Supplemental Material

1. Supplemental Methods

The following direct measurements were obtained from the two consecutively renal biopsy sections that were scanned into the image files used for analyses:
1. The area of cortex on the periodic acid-Schiff (PAS) and trichrome (TRI) stained sections.
2. The number and total area of complete non-sclerotic glomeruli (NSG) on the PAS stained section.
3. The number and total area of partial NSG on the PAS stained section. These are glomeruli that have been transected by the biopsy needle and were counted as half a glomerulus.
4. The number of globally sclerotic glomeruli (GSG) on the PAS and trichrome stained sections.
5. Luminal boundary and intimal-media boundary of the most orthogonal artery (if artery present) on the trichrome stained section.
6. Area of non-tubular regions within 5 consecutive circles (totaling 1,000,000 μm^2) along the sectioned cortex. The excluded non-tubular area included glomeruli and vessels.
7. The number of complete tubules within these 5 circles.
8. The number of partial tubules within these 5 circles (partial tubules were counted as half a complete tubule).

On review of all donor biopsy sections, we did not find a single glomerulus with segmental glomerulosclerosis. Thus, we assumed sectioned profiles of glomeruli that appeared to be NSG were truly NSG and sectioned profiles that appeared to be GSG were truly GSG. The total number of NSG was obtained by summing the numbers of complete and partial NSG (counted as 0.5 NSG). Mean area of NSG was obtained by dividing a total area of all NSG by a total number of NSG per biopsy section. The consecutive PAS and TRI sections were both used to estimate % globally sclerotic glomeruli. NSG (or GSG) area densities were determined by the total number of NSG (or GSG) divided by the area of cortex (in mm^2). The volume of NSG (mm^3) (**Eq. 1**), the NSG density (per mm^3 of cortex) (**Eq. 2**), and the GSG density (per mm^3 of cortex) (**Eq. 3**) were calculated using the Weibel-Gomez stereological models.[1]

(**Eq. 1**) **Non-sclerotic glomerular volume (mm^3)** = $$\frac{1.382 \times (\text{Mean area of NSG})^{\frac{3}{2}}}{1.01}$$

(**Eq. 2**) **Non-sclerotic glomerular density (NSG per mm^3 of cortex)** = $$\frac{1}{1.382} \times \sqrt[2]{\frac{(\frac{\text{Total number of NSG}}{\text{Area of cortex}})^3}{\frac{\text{Total area of NSG}}{\text{Area of cortex}}}}$$

(**Eq. 3**) **Mean Globally-sclerotic glomerular density (GSG per mm^3 of cortex)**

$$= \frac{1}{2} \times [(\frac{1}{1.382} \times \sqrt[2]{\frac{(\frac{\text{Number of PAS GSG}}{\text{Area of PAS cortex}})^3}{\frac{\text{Total area of PAS GSG}}{\text{Area of PAS cortex}}}}) + (\frac{1}{1.382} \times \sqrt[2]{\frac{(\frac{\text{Number of TRI GSG}}{\text{Area of TRI cortex}})^3}{\frac{\text{Total area of TRI GSG}}{\text{Area of TRI cortex}}}})]$$

Due to the relative infrequency of GSG, the GSG density was averaged between then two consecutive sections (PAS and trichrome stained). NSG volume and NSG density was based on one section (PAS stained).

