The Geometry of Arteriovenous Fistulas Using Endothelial Nitric Oxide Synthase Mouse Models

Isabelle Falzon,1,2 Hannah Northrup,1,2 Lingling Guo,3 John Totenhagen,4 Timmy Lee,3,5 and Yan-Ting Shiu2,6

Abstract

Background Arteriovenous fistula (AVF) maturation failure is a significant clinical problem in the hemodialysis population. Geometric parameters of human AVFs were associated with AVF development, but causative studies are lacking. We characterized mouse AVF geometry using endothelial nitric oxide synthase (NOS3) mouse models.

Methods Carotid-jugular AVFs were created in NOS3 overexpression (OE), knockout (KO), and wild-type (WT) mice. At 7 and 21 days postcreation, black-blood magnetic resonance images of AVFs were acquired and used to build three-dimensional reconstructions of AVF lumens. We used these reconstructions to calculate the lumen area, lumen centerline, and centerline-derived parameters: anastomosis angle, tortuosity, nonplanarity angle, and location of maximal distance between the feeding artery and AVF vein. Inter- and intrauser variabilities were also determined.

Results When all mice were considered, increased minimum AVF venous lumen area was accompanied by increased venous tortuosity and increased distance between the artery and vein, with both remaining in plane with the anastomosis. At day 7, the lumen area of AVFs from all strains was 1.5- to 2.5-fold larger than native veins. Furthermore, at day 21, AVF lumen in NOS3 OE (4.04±1.43 mm²) was significantly larger than KO (2.74±1.34 mm²) (P<0.001) and WT (2.94±1.30 mm²) mice (P<0.001). At day 21, the location of maximal artery-vein distance on the vein was further away from the anastomosis in OE (4.49±0.66 mm) than KO (2.87±0.38 mm) (P=0.01). Other geometric parameters were not significantly different between mouse strains or time points. Inter- and intrauser variabilities were small, indicating the reliability and reproducibility of our protocol.

Conclusions Our study presents a detailed characterization of mouse AVF geometry, and a robust protocol for future mechanistic studies to investigate the role of molecular pathways in AVF geometry. Identifying a geometry related to desired AVF remodeling can help inform surgery to enhance AVF maturation.

1Department of Biomedical Engineering, University of Utah, Salt Lake City, Utah
2Division of Nephrology and Hypertension, Department of Internal Medicine, University of Utah, Salt Lake City, Utah
3Division of Nephrology, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama
4Department of Radiology, University of Alabama at Birmingham, Birmingham, Alabama
5Veterans Affairs Medical Center, Birmingham, Alabama
6Veterans Affairs Medical Center, Salt Lake City, Utah

Correspondence: Dr. Yan-Ting Shiu, Division of Nephrology and Hypertension, University of Utah, 295 Chipeta Way, Suite 4000, Salt Lake City, UT 84109. Email: y.shiu@hsc.utah.edu
lumen expansion in the AVF vein (2). One mechanism of lumen expansion is nitric oxide (NO)–mediated vasodilation by the relaxation of vascular smooth muscle cells (13). Endothelial NO synthase (NOS3) is an enzyme that catalyzes the production of NO (13). Previously, our group has shown that genetically modifying NOS3 in mice influences AVF venous lumen area and hemodynamics for $n=1$ at day 7 postsurgery (14). To build on this previous study (14), we (1) increase the number of animals of all genetic modifications at the day 7 time point, (2) add a day 21 postsurgery time point, (3) investigate whether/how NOS3 expression levels influence AVF geometry, and (4) investigate whether/how NOS3 expression levels influence the lumen of no-surgery veins. The investigation of AVF geometry is relevant because geometry has been shown to influence hemodynamics in simulations using idealized human AVF geometric models (3–6). Additionally, understanding the geometry of rodent AVFs is important for future work and mechanistic studies using rodent models. This paper aimed to (1) establish a protocol to quantify AVF geometric parameters, using high-resolution 3D images of mouse AVFs; and (2) investigate AVF geometry over time in mice with different expression levels of NOS3. Specifically, we quantified the lumen cross-sectional area, anastomosis angle, tortuosity, nonplanarity angle, distance between the feeding artery and the AVF vein, and the location of maximal distance between the feeding artery and AVF vein. Inter- and intrauser variabilities were also determined. We hypothesized that genetic manipulation of NOS3 results in an altered AVF geometry over time.

