Appendix 1: Methods for Vascular Function Studies

Vascular function studies were performed by trained, certified study personnel within the 45 days prior to AVF creation surgery. Participants fasted for at least 6 hours prior to the studies and refrained from exercise starting at midnight before the studies. When possible, the studies were performed on a single day and the order for studies that were performed on the same day was: (1) venous occlusion plethysmography, (2) arterial pulse wave velocity, (3) brachial artery flow-mediated dilation, and (4) brachial artery nitroglycerin-mediated dilation. Prior to the first vascular function study on a given day, blood pressure and heart rate were measured after a 10-minute period of rest in a supine position. Three measurements were made using a SunTech 247 device (SunTech Medical) with at least one minute between readings. The arm intended for AVF creation was used for the vascular function studies, unless there was a patent arteriovenous vascular access in the arm.

Venous Occlusion Plethysmography

Participants were placed in a supine position with the arm supported and elevated above the level of the heart. The Hokanson EC5 strain gauge plethysmography device with NIVP3 software was used for waveform acquisition and analysis (D.E. Hokansan, Inc). A strain gauge of an appropriate size was placed around the forearm at the position of greatest diameter to measure change in forearm circumference. An SC10D arm cuff (Hokansan, Inc) placed on the upper arm was inflated for 3 minutes to the designated pressure and then deflated using an automatic rapid inflator device. Waveforms were acquired while the cuff was inflated and for 5 seconds after deflation was initiated. The procedure was performed at cuff inflations to 20 mm Hg, 30 mm Hg, 40 mm Hg, 50 mm Hg, and 60 mm Hg in succession. The linear regression equation for the relationship between the venous pressure (estimated as cuff pressure) and the change in forearm volume (ml/100ml forearm) was generated and the slope, expressed as % increase/mm Hg, was used as the indicator of venous capacitance. The maximum venous outflow (ml/100ml/min) after deflation was determined at each cuff pressure. The linear regression equation for the relation between venous pressure and the maximum venous outflow was generated, and the slope of the regression line was used as an indicator of venous outflow.

Carotid-Radial and Carotid-Femoral Arterial Pulse Wave Velocity

Pulse wave velocity (PWV) was measured using the SphygmoCor device (Atcor Medical). The carotid-radial distance was computed as the distance from the sternal notch to the radial pulse minus the distance from the sternal notch to the carotid pulse. The carotid-femoral distance was computed as the distance from the sternal notch to the femoral pulse minus the distance from the sternal notch to the carotid pulse. Pulse waveforms were recorded using applanation tonometry at the carotid followed by the radial sites for the carotid-radial PWV determination, and at the carotid followed by the femoral sites for carotid-femoral PWV determination. Waveform acquisition was repeated if the standard deviation for a set of 10 waveforms was >10%. The QRS complex from electrocardiogram leads served as the reference for the origin of the pulse waveform. Pulse wave velocities are expressed as m/sec.

Brachial Artery Flow-Mediated Dilation and Nitroglycerin-Mediated Dilation

After placement of a Custom Hokanson 3.25" X 22" blood pressure cuff with a quick release sphygmomanometer (Hokansan, Inc.) on the upper arm and 10 minutes of rest in a supine position, a high-resolution linear ultrasound probe (at least 7.5 mHz) was used to obtain 2-dimensional (2D) images of the brachial artery and pulsed wave Doppler signals. The blood pressure cuff was then inflated to 200 mmHg or 50 mmHg above the systolic blood pressure, whichever value was higher. After 5 minutes of inflation, the cuff was deflated. Fifteen seconds after deflation, brachial artery Doppler signals were obtained. 2D images gated on the R-wave were obtained from 55-65 seconds after deflation to determine flow-mediated dilation (FMD).

Following a 10-minute period of rest in a supine position, 2D ultrasound imaging of the brachial artery was performed at the same location used for the measurement of FMD. Image acquisition was repeated 3 minutes after administration of sublingual nitroglycerin 0.4 mg to determine nitroglycerin-mediated dilation (NMD). Nitroglycerin was not administered and NMD was not assessed if any of the systolic blood pressure readings were <100 mm Hg, if there was use of a phosphodiesterase type 5 inhibitor within the past 7 days, or if there was a history of migraine headaches or a history of nitroglycerin intolerance.

The 2D images were used for measurement of brachial artery diameter using customized software and the Doppler signals were used for determination of flow. Image analysis was performed at the HFM Vascular Function Core facility at Boston University. FMD and NMD are expressed as the post-ischemia percentage increase and post-nitroglycerin percentage increase in brachial artery diameter, respectively. Resting and hyperemic flow were determined from the Doppler signals.

Appendix 2: Statistical Appendix

Part 1: Description of Multiple Imputation Procedure.

Preparatory to all analyses, we first deleted all US measurements occurring after thrombosis and interventions to the AVF. The numbers of patients with non-missing VFT and AVF blood flow measurements at the respective follow-up US assessments, after these deletions, are provided in Appendix Table A1.