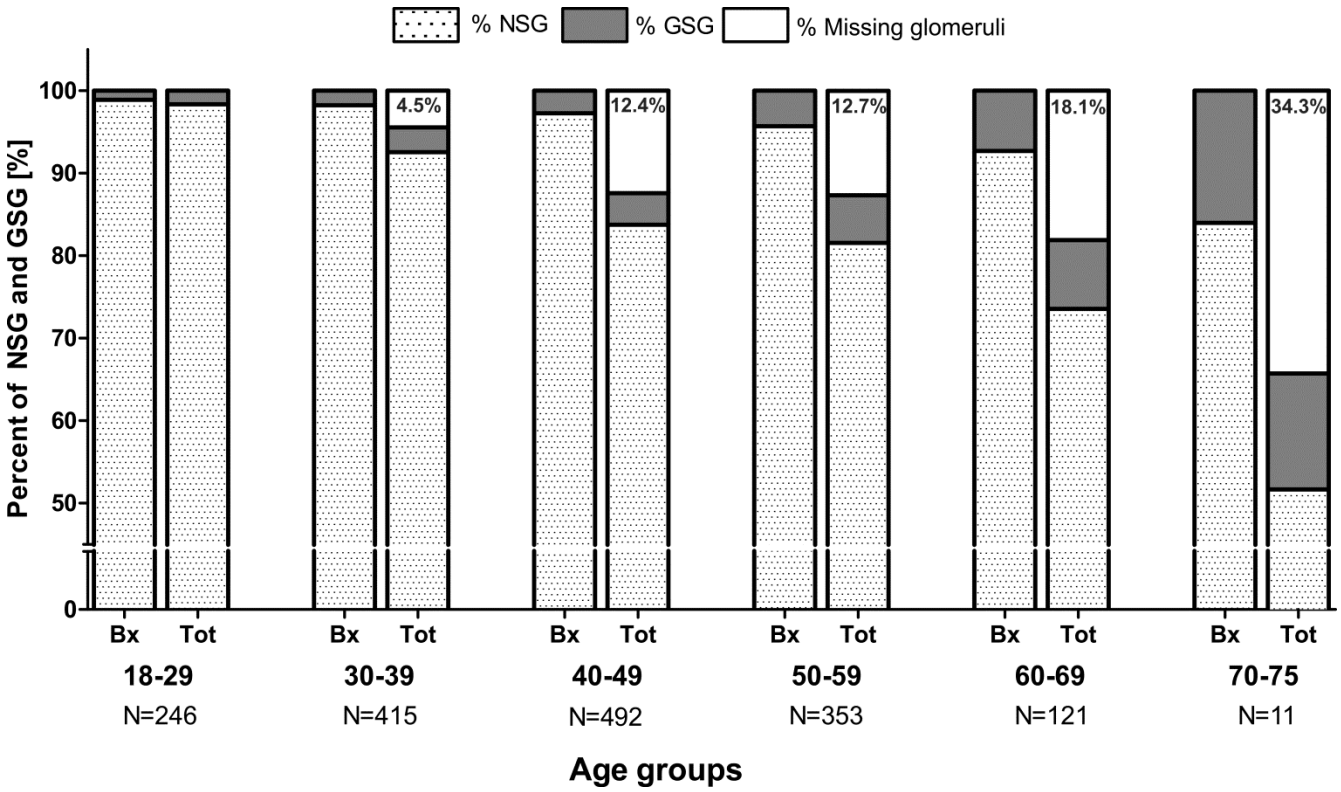
% Luminal stenosis was the area of intima relative to the total area of intima and lumen (**Eq. 4**):

(Eq. 4) % Luminal stenosis = $$\frac{\text{Intima to media boundary area} - \text{intimal to luminal boundary area}}{\text{Intima to media boundary area}}$$

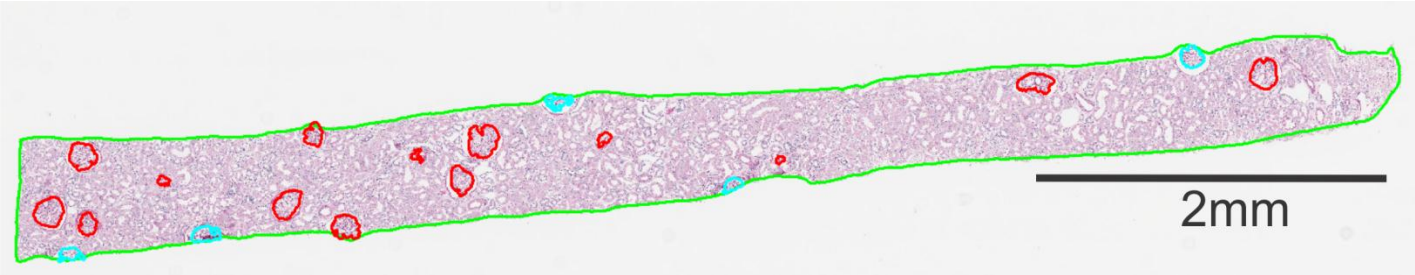
The mean profile tubular area estimates the average cross-sectional area of a tubule by counting the number of tubules in 1,000,000 μm^2 defined area of cortex after excluding all non-tubular structures (**Eq 5).**[2]

(Eq. 5) Mean profile tubular area (μm^2) = $$\frac{1{,}000{,}000 - \text{area of nontubular structures}}{\text{Number of complete tubules} + 0.5 \text{x} \text{Number of partial tubules}}$$

2. Supplemental Figures



Supplemental Figure 1. Percentage of each glomeruli type as determined by biopsy alone (Bx) or relative to the estimated total number of glomeruli (Tot). For each age group, % non-sclerotic glomeruli (NSG) and % globally sclerotic glomeruli (GSG) among detected glomeruli on biopsy is compared to the estimated % NSG, % GSG, and % missing glomeruli among the total number of glomeruli. Missing glomeruli were estimated from the number of NSG + GSG in 18-29 year olds minus the number of NSG + GSG in each other age group.



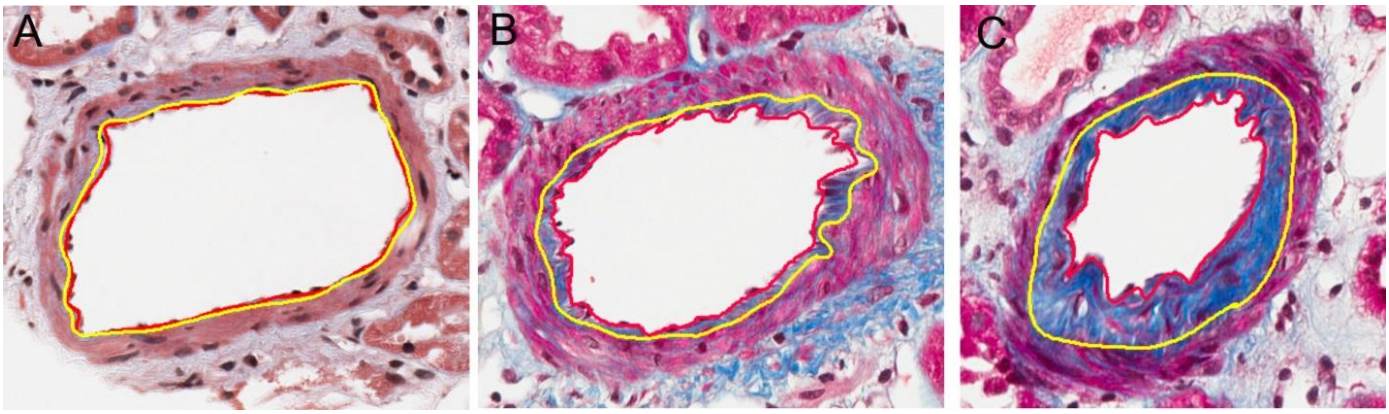
Total number of NSG = **number of complete NSG + 0.5 × number of partial NSG** = 14 + 0.5×5 = **16.5**