Materials and Methods
Mouse Model and Magnetic Resonance Image Acquisition
Carotid-jugular AVFs (Figure 1A) were created in C57BL/6 mice (wild type, WT), and NOS3 overexpression (OE) and homozygous knockout (KO) mice on a C57BL/6 background, as previously described (15). Young male mice were used. One surgeon created AVFs for all mice. WT and KO mice were obtained from Jackson Laboratories (Bar Harbor, ME). OE mice were provided by Dr. Christopher Kevil. The varying NOS3 levels between strains was confirmed by Western blot and cyclic guanosine monophosphate levels (14). At 7 and 21 days post-AVF creation, mice were subject to two-dimensional, T2-weighted fast spin echo sequence with black-blood double inversion magnetic resonance imaging (MRI) to visualize blood vessel lumens, as previously described (15). Briefly, scans were performed using a 9.4 Tesla Bruker MRI machine (Bruker Biospin, Billerica, MA) (Figure 1B), and the black-blood MRI scan consisted of 35 continuous 0.5-mm-thick axial slices with an in-plane resolution of 0.1 mm. There were six conditions of mice in total: three mouse strains (WT, OE, and KO) and two time points (day 7 and day 21 after AVF creation). The number of mice was five for WT day 7, two for WT day 21, four for KO day 7, three for KO day 21, three for OE day 7, and two for OE day 21. The AVF creation, MRI scan acquisition, segmentation, and 3D reconstruction steps are shown in Figure 1.
and four for OE day 21. Animal studies and experiments were approved by the University of Alabama at Birmingham (UAB) Institutional Animal Care and Use Committee, and performed in accordance with the National Institutes of Health guidelines.

**Reconstruction**

Reconstructions of the AVF lumen and contralateral, no-surgery vessels were done using the black-blood MRI scans in Amira 5.2.1 (Visage Imaging, Inc., San Diego, CA) (15). Briefly, images were segmented using a thresholding tool and then adjusted manually (Figure 1C); this series of two-dimensional slices was used to generate a 3D surface, which was then smoothed into the final lumen reconstruction (Figure 1D). VMTK 1.4.0 (www.vmtk.org) was used to calculate centerlines of vessel lumens (Figure 1E) at 0.1-mm increments, through the proximal artery, distal artery, AVF vein, and the anastomosis origin. The anastomosis origin was defined as the point where a centerline through the distal artery would diverge into the AVF vein and proximal artery. The x, y, and z coordinates of these centerlines and anastomosis origin were used in the calculation of geometric parameters.

**Cross-Sectional Area Calculation**

The cross-sectional area of the vessel lumens was calculated in Amira 2019.1, using the reconstructed lumen and centerline. Cross-sections were created perpendicular to the centerline every 0.2 mm, starting from the anastomosis. For the proximal and distal artery, the cross-sectional area was calculated for the whole reconstruction, which was 4.6–6.6 mm for proximal artery and 1.4–2.4 mm for distal artery; for the vein, the cross-sectional area was calculated before the first vein branch, which was 5.2–6.8 mm. The venous length was from the first slice at the anastomosis to the last slice before the vein branch. This cross-sectional area calculation method was validated using a cylindric tube of known size. Previously, our group used a Matlab code to calculate the cross-sectional area every 0.1 mm, starting from the anastomosis (14,15). Since then, we have made it more efficient to do this calculation in Amira.