In order to incorporate information from available non-missing data, reduce risk of bias, and properly account for uncertainty resulting from the missing data, we used multiple imputation to impute missing VFT and ultrasound measurements prior to subsequent statistical analysis. Multiple imputation has been shown to provide superior statistical performance compared to strategies such as single imputation and complete-case analysis, where only subjects with complete data are included in the analysis[1,2].

The multiple imputation procedure required 4 steps:

Step 1. We determined which variables to include in the imputation model. Following standard guidelines[1,2]. we included in the imputation model all variables that were included as exposure variables, covariates, or outcome variables in the regression analyses presented in this report. These variables included each of the five pre-operative VFT variables, the post-operative 1-day, 2-week and 6-week AVF blood flow and AVF vein diameters, the pre-operative inflow artery diameter, brachial artery flow, and the minimum draining vein diameter, the four demographic covariates (age, sex, race, and dialysis status at surgery), clinical center, and fistula location (upper arm vs. forearm). In addition to these variables from our analytic models, the imputation model also included additional auxiliary variables that were deemed to be potential predictors of the missing VFT or US measurements or to be predictors of the pattern of missing responses. The full set of variables included in the imputation model is listed in Appendix Table A2. Key variables, including those used in the regression models considered in this manuscript, were forced into the imputation model as indicated in the second column of the table. Due to positive skewness, several variables were either log transformed or square root transformed prior to carrying out the multiple imputations. To prevent loss of precision from over-parameterization of the imputation models, other variables were included as predictor variables for imputation only of those variables with which their Spearman correlations exceeded 0.2. All variables in the imputation models, including clinical center, were treated as fixed effects.

Step 2. We applied the R package MICE to generate 10 imputed data sets. The multiple imputations were performed under a fully sequential imputation approach[3], in which 10 replacement values for each missing value were drawn randomly using predictive mean matching for continuous variables and logistic regression for categorical variables. As described in the methods section of the manuscript, all imputed measurements following thrombosis or death were however set to missing to avoid interpretational paradoxes.

Step 3. Each statistical analysis presented in the manuscript was performed separately for each of the 10 imputed data sets.

Step 4. We combined results of these 10 analyses using Rubin's formulae[1], thereby incorporating information on variation between the 10 imputed data sets to account in our analyses for uncertainty associated with the missing data.

Part 2: Details of Regression Models.

Each of the generalized linear mixed effects regression models that were used to relate US outcomes to VFT predictor variables included a random effect for clinical center to account for center-to-center variation in both the US outcomes and VFTs. Linear and quadratic terms for time from surgery to the US measurement were also included as additional covariates in the models for the US outcomes measured at the 1-day assessment. For models with average draining vein diameter as the outcome, we employed a restricted pseudo-maximum likelihood algorithm under a normal outcome model with a linear link function to relate the mean draining vein diameter to the predictor variables. For models with AVF flow as the outcome, we used a Gaussian quadrature algorithm with a minimum of 20 quadrature points under a gamma distributed outcome model with a logarithmic link function.

As described in the primary manuscript, the regression models controlled for fistula location as well as pre-operative US measurements of minimum vein diameter, inflow artery diameter, and brachial artery flow, plus age, race (Black vs. other), and sex and an indicator variable for whether chronic hemodialysis had been initiated prior to AVF placement. Each of the three pre-operative US measures were represented by natural cubic splines with 4 equally spaced knots to accommodate potential nonlinear relationships. Additional covariates were defined as pairwise interactions between the pre-operative venous and artery diameters and the upper arm flow, and between the pre-operative US measurements and fistula location. An additional interaction term between artery and minimum vein diameter was not included in the models as this term was found to be highly correlated with the other terms in the model.

[1] Rubin, Donald B. (1987) *Multiple Imputation for Nonresponse in Surveys.* New York: Wiley.

[2] Schafer, Joseph L. "Multiple imputation: a primer." *Statistical methods in medical research* 8.1 (1999): 3-15.

[3] Van Buuren, Stef, et al. "Fully conditional specification in multivariate imputation." *Journal of statistical computation and simulation* 76.12 (2006): 1049-1064.

Table A1: Numbers of non-missing measurements in analyses of VFT parameters

VFT Parameter	Number of non-missing VFT measurements[1]	Number of subjects (%) included in analyses after multiple imputation of both missing US values and missing VFT measurements[1]			Number of subjects with non-missing AVF-blood flow and VFT values			Number of subjects with non-missing AVF-vein diameter and VFT values		
		Day 1	Week 2	Week 6	Day 1	Week 2	Week 6	Day 1	Week 2	Week 6
Brachial Artery FMD	549 (91%)	587 (98%)	570 (95%)	555 (92%)	494 (82%)	479 (80%)	446 (74%)	497 (83%)	477 (79%)	446 (74%)
Brachial Artery NMