerular tuft volume by average capillary length. Lastly, the average capillary radius was estimated by taking the square root of glomerular capillary CSA divided by Π (34).

Statistical Analysis

Effects of sex and UNX on body weight, kidney weight, glomerular tuft volume (V_G), capillary radius ($R_{CAP)}$, and capillary length ($L_{CAP)}$ were assessed with a 2-way ANOVA with Sidak post hoc analysis or unpaired T-test depending on the number of independent variables. All data are mean±SEM where $P<0.05$ was considered statistically significant.

Results

I. Rats with Intact Kidneys

Body Weight and Kidney Weight

Our first goal was to assess whether sex differences in glomerular capillary morphology were evident in rats of a similar size. We therefore specified all rats to arrive at ETSU with a body weight of 200-225 grams. Males (n=4) were 7-wks-old and females (n=4) were 10-wks-old upon arrival at ETSU's animal facility. Rats were provided one week to acclimate to the ETSU animal facility prior to kidneys being perfusion-fixed and harvested. Of note, one male rat was excluded from analysis because of poor perfusion-fixation of the kidney, which is critical when assessing glomerular capillary morphological parameters via stereology (29).

Despite male and female rats having a similar body weight upon arrival at ETSU, body weight was 1.25-fold greater (**Fig. 1A**) in males one week later when kidneys were harvested. Similarly, the absolute weight of the left kidney was 1.30-fold greater (**Fig. 1B**) in males. Because kidney weight is largely dependent on body weight (36), we also assessed normalized kidney weight (grams of kidney weight / kilograms of body weight). As expected, no significant difference in normalized kidney weight was observed (**Fig. 1C**) between sexes (5). In summary, although male and female rats arrived at ETSU with similar body weights, males exhibited significantly greater body and absolute kidney weights one week later, which is likely a result of the faster rate of growth in male rats at this age range.

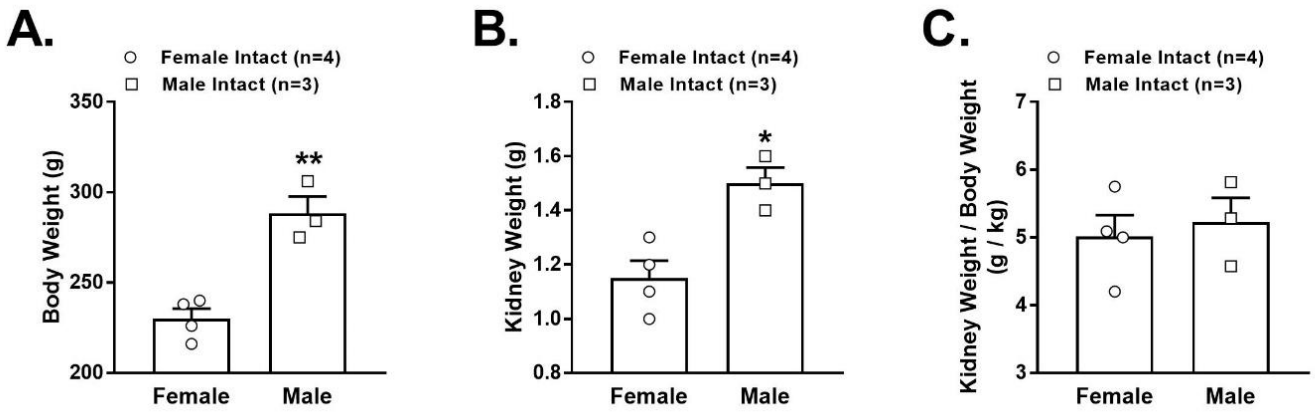

Figure 1: Body and kidney weight in rats with intact kidneys.

In rats with intact kidneys, males (n=3) exhibited a greater A) body weight and B) absolute kidney weight as compared to females (n=4). In contrast, no significant differences were observed in normalized kidney weight (grams kidney weight / kilograms body weight) between sexes. Values are expressed as mean ± SEM. Unpaired t-tests were used to compare male and female rats. *p<0.05, **p<0.01, ***p<0.001.

Glomerular Tuft Volume, Number of Glomerular Capillaries, and Fractional Capillary Volume

Next, we assessed whether sex differences in the glomerular tuft volume, number of glomerular capillaries, and fractional capillary volume were evident in rats with intact kidneys. The number of glomerular capillaries represents the number of capillary lumen profiles in a single 1 µm section. Fractional capillary volume is a measurement of the volume of the glomerulus occupied by capillary lumina. There was no significant sex difference in glomerular tuft volume, (**Fig. 2A**) number of capillaries (**Fig. 2B**), or fractional capillary volume (**Fig. 2C**). In summary, although kidney mass was modestly, but significantly, greater in males, there were no significant sex differences in glomerular tuft volume or capillary numeracy. This suggests that sex differences in kidney mass is due to differences in regions of the kidney other than the glomerulus, such as renal tubules or interstitium (29, 37).

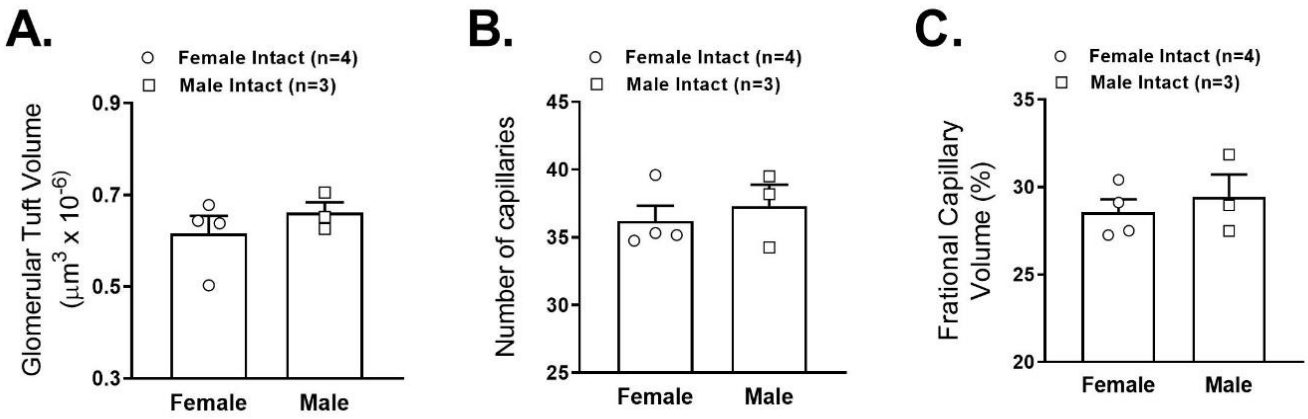

Figure 2: Glomerular tuft volume, number of capillaries, and fractional capillary volume.

In rats with intact kidneys, there was no significant difference in A) glomerular tuft volume, B) number of capillaries, and C) fractional capillary volume in males (n=3) as compared to females (n=4). Values are expressed as mean ± SEM. Unpaired t-tests were used to compare male and female rats.

Glomerular Capillary Radius and Length

Finally, we assessed if sex differences in glomerular capillary radius and length were evident in rats with intact kidneys. Although there was a tendency of greater capillary length in males (P = 0.1), no significant sex difference was observed with regards to either glomerular capillary radius (**Fig. 3A**) or length (**Fig. 3B**). These results are consistent with the data shown in Fig. 2 and suggests the greater kidney mass in male rats is likely due to a greater volume of extraglomerular structures.

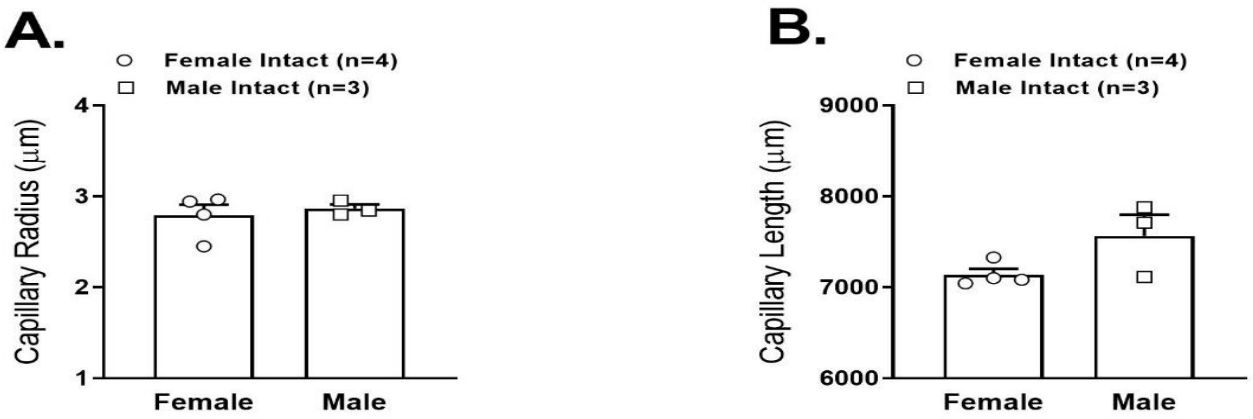

Figure 3: Glomerular capillary radius and length.

In rats with intact kidneys, there were no significant differences in A) glomerular capillary radius and B) glomerular capillary length in males (n=3) as compared to females (n=4). Values are expressed as mean ± SEM. Unpaired t-tests were used to compare male and female rats.

II. Rats with Uninephrectomy (UNX)

Body Weight and Kidney Weight

Our second goal was to assess whether differences in glomerular capillary morphology were apparent in male (n=4) and female (n=4) rats at six weeks following a uninephrectomy with subsequent compensatory hypertrophy of the remaining kidney.

Body weight was 2.1-fold significantly greater in males as compared to females (**Fig. 4A**). Likewise, kidney weight was 2.3-fold significantly greater in males as compared to females (**Fig. 4B**). In contrast, no significant sex difference was found in normalized kidney weight (**Fig. 4C**), which was expected given the strong association between kidney and body weight. In summary, although absolute body and kidney weights were greater in male rats at six weeks following UNX, the extent of renal compensatory hypertrophy was similar when normalized to body weight.

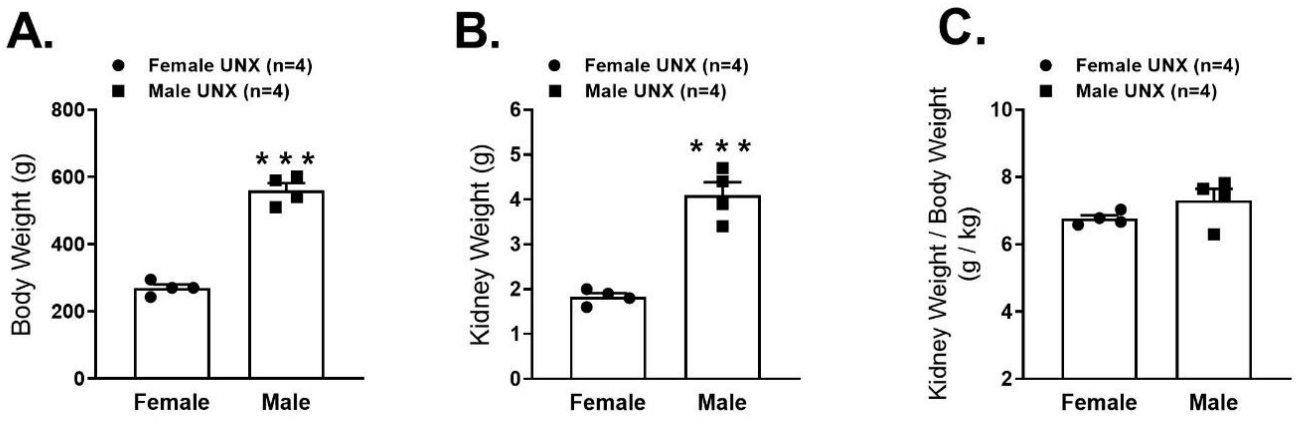

Figure 4: Body and kidney weight in UNX rats.

In UNX rats, males (n=4) exhibited a greater A) body weight and B) absolute kidney weight as compared to females (n=4). In contrast, no significant differences were observed in C) normalized kidney weight between sexes. Values are expressed as mean ± SEM. Unpaired t-tests were used to compare male and female rats. *p<0.05, **p<0.01, ***p<0.001.

In rats subjected to UNX, body weight was assessed at the time of UNX and six weeks later in the same rats. In addition, the weight of the excised right kidney was recorded at the time of surgery as was the weight of the remaining left kidney six weeks later from the same rat. As such, we compared the ratio of post-UNX to pre-UNX body weight, kidney weight, and normalized kidney weight in male and female rats to confirm that absolute increases in kidney weight are proportional to increases in body weight in both sexes. Importantly, body weight at the time of UNX was 268±9 g in males and 225±4 g in females, which is similar to the respective body weights of male and female rats with intact kidneys and of a similar age (see **Fig. 1A**). Thus, the significantly greater final body weights of rats subjected to UNX (**Fig. 4A**) vs. those with intact kidneys (**Fig. 1A**) was not a result of growth associated factors as opposed to greater initial body weights. The post-UNX to pre-UNX body weight ratio was 1.8-fold significantly greater in males vs. females (**Fig. 5A**). Similarly, the post-UNX to pre-UNX kidney

weight ratio was 1.7-fold significantly greater in males vs. females (**Fig. 5B**). Lastly, no significant sex difference was found after normalizing the post-UNX to pre-UNX kidney weight ratio to the body weight ratio (**Fig. 5C**). These results support that the greater increase in kidney weight after UNX in male rats is most likely due to their greater body weights as compared to female rats.

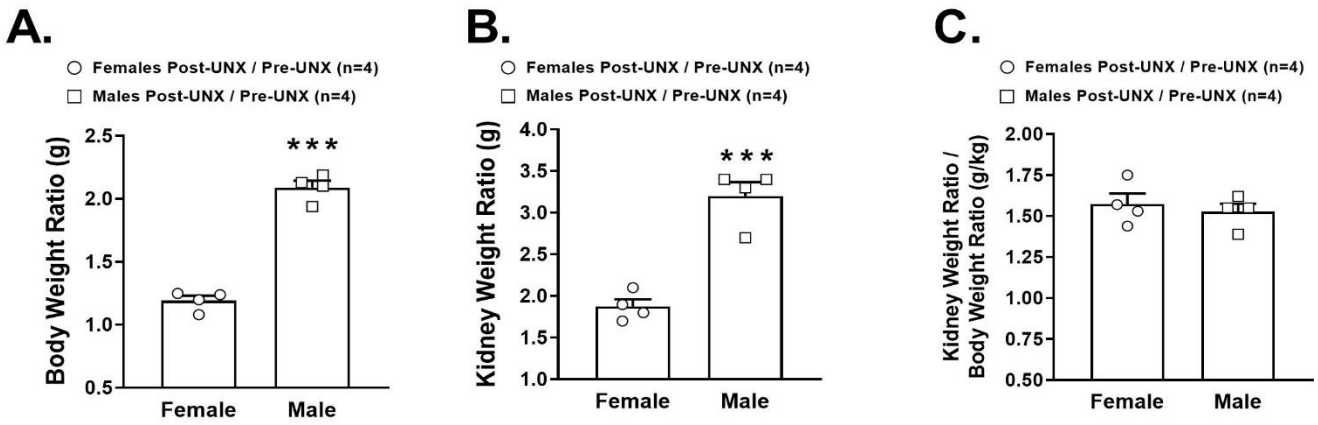

Figure 5: ratio comparison of body weight and kidney weight from pre-UNX to six weeks post-UNX.

Males exhibited a greater A) body weight ratio and B) kidney weight ratio. However, no significant difference was found in C) normalized kidney weight ratio between sexes. Values are expressed as mean ± SEM. Unpaired t-tests were used to compare male and female rats. *p<0.05, **p<0.01, ***p<0.001.

Glomerular Tuft Volume, Number of Glomerular Capillaries, and Fractional Capillary Volume

To determine whether the compensatory increases in glomerular tuft and capillary morphology differ between sexes, we assessed glomerular tuft volume, number of glomerular capillaries, and fractional capillary volume in the remnant kidney six weeks following UNX. Glomerular tuft volume was 1.3-fold significantly greater in males **(Fig. 6A**). No significant sex difference in number of capillaries was observed (**Fig. 6B**); however, females exhibited a 1.12-fold significantly greater fractional capillary volume as compared to males (**Fig. 6C**). While the

reasons for the difference in fractional capillary volume remain to be determined, these results indicate significant sex differences in the compensatory increase in glomerular capillary volume vs. extra-capillary volume (e.g., mesangial cells, extracellular matrix) following UNX.

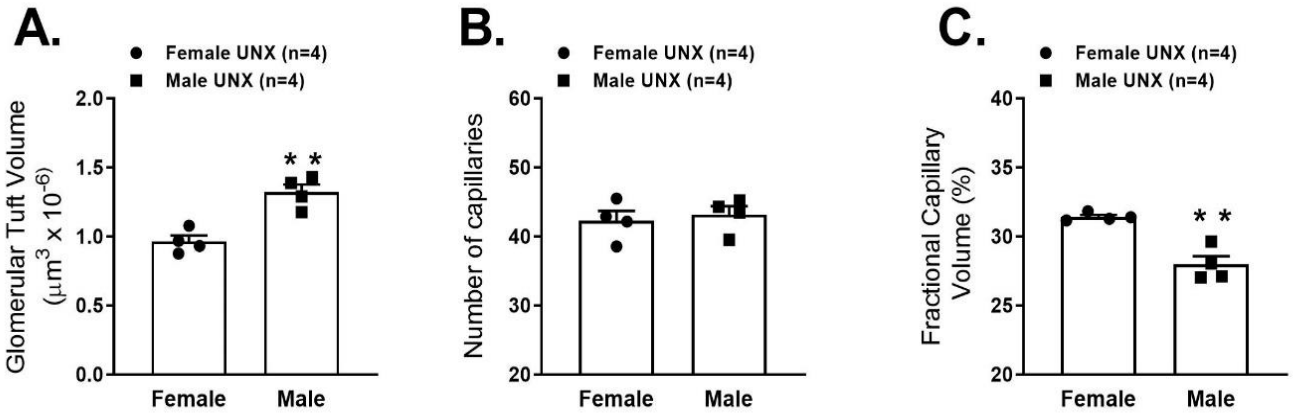

Figure 6: Glomerular tuft volume, number of capillaries, and fractional capillary volume.

In UNX rats, a significant sex difference was found in A) glomerular tuft volume, but not in the B) number of capillaries. C) fractional capillary volume was significantly greater in females (n=4) vs. males (n=4). Values are expressed as mean ± SEM. Unpaired t-tests were used to compare male and female rats. *p<0.05, **p<0.01, ***p<0.001.

Glomerular Capillary Radius and Length

Next, we assessed the extent of sex differences in glomerular capillary radius and length in rats following UNX. No significant difference in capillary radius was found between males and females (**Fig. 7A**). However, males had a 1.13-fold significantly greater capillary length than their female counterparts (**Fig. 7B**). As our capillary length measurement includes the total length of all capillaries in the glomerular tuft, a greater total length of capillaries can be due to either lengthening of existing capillaries or sprouting and development of new capillaries. When viewed in context with the data in Fig. 6B and Fig. 6C, the data in Fig. 7 suggests that there may be sex differences in the mechanism of compensatory responses of glomerular capillaries

following UNX. That is, the compensatory increase in capillary length in male rats may mainly be lengthening of existing capillaries, whereas female rats may exhibit more capillary sprouting resulting in the growth of new capillaries. Future experiments will be required to determine if there are indeed sex differences in the mechanism of compensatory glomerular capillary growth after UNX.

Figure 7: Capillary radius and length within the glomerulus.

In UNX rats, males (n=4) exhibited no significant difference in A) glomerular capillary radius as compared to females (n=4). However, B) glomerular capillary length was significantly greater in male vs. female rats. Values are expressed as mean ± SEM. Unpaired t-tests were used to compare male and female rats. *p<0.05, **p<0.01, ***p<0.001.

III. Intact vs. UNX Comparison

Fig.'s 8-10 show the combined data of intact and UNX groups. It is important to keep in mind that rats subjected to UNX were six weeks older than their intact counterparts. Nevertheless, summarizing all groups in the same graph is a useful exercise and provides a comprehensive summary of the data.

Body Weight and Kidney Weight

The body weight of rats subjected to UNX vs. rats with intact kidneys was 1.9-fold significantly greater in males; however, there was no significant difference in female rats with UNX vs. intact kidneys (**Fig. 8A**). Absolute kidney weight (**Fig. 8B**) was 2.7-fold significantly greater in male rats with UNX vs. those with intact kidneys. In female rats, absolute kidney weight was 1.6-fold significantly greater in rats with UNX vs. those with intact kidneys. Lastly, normalized kidney weight was 1.4-fold significantly greater in rats with UNX vs. those with intact kidneys in both sexes (**Fig. 8C**).

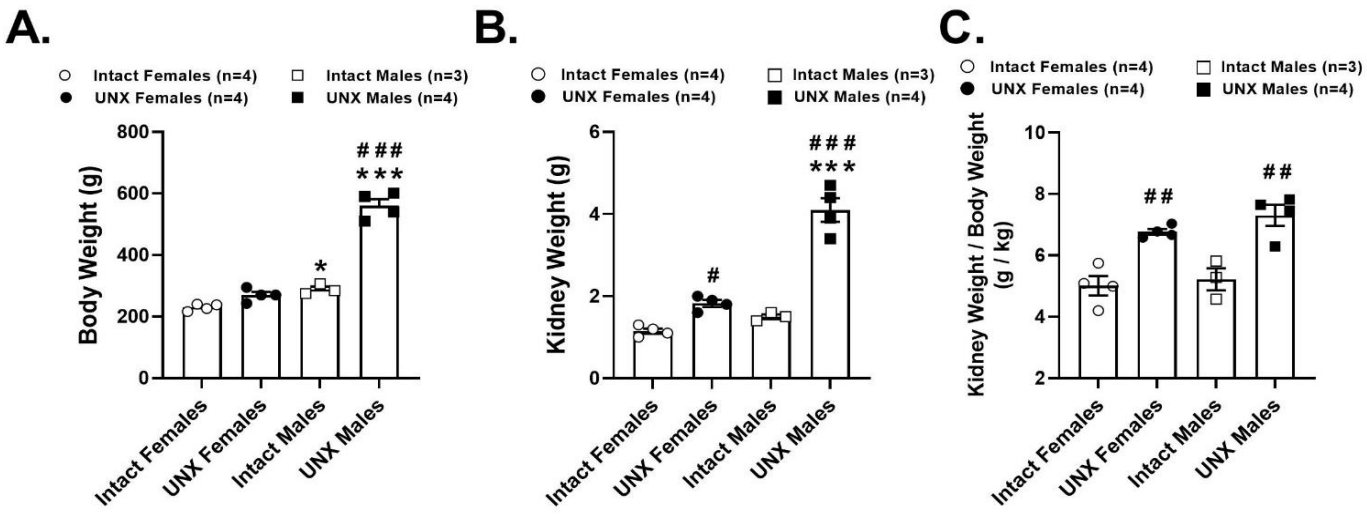

Figure 8: Body and kidney weight of intact and UNX groups of female and male rats.

A) body weight, B) kidney weight, and C) normalized kidney weight in male (n=3-4/group) and female (n=4 group) rats with intact or UNX kidney treatments. Asterisk represents significance between sex within the same kidney group. Pound represents significance between kidney groups within the same sex. All were analyzed via 2-way ANOVA with Sidak post-hoc analysis. Values are expressed as mean ± SEM. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$. *p<0.05, **p<0.01, ***p<0.001.

Glomerular Tuft Volume, Number of Glomerular Capillaries, and Fractional Capillary Volume

Glomerular tuft volume was 2-fold significantly greater in male rats following UNX vs. those with intact kidneys (**Fig. 9A**). Likewise, glomerular tuft volume was 1.6-fold significantly

greater in female rats following UNX vs. those with intact kidneys **(Fig. 9A**). The number of capillaries was 1.2-fold significantly greater in both male and female rats with UNX vs. respective rats with intact kidneys (**Fig. 9B**). Lastly, while no significant difference in fractional capillary volume was found between male rats following UNX vs. those with intact kidneys, female rats after UNX treatment exhibited a 1.1-fold significantly greater fractional capillary volume as compared to female rats with intact kidneys (**Fig. 9C**).

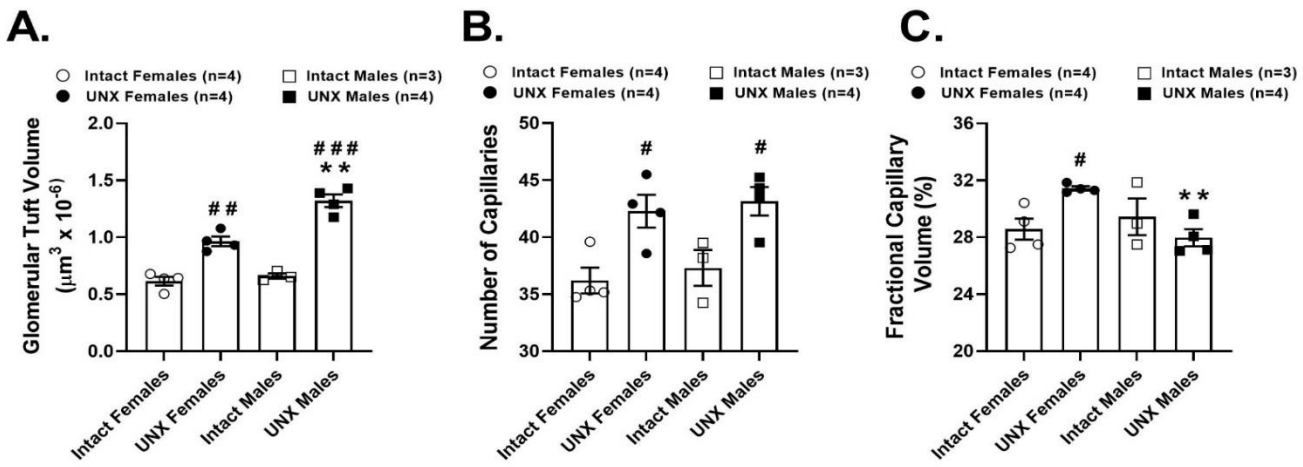

Figure 9: Glomerular tuft volume, number of capillaries, and fractional capillary volume of intact and UNX groups of female and male rats.

A) glomerular tuft volume, B) number of capillaries, and C) fractional capillary volume in male (n=3-4/group) and female (n=4 group) rats with intact or UNX kidney treatment. Asterisk represents significance between sex within the same kidney group. Pound represents significance between kidney groups within the same sex. All were analyzed via 2-way ANOVA with Sidak post-hoc analysis. Values are expressed as mean ± SEM. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$. *p<0.05, **p<0.01, ***p<0.001.

Glomerular Capillary Radius and Length

Glomerular capillary radius was 1.2- to 1.3-fold significantly greater in male and female rats following UNX vs. their counterparts with intact kidneys (**Fig. 10A**). The difference in glomerular capillary length between rats following UNX and rats with intact kidneys was highly significant for both sexes with male and female rats exhibiting a 1.5-fold and 1.4-fold,

respectively, greater length following UNX as compared to their respective counterparts with intact kidneys (**Fig. 10B**).

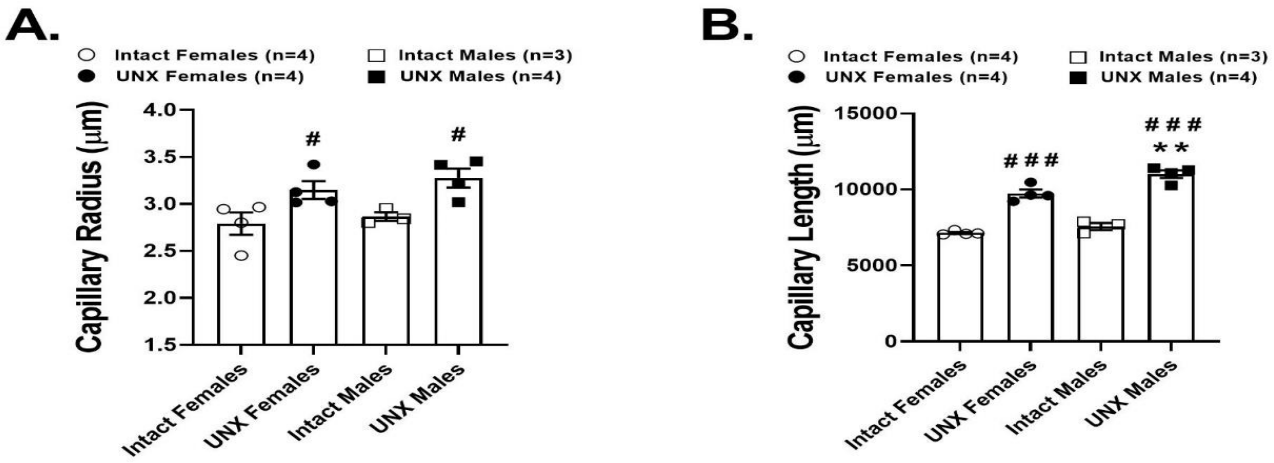

Figure 10: Glomerular capillary radius and length of intact and UNX groups of female and male rats.

A) glomerular capillary radius and B) glomerular capillary length in male (n=3-4/group) and female (n=4 group) rats with intact or UNX kidney treatments. Asterisk represents significance between sex within the same kidney group. Pound represents significance between kidney groups within the same sex. Both were analyzed via 2-way ANOVA with Sidak post-hoc analysis. Values are expressed as mean ± SEM. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$. *p<0.05, **p<0.01, ***p<0.001.

Discussion

CKD affects males and females similarly by inducing hypertrophy of the remnant, uninjured nephrons. However, there remains a lack of understanding as to why progression from CKD to ESRD is significantly more rapid in males. The goal of the present study was to assess whether sex differences exist in capillary radius or length, which may contribute, in part, to the greater progression of CKD in males. While both male and female rats with UNX exhibited significant greater glomerular capillary length and radius as compared to respective rats with intact kidneys, male rats with UNX exhibited a significantly greater capillary length as compared to females. These data indicate that sex differences in the compensatory increase in glomerular capillary length following the loss of nephrons may contribute, in part, to the observed sex difference in the rate of CKD progression.

Previous studies examining the effect of UNX in rats support the findings from our study. Glomerular tuft volume has been shown to increase following UNX in rats, which is an adaptive response to the 50% reduction in nephron number (29, 34, 38). Consistent with the increase in glomerular tuft volume, an increase in glomerular capillary volume is also typically observed following UNX. However, hypertrophy-induced increases in capillary radii does not appear to contribute to the increased glomerular tuft volume found in males (27). Instead, compensatory increases in glomerular capillary length, as shown in the present study and previous literature (27, 29, 34), is the major contributor to the increase in capillary volume. The increase in length could be associated with the lengthening of existing capillaries or branching of new capillaries, as suggested by Nyengaard (34). However, the methods used in our study are not adequate to distinguish between such mechanisms of capillary lengthening. Utilization of a physical

dissector/fractionator approach, in which total nephron number is assessed, would be required for this type of analysis.

Both males and females will exhibit renal hypertrophy following UNX. Our study showed that males exhibit greater increases in the percent change of kidney weight post UNX, consistent with previous literature (38). Similarly, males have also been shown to exhibit a significantly greater percent change of glomerular volume post UNX (38). The greater body weight is typically much larger in males and likely a contributor to the greater kidney weight (36). Current literature lacks studies identifying sex differences in capillary length and radius. However, the present study found capillary radius to be similar between sexes but length greater in males post UNX.

The present study also found fractional capillary volume significantly greater in UNX females as compared to UNX males. As a reminder, fractional capillary volume is an approximate percentage of the Bowman's capsule occupied by the glomerulus. Two potential explanations arise from this discovery since both sexes began at similar body weights. One, the discrepancy in occupied space could be a result of less hypertrophy of other, capillary independent, structures within the glomerular tuft, such as mesangial cells or extracellular matrix. On the other hand, it is possible that kidneys of female rats exhibit greater branching of capillaries during compensatory hypertrophy. Future studies will be required to determine the mechanism contributing to the greater fractional capillary volume in female vs. male rats following UNX.

As mentioned previously, males are more susceptible to hypertensive glomerular injury exhibit and exhibit an expeditated rate of CKD progression. Our study indicates that the significantly greater compensatory increase in capillary length may be contributing to sex

differences in CKD progression via a reduction in podocyte density. Podocytes are terminally differentiated epithelial cells that surround glomerular capillaries that provide structural support against the relatively high capillary pressures (39). During compensatory hypertrophy, podocytes hypertrophy and are required to support a greater area of the glomerular capillary, which promotes podocyte stress (6). Glomerular capillary hypertension in the presence of glomerular capillary hypertrophy and reduced podocyte density can lead to podocyte detachment, capillary injury, and CKD progression (25). As a result, podocytes must hypertrophy and damage irreparably until detachment (6). The significantly greater capillary length in male rats observed in the present study indicate that podocytes would be forced to support a larger area of the capillary wall, which would subsequently decrease podocyte density and increase the stress on the cells. As such, a significant increase in length in males as compared to females could be the key factor leading to the sex-based discrepancy in the rate of CKD progression. Further research in our lab will assess whether sex differences in podocyte density exist following UNX in rats.

Limitations

In the present study, rats with intact kidneys were six weeks younger than rats with UNX. Thus, age-related increases in glomerular volume and capillary size also contributed, in part, to the greater capillary radii and length in rats with UNX vs. those with intact kidneys. Our lab is currently accounting for this by analyzing glomerular capillary morphology in age-matched rats with intact kidneys and following UNX. As previously mentioned, it is difficult to deduce the extent to which the significantly greater capillary length in rats with UNX vs. intact kidneys was due to the lengthening of existing capillaries or the sprouting of new capillaries (34). Our study had relatively small group numbers (n=4), which may have led to a reduced ability to detect

significant differences between groups. In this regard, our lab does plan to perform additional studies to increase the number of rats in all groups.

In conclusion, the data in this study supports the concept that greater increases in capillary length during compensatory renal hypertrophy in male rats may contribute to their greater rate of CKD progression via a reduction in podocyte density.

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