Area of cortex = 4.685 mm^2

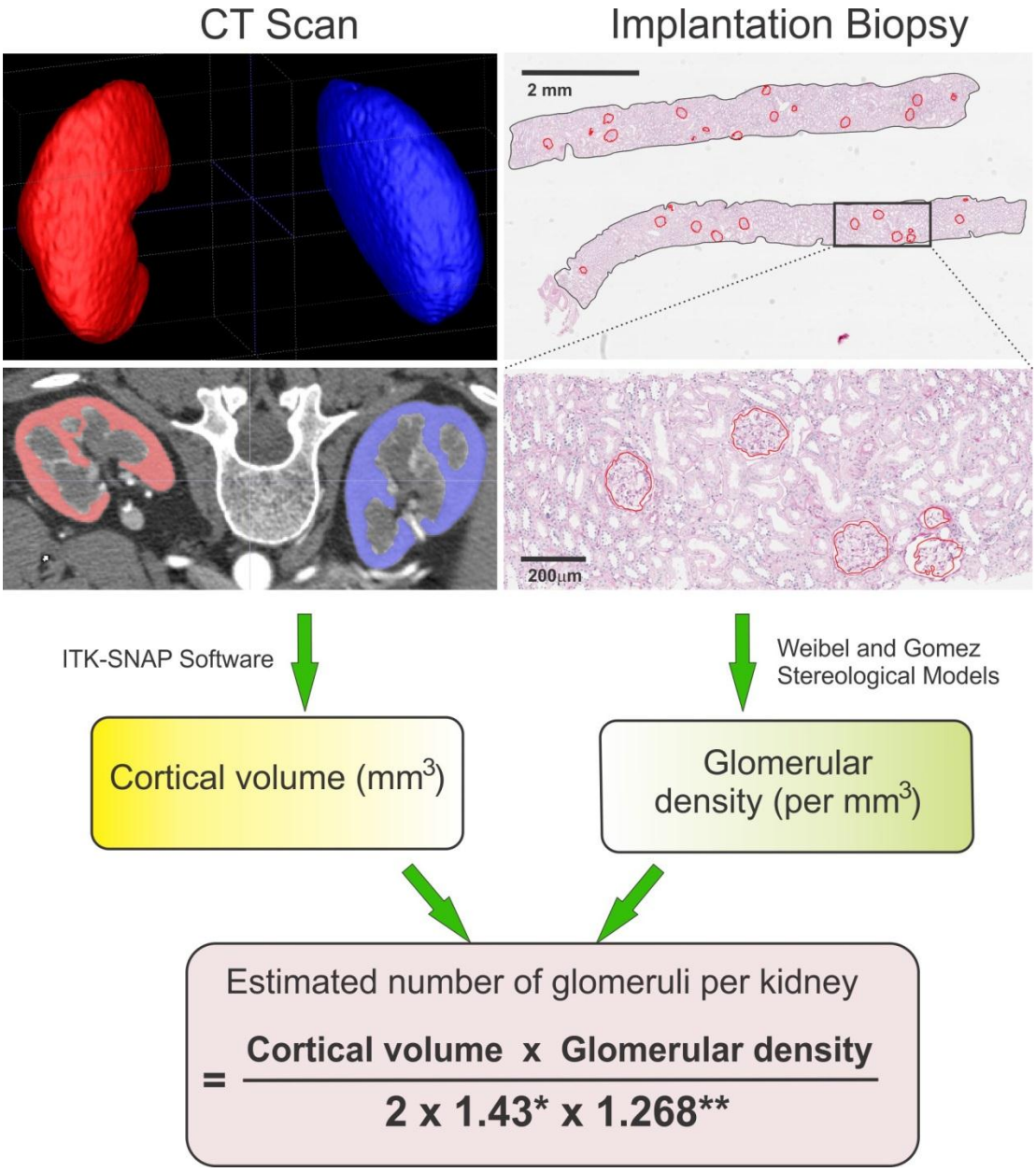
Total area of NSG = area of complete NSG + area of partial NSG **=** 176,229 μm^2 + 40,877 μm^2 **= 0.217 mm^2**

NSG density (per mm^3 of cortex) $= \frac{1}{1.382} \times \sqrt[2]{\frac{(\frac{\text{Total number of NSG}}{\text{Area of cortex}})^3}{\frac{\text{Total area of NSG}}{\text{Area of cortex}}}} = \frac{1}{1.382} \times \sqrt[2]{\frac{(\frac{16.5}{4.685})^3}{\frac{0.217}{4.685}}}$ **= 22.2 NSG per mm^3 of cortex**

Supplemental Figure 2. An example calculation of non-sclerotic glomerular (NSG) density. Renal biopsy section was stained with periodic acid-Schiff and scanned into high-resolution image. Annotations for cortical area (green), 14 complete NSG (red) and 5 partial NSG (cyan) are shown.



Supplemental Figure 3. Representative images of luminal stenosis. The % luminal stenosis was defined by the area of intima (between red and yellow boundaries) divided by the area of intima and lumen (within yellow boundary). Different degrees of luminal stenosis are shown: A) 0% luminal stenosis, B) 22% luminal stenosis and C) 53% luminal stenosis. Mason trichrome stain at 20x magnification shown.



Supplemental Figure 4. A schematic summary of the method used to estimate the number of glomeruli. *correction factor that accounts for 43% tissue volume shrinkage due to formalin fixation and paraffin embedding of biopsy sections;[3] **correction factor that accounts for 26.8% tissue volume shrinkage due to loss of perfusion following renal biopsy.[4]

3. Supplemental Tables

Supplemental Table 1. Percentage difference in nephron number, cortical volume, and globally sclerosed glomeruli (GSG) in older age groups relative to 18-29 year olds.