---

**Figure 2.** AVF geometric parameter calculation. Points, lines, planes, and vectors used for calculating the (A) anastomosis angle, (B) tortuosity, (C) nonplanarity angle, and (D) maximum distance between the vein and artery. In all panels, the red circle indicates the point of the anastomosis origin. In (A–C), solid gray lines indicate lumen centerlines. In (A and C), yellow squares are points in the centerlines that are 1-mm, straight-line distances from the anastomosis origin, and the dashed arcs represent the anastomosis or nonplanarity angle, respectively. In (A), a and b are vectors from the anastomosis origin to 1-mm distances on the AVF vein and proximal artery centerline, respectively. In (B and C), the blue triangle is the maximum distance point. In (B), D₁ and L₁ are the straight-line distance and along-the-centerline distance from the anastomosis origin to the point of maximum distance, respectively. In (C), c and d are vectors from the 1-mm distance on the distal artery of AVF vein, respectively, to the 1-mm distance on the proximal artery centerline. Also in (C), e is a vector from the anastomosis origin to the maximum distance point, and the yellow plane represents the anastomosis plane. In (D), the black dots are the points the centerline is composed of, the plane indicated by the teal rectangular outline is normal to the proximal artery centerline and contains the teal cross-sections, and L₂ and L₃ are the along-the-centerline distances in the AVF vein and proximal artery, respectively, to the maximum distance between the artery and the vein, indicated by the blue star and dashed line.
two changes. First, we switched from the Matlab code to Amira, because the latter is less prone to user error/bias for rodents. Second, we performed sensitivity analysis and found that the 0.1-mm and 0.2-mm intervals resulted in similar average cross-sectional area results (differences were <10%) for the AVF vein, and the 0.2-mm interval required approximately half the computational time of the 0.1-mm intervals. The average cross-sectional area was the average of all slices taken from all mice within a condition. The minimum or maximum cross-sectional area was the average of each animal’s minimum or maximum cross-sectional area value from all mice within a condition.

Anastomosis Angle
The anastomosis angle was calculated using two vectors with the same length, a and b, in Figure 2A and Equation 1. These vectors were taken from the anastomosis origin to a straight-line distance of 0.5, 1, or 1.5 mm on the centerline into the vein (a) and into the proximal artery (b). In Figure 2A, the yellow squares indicate the 1-mm distance from the anastomosis origin (red circle). In Equation 1, \( \cos = \cos \), the \( \cdot \) indicates a dot product, and the coefficient 57.3 was used to convert from radians to degrees.

\[
anastomosis\ angle = 57.3 \cos^{-1}\left(\frac{a \cdot b}{|a||b|}\right)
\]  

(1)

Tortuosity
As shown in Figure 2B and Equation 2, tortuosity was calculated using a ratio of the along-the-centerline distance, curved length (L_t), to the straight-line distance (D_t) starting from the anastomosis origin to the same end point (blue triangle) further along the AVF vein centerline. This equation has been previously used to analyze the tortuosity of the carotid artery (16), brachial artery, and cephalic vein (17) in patients.

\[
tortuosity = \frac{L_t}{D_t} - 1
\]  

(2)

To determine the optimal end point for L_t and D_t, we considered end points of 1, 2, 3, and 4 mm straight-line distances from the anastomosis origin, and the last point (maximum distance point, the blue triangle in Figure 2B) on the AVF vein centerline before any vein branches occur, which was 6–10 mm, depending on the mouse.

This approach was also used for calculating arterial tortuosity. For the artery, the maximum distance point was the last point on the proximal or distal artery centerline. Due to the short length of the distal artery, limited by the available MRI scans for reconstruction, tortuosity could only be calculated for a 1-mm end point and the maximum distance point.

Nonplanarity Angle
As shown in Figure 2C and Equation 3, nonplanarity is a measure of how much the downstream AVF vein (defined by the vector e) deviates from the near-anastomosis plane (defined by the vectors c and d). In Equation 3, \( \sin = \sin \), and \( \times \) indicates the cross product.

\[
\text{nonplanarity angle} = 57.3 \sin^{-1}\left(\frac{(c \times d) \cdot e}{|c \times d||e|}\right)
\]  

(3)

e is a vector from the anastomosis origin to a point on the vein centerline; D_1 in Figure 2B is the length of the vector e. Thus, the same different lengths of D_1 were used to determine optimal e as well. The yellow squares in Figure 2C are the same as those in Figure 2A. c and d are vectors from the squares on the distal artery and the vein centerline, respectively, to the square on the proximal artery. Thus, the same different lengths for a and b were used to determine optimal c and d as well. This parameter was only calculated for the vein, not the artery.

Distance between Vein and Artery
The AVF lumen reconstruction, the proximal artery centerline, and the AVF vein centerline were opened in Amira 2019.1 to determine the distance between the artery and vein. A plane (teal rectangle in Figure 2D) was created perpendicular to the proximal artery centerline and moved across the proximal artery centerline, starting from the anastomosis origin. The centerline points on the proximal artery (x_{artery}, y_{artery}, z_{artery}) and AVF vein (x_{vein}, y_{vein}, z_{vein}) that the plane intersected simultaneously were visually observed and recorded. The coordinates of these two points were used to calculate the artery-vein distance using Equation 4. This was done for all points on the proximal artery centerline, which has an average of 105 points spaced at 0.05 mm. We also determined the location of the maximum artery-vein (max A-V) distance (star in Figure 2D), how far it was along both centerlines of the AVF vein (L_2 in Figure 2D) and the proximal artery (L_1 in Figure 2D) from the anastomosis.

\[
distance = \sqrt{(x_{vein} - x_{artery})^2 + (y_{vein} - y_{artery})^2 + (z_{vein} - z_{artery})^2}
\]  

(4)

Inter- and Intrauser Variability
Inter- and intrauser variability was assessed for average cross-sectional area, the anastomosis angle at 1 mm for a and b in Equation 1, tortuosity at D=4 mm in Equation 2, and nonplanarity angle using the 1 mm for c and d, and 4 mm for e in Equation 3. Two users (I.F., M.E.) analyzed all available mice. User 1’s (I.F.) reconstructions are presented in the results of this paper. User 2’s (M.E.) reconstructions were used to compare to user 1’s for determining interuser variability. A random number generator was used to choose one mouse from each strain and time point condition. User 1 then reconstructed and analyzed these six mice again, and these reconstructions were used for determining intrauser variability. There were 3–5 months between user 1’s initial reconstruction of all available mice and the second reconstruction of six mice for intrauser variability.

Statistical Analyses
Statistics were computed in GraphPad Prism 8 (GraphPad Software, San Diego, CA). For both anastomosis angle and the tortuosity of the vein and proximal artery, data were grouped by vector length and then tested with one-way ANOVA with a post-test for a linear trend. Distal arterial
tortuosity data was tested using a t test, because only two vector lengths were able to be calculated. For correlation, the parameters were compared in pairs using Pearson correlation tests. Linear trends were considered significant if \( P<0.05 \). Differences for inter- and intrauser variability were assessed using paired t tests and considered significant if \( P<0.05 \). One-way ANOVA with a Tukey–Kramer test was used to compare geometric parameters between strains and time points, and differences were considered significant if \( P<0.05 \).

**Results**

In this investigation, both the artery and vein were analyzed. The focus of our presentation is on the AVF vein because it is the site of cannulation for dialysis and has received much more attention in the literature than the arterial limb of the AVF. Arterial data are referenced in text, and their figures are presented in the Supplemental Figures 1–6.

**Determining Geometric Analysis Protocol**

There is a total of six conditions, from three mouse strains and two postsurgery time points. Representative lumen reconstructions of AVFs, one for each of the six conditions, from user 1 are shown in Figure 3. The length of the vessel may affect the value of the geometric parameters but, currently, there is not a standard length used for calculation in mice. Therefore, we considered multiple vectors and distances to determine an optimal length independent of biology. As such, all animals are grouped together, with \( n=21 \), without distinguishing mice by strain or time point. The results are presented in Figures 4 and 5.

The anastomosis angle (mean±SD) for \( a \) vectors of 0.5, 1.0, and 1.5 mm were 89.5°±16.2°, 94.3°±11.6°, and 90.0°±8.86°, respectively, and were similar \((P>0.05)\) (Figure 4A). Therefore, the middle value of 1.0 mm was used for comparing between strains and time points. Note that the anastomosis angle in our mouse AVF is similar to the median anastomosis angle, 90.0°, in a cohort of patients with brachial-cephalic AVFs (8).

The venous tortuosity (mean±SD) for the lengths of \( D_1 \) of 1, 2, 3, and 4 mm, and the length to the maximum distance point (max distance length) were 0.155±0.075, 0.132±0.050, 0.279±0.140, 0.422±0.160, and 0.568±0.112, respectively (Figure 4B). The tortuosity at lengths 1 and 2 mm were similar \((P=0.62)\), and yet comparisons of all other lengths (3 mm, 4 mm, max distance length) were significantly different. The increase of tortuosity from 2 mm to max distance length was linear \((P<0.001)\). Thus, we decided to use max distance length possible, and we found the average venous tortuosity for all mice was 0.568±0.112. The average arterial tortuosity for all mice using the max distance length possible was 0.019±0.008 for the proximal artery, and 0.062±0.026 for the distal artery, much smaller than the venous tortuosity, likely because the artery is relatively straight. Although the tortuosity decreased when a longer length of the artery was considered (Supplemental Figures 1A and 4A), this negative trend may not be of physiologic significance due to the small value of arterial tortuosity.

The calculation of the nonplanarity angle requires \( c, d, e \) vectors, and we considered the absolute value (magnitude) of the nonplanarity angle for various combinations of these vectors. As mentioned above, the same different lengths for \( a \) and \( b \) were used to determine optimal \( c \) and \( d \), and the same different lengths of \( D_1 \) were used to determine optimal \( e \). Nonplanarity angle magnitude decreased with increasing vectors \( a \) and \( b \), and, for most cases, also decreased with increasing \( D_1 \) (Figure 4C). Thus, we decided to use 1 mm for \( a \) and \( b \) (same as anastomosis angle) and to use the max

![Figure 3](image-url) | Representative AVF lumen reconstructions show heterogeneity in AVF geometry between mouse strains and postsurgery time points. Representative geometries from all mouse strains at two postsurgery time points. The 1-mm scale bar applies to all geometries. Solid black lines: lumen centerline. Red circle: anastomosis origin. Yellow square: point of 1-mm, straight-line distance from anastomosis origin onto each centerline. Blue triangle: maximum distance point on the AVF vein centerline before any branch.
distance point for D1 (same as tortuosity). At this combination, the nonplanarity angle magnitude for all mice was 5.01° ± 3.87° (mean ± SD). To the best of our knowledge, there is no published venous nonplanarity angle of human AVF in the literature for comparison. Pearson correlation was done to identify any significant corrections, in pairs, between nine geometric parameters: average venous area, minimum venous area, maximum venous area, anastomosis angle, venous tortuosity, nonplanarity angle, nonplanarity angle magnitude, max A-V distance, and vein length at max A-V distance. There were 35 paired combinations total. Six combinations were significant (Figure 5). The AVF vein area is among the most critical in determining AVF maturation. We found that the average venous area was positively associated with the maximum venous area (Figure 5A), although it only trended to being positively associated with the minimum venous area (P = 0.10). When the max A-V distance was considered, as it increased, the minimum venous area increased (Figure 5B), venous tortuosity increased (Figure 5C), vein length at where the max A-V distance occurred increased (Figure 5D), but yet nonplanarity angle magnitude decreased (Figure 5E). When the vein length at where the max A-V distance occurs was considered, as it increased, nonplanarity angle magnitude decreased (Figure 5F), which supports the correlations shown in Figure 5, D and E. Together, Figure 5, B–F, shows that, when all mice were considered, increased minimum venous area was accompanied by increased venous tortuosity and increased distance between the proximal artery and AVF vein, with both still remaining on the same plane as the anastomosis.

**Intra- and Interuser Variability of the Geometric Analysis**

For inter- and intrauser variability, users used 1 mm for vectors a, b, c, d, and 4 mm for vector e and length D1. In both the inter- and intrauser variability assessment, no significant difference was found for average venous area, anastomosis angle, venous tortuosity, or nonplanarity angle magnitude (Figure 6). For the proximal and distal arterial area, no difference was observed for intrauser variability, but there was a significant difference (P < 0.001) for interuser variability, where user 2 consistently had a larger average area than user 1 (Supplemental Figures 2 and 5). This difference in the arterial area could be because the artery lumen was very small (usually 15 pixels), and so even a small difference would lead to a big error. In contrast, the vein was bigger (usually 400 pixels), and so was not as sensitive to small differences.

**Figure 4.** Venous AVF geometric parameters may depend on the length of the vector used for calculation. (A) Anastomosis angle, (B) tortuosity, and (C) nonplanarity angle magnitude. In (A and B), the short horizontal line shows the mean. In (C), the bar height indicates the mean. n = 21. Max, maximum.

**Geometric Parameters of AVFs with Different NOS3 Levels and Postsurgery Time Points**

We have previously calculated the AVF lumen area for a limited number of animals (n = 5) (14,15). This paper increased the animal numbers, modified the method for calculating area, and investigated area over time in 21 animals. Using our previous and current methods, we obtained a comparable lumen area and reached the same conclusions that NOS3 OE leads to a larger lumen area than WT or KO, but the current method is less prone to user bias/error.

The cross-sectional lumen areas (mean ± SD) of the contralateral, no-surgery external jugular veins in WT (2.90 ± 0.80 mm²),
KO (2.39±0.71 mm$^2$), and OE (3.26±0.77 mm$^2$) mice differed significantly (P<0.001), indicating the influence of the whole-body NOS3 expression levels in the vascular lumen. At day 7, the venous area of AVFs from all strains was 1.5- to 2.5-fold bigger than native veins. At day 7, the average venous area of OE (3.51±1.30 mm$^2$) and WT (3.02±1.28 mm$^2$) was significantly greater than KO (2.30±1.20 mm$^2$) (Figure 7A). By day 21, the average venous area of OE (4.04±1.43 mm$^2$) was significantly greater than both WT (2.94±1.30 mm$^2$) and KO (2.74±1.34 mm$^2$) (Figure 7A). It is important to point out that the venous area was not uniform, being narrow when at and near anastomosis, and getting bigger when moving away from the anastomosis (Figure 7B). We found that the difference between strains was greater further away from the anastomosis. Additionally, at day 7, the peak area for WT, KO, and OE occur at 3.2, 2.6, and 3.0 mm, respectively, along the venous centerline (Figure 7B, left panel); at day 21, the peak area for WT, KO, and OE are at 2.0, 2.0, and 3.2 mm, respectively, along the venous centerline (Figure 7B, right panel). There was no difference in the maximum venous area (Figure 7C). We also investigated the minimum venous area, because this parameter is used in the clinical setting. At day 21, the minimum venous area was significantly higher in OE (0.43±0.15 mm$^2$) than WT (0.12±0.03 mm$^2$) (P=0.04), and trending bigger than KO (0.20±0.09 mm$^2$) (P=0.09) (Figure 7D).

The anastomosis angle, venous tortuosity, nonplanarity angle magnitude, and max A-V distance did not differ significantly among mouse strains or time points (Figure 8, A–D), but the distance along the vein centerline where the max A-V distance occurred did (Figure 8F). When compared with day 7, by day 21, this distance became significantly closer to the anastomosis for both WT (3.10±0.78 mm at day 21 versus 4.64±0.51 mm at day 7) and KO (2.87±0.38 mm at day 21 versus 4.55±0.38 mm at day 7), whereas it trended to being further away from the anastomosis for OE (4.49±0.66 mm at day 21 versus 3.65±0.44 mm at day 7). We observed the same qualitative pattern for the distance along the proximal artery centerline, where the max A-V distance occurred, although this pattern did not reach statistical significance (Figure 8E).

The AVF vein has received more focus than the AVF artery in the literature because it is cannulated for dialysis. We analyzed the artery to fill the gap of knowledge regarding the AVF arterial limb. For the proximal artery, at day 7, both KO (0.16±0.05 mm$^2$) and OE (0.16±0.05 mm$^2$) had greater arterial areas than WT (0.14±0.04 mm$^2$); at day 21, OE (0.17±0.05 mm$^2$) had significantly greater proximal arterial area than both WT (0.15±0.04 mm$^2$) and WT (0.14±0.05 mm$^2$), but the difference was very small, being only 0.02–0.03 mm$^2$ (Supplemental Figure 3A). Whereas the area of the proximal artery was not uniform along the centerline, there was not a clear trend for the area with respect to the anastomosis (Supplemental Figure 3B), and both the maximum and minimum areas are very small, being 0.10–0.25 and 0.05–0.15 mm$^2$, respectively (Supplemental Figure 3, C and D). This may be because the arterial area was very small throughout and, therefore, the maximum and minimum areas were very close. Indeed, there

Figure 5. | Some geometric parameters are significantly correlated with each other. (A) Average venous cross-sectional area is positively correlated with the maximum venous cross-sectional area. The maximum distance between the artery and vein (max A-V distance) is positively correlated with (B) the minimum venous cross-sectional area, (C) venous tortuosity, and (D) the vein length where the max A-V distance occurs, but is negatively correlated with (E) the nonplanarity angle magnitude. (F) The vein length where the max A-V distance occurs is negatively correlated with nonplanarity angle magnitude. The strain and time point of the animal is specified by the color and symbol key. n=21. D7, day 7 postsurgery; D21, day 21 postsurgery; KO, knockout; OE, overexpression; WT, wild type.
was no difference in minimum or maximum arterial area with respect to strain or time point (Supplemental Figure 3, C and D). Finally, the proximal arterial tortuosity did not vary significantly with respect to mouse strains or time points (Supplemental Figure 1B). For the distal artery, the area (Supplemental Figure 6) and tortuosity (Supplemental Figure 4B) were small and similar among mouse strains or time points.

Discussion
This study presented a protocol to quantitatively describe mouse AVF. Considering the small size of the mice, we performed inter- and intrauser variability analysis and found reproducibility of the geometric parameters, indicating the reliability of our protocol. Several significant correlations between geometric parameters were found. First, as the average venous area increased, the maximum venous area increased. This indicates that the maximum venous area is reflective of the vein’s overall expansion as opposed to a single outlier. Second, when all mice were considered, an increased minimum venous area was accompanied by increased venous tortuosity and increased distance between the proximal artery and AVF vein, with both remaining in-plane with the anastomosis. A potential explanation for this could be that, as the venous limb of the AVF expands, it needs to move away from the artery to have space to expand; expansion and turning away from the artery result in increased venous tortuosity, with optimal separation occurring in plane with the anastomosis.

We found that, at day 21, AVF lumen in NOS3 OE mice was bigger than NOS3 KO and WT mice, and the location of max A-V distance was further away from the anastomosis in NOS3 OE than NOS3 KO and WT. Our results indicate that OE of NOS enhances lumen expansion and that the separation of arterial and venous limbs occurs gradually downstream, as opposed to abruptly at the anastomosis, in NOS OE mice. However, contrary to our hypothesis, anastomosis angle, tortuosity, and nonplanarity did not differ significantly between mouse strains. We built on our previous
research (14), which had established a relationship between NOS3 expression and the AVF’s lumen area and hemodynamics, by (1) increasing the number of animals at the postsurgery day 7 time point; (2) adding a postsurgery day 21 time point; (3) analyzing the AVF geometry because previous literature (3–6) had established a relationship between geometry and hemodynamics; and (4) quantifying the area of the contralateral, no-surgery veins. Our finding that NOS3 expression did not significantly alter the anastomosis angle, venous tortuosity, or nonplanarity angle magnitude indicates that the change in hemodynamics with respect to NOS3 expression we observed (14) was not due to the effect of NOS3 on AVF geometry.

Only one surgeon performed all surgeries in this study. Therefore, this may indicate that our surgeon was successful in creating consistent AVFs for all mice in an unbiased fashion, and the consistent surgery may have prevented some potential geometric differences due to genetic manipulation of NOS3. Another explanation is the limitation of our centerline-based approach. It is possible that the outline of a 3D shape changes without moving the centerline. We chose a centerline-based approach because it has been extensively used in the AVF and non-AVF settings in the literature (3–12,16–18). Future research can consider non–centerline-based approaches such as particle-based shape analysis, which involves distributing correspondence points onto shapes to identify how a shape deviates from a control shape (19,20). An additional limitation of this study is the small sample size of mice (five for WT day 7, WT day 21, KO day 7, KO day 21, OE day 7, and OE day 21, the animal number \( n \)=5, 2, 4, 3, 3, and 4, respectively. \( *P < 0.05 \).