	Nephron number		Cortical volume, mm^3		%GSG	
Age Group	Mean (95% CI)	% Difference	Mean (95% CI)	% Difference	Mean (95% CI)	% Difference
18-29	990,661 (937,150 – 1,044,172)	-	217,856 (212,736 – 222,976)	-	1.1 (0.8 – 1.5) %	-
30-39	932,215 (897,150 – 967,279)	-5.9	217,707 (214,730 – 222,685)	+0.4	1.9 (1.5 – 2.4) %	+0.8
40-49	843,751 (811,571 – 875,932)	-15	208,721 (204,727 – 212,715)	-4.2	2.9 (2.4 – 3.4) %	+1.8
50-59	821,673 (782,715 – 860,631)	-17	198,449 (194,150 – 202,747)	-8.9	4.3 (3.9 – 4.9) %	+3.2
60-69	740,836 (682,875 – 798,797)	-25	191,607 (184,269 – 198,945)	-12	6.9 (5.2 – 8.6) %	+5.8
70-75	520,410 (363,958 – 676,861)	-48	182,664 (156,024 – 209,304)	-16	16 (6.2 – 25) %	+15

Supplemental Table 2. Clinical characteristics as predictors of nephron number (unadjusted and adjusted for each other characteristic)

	Unadjusted		Multivariable-adjusted	
Variable	Difference in nephron number	P Value	Difference in nephron number	P Value
Age per 10 years	-62,074	**<0.0001**	-45,885	**<0.0001**
Male	85,205	**<0.0001**	50,808	0.11
Body mass index per SD	-10,354	0.27	-9,938	0.35
Height per SD	48,290	**<0.0001**	42,407	**0.003**
Mild hypertension	-126,938	**<0.0001**	-33,165	0.32
Family history of ESRD*	-23,601	0.21	-56,175	**0.006**
Uric acid per SD	-7,862	0.41	-30,498	**0.018**
Measured GFR per SD	75,040	**<0.0001**	58,164	**<0.0001**
24h urine albumin per SD	11,508	0.26	6,543	0.52

*Donors with a family history of end-stage renal disease (often an offspring donating to a parent) were a mean 5 years younger than donors without a family history of end-stage renal disease. SD = Standard Deviation

Supplemental Table 3. Sensitivity analysis of clinical characteristics as predictors of nephron number (adjusted for each other characteristic).

Characteristic	Original analysis (N=1638)		At least 10 glomeruli (N=1281)		Excluding donors >70 years old (N=1627)		Excluding donors with imputed cortical volume (N=1484)	
	Difference in nephron number	P Value	Difference in nephron number	P Value	Difference in nephron number	P Value	Difference in nephron number	P Value
Age per 10 years	-45,885	**<0.0001**	-44,252	**<0.0001**	-42,772	**<0.0001**	-50,510	**<0.0001**
Male	50,808	0.11	53,060	0.11	52,785	0.09	66,840	**0.04**
Body mass index per SD	-9,938	0.35	-9,318	0.42	-11,000	0.31	-12,049	0.29
Height per SD	42,407	**0.003**	50,283	**0.001**	40,754	**0.004**	38,000	**0.01**
Mild hypertension	-33,165	0.32	-18,692	0.61	-32,314	0.34	-33,163	0.35
Family history of ESRD	-56,175	**0.006**	-56,777	**0.01**	-55,242	**0.008**	-58,789	**0.006**
Uric acid per SD	-30,498	**0.018**	-37,781	**0.006**	-30,142	**0.02**	-32,486	**0.02**
Measured GFR per SD	58,164	**<0.0001**	52,566	**<0.0001**	57,907	**<0.0001**	57,846	**<0.0001**
24h urine albumin per SD	6,543	0.52	8,813	0.44	10,260	0.33	8,958	0.40

References:

1. Weibel, ER, Gomez, DM: A principle for counting tissue structures on random sections. *J Appl Physiol,* 17**:** 343-348, 1962.
2. Elsherbiny, HE, Alexander, MP, Kremers, WK, Park, WD, Poggio, ED, Prieto, M, Lieske, JC, Rule, AD: Nephron hypertrophy and glomerulosclerosis and their association with kidney function and risk factors among living kidney donors. *Clin J Am Soc Nephrol,* 9**:** 1892-1902, 2014.
3. Fulladosa, X, Moreso, F, Narvaez, JA, Grinyo, JM, Seron, D: Estimation of total glomerular number in stable renal transplants. *J Am Soc Nephrol,* 14**:** 2662-2668, 2003.
4. Lerman, LO, Bentley, MD, Bell, MR, Rumberger, JA, Romero, JC: Quantitation of the in vivo kidney volume with cine computed tomography. *Invest Radiol,* 25**:** 1206-1211, 1990.