**Figure 7.** | Venous cross-sectional area may differ between mouse strains and postsurgery time points. (A) The average cross-sectional area along the centerline between the first slice after the anastomosis and before any branch, at 0.2 mm intervals. The slice number for individual mice ranges from 24 to 35 per mouse. (B) The cross-sectional area along the centerline, where 0 mm is at the first slice after the anastomosis. (C) Minimum and (D) maximum cross-sectional area. Data in (A, C, and D) are presented as mean±SEM. For WT day 7, WT day 21, KO day 7, KO day 21, OE day 7, and OE day 21, the animal number \( n \)=5, 2, 4, 3, 3, and 4, respectively. \( *P < 0.05 \).
AVF development. Yet, a few clinical studies found that the angle did not affect maturation (12), or stenosis (9,10). To the best of our knowledge, there is no literature on the tortuosity or nonplanarity angle of the human AVF vein. This study characterizes mouse AVF geometry in detail in strains with varying NOS3 expression and provided a robust protocol for future mechanistic studies to investigate the role of molecular pathways in AVF geometry. To treat the clinical problem of AVF maturation failure, both optimal geometric parameters and methods for controlling them to achieve these optimal values will be needed. For example, the anastomosis angle can be controlled with surgical technique (4) or with devices (21,22). Additionally, this protocol may be used to investigate other molecular pathways that have the potential to modify AVF geometry.

Disclosures
T. Lee is a consultant for Merck and Boston Scientific. All remaining authors have nothing to disclose.

Funding
T. Lee was supported by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) grant R44DK109789, National Heart, Lung, and Blood Institute grant R01HL139692, and U.S. Department of Veterans Affairs merit award I01BX003387. Y. Shiu was supported by NIDDK grants R01DK100505 and R01DK121227, and by U.S. Department of Veterans Affairs merit award I01BX004133.

Acknowledgments
The authors thank Dr. Yong He for his input on the mathematic calculation of geometric parameters, Mr. Mark Eisele (M.E.) for assisting interuser variability analysis, UAB small animal imaging core facility for mouse MRI scans, and UAB—University of California San Diego O’Brien Core Center for AKI Research (P30 DK079337) for performing all mouse AVF surgeries. This work was presented as a poster at the Annual Meeting of American Society of Nephrology in Washington D.C. in 2019. This work was a part of Ms. Isabelle Falzon’s Master’s Thesis at the University of Utah.

Author Contributions
I. Falzon was responsible for data curation and formal analysis; I. Falzon, T. Lee, H. Northrup, and Y. Shiu conceptualized the study, wrote the original draft, and reviewed and edited the manuscript; I. Falzon and H. Northrup were responsible for investigation; T. Lee and Y. Shiu were responsible for funding acquisition, project administration, resources, software, and supervision; and all authors were responsible for methodology.

Supplemental Material
This article contains the following supplemental material online at http://kiddney360.asnjournals.org/lookup/suppl/doi:10.34067/KID.K3602020000183/DCSupplemental.

Supplemental Figure 1. Proximal arterial tortuosity calculation.
Supplemental Figure 2. Inter- and Intra-user variability for proximal arterial cross-sectional area.
Supplemental Figure 3. Comparisons of proximal arterial cross-sectional area between mouse strains and post-surgery time points.
Supplemental Figure 4. Distal arterial tortuosity calculation.
Supplemental Figure 5. Inter- and Intra-user variability for distal arterial cross-sectional area.
Supplemental Figure 6. Comparisons of distal arterial cross-sectional area between mouse strains and post-surgery time points.

$$\text{Figure 8. Geometric parameters were mostly similar between mouse strains and postsurgery time points. (A) Anastomosis angle, (B) venous tortuosity, (C) nonplanarity angle magnitude, (D) maximum A-V distance. (E and F) Location of max A-V distance along the (E) artery or (F) vein centerline. Data are presented as mean±SEM. For WT day 7, WT day 21, KO day 7, KO day 21, OE day 7, and OE day 21, the animal number (n)=5, 2, 4, 3, 3, and 4, respectively. }^*P<0.05.$$
References


Received: April 6, 2020 Accepted: July 30, 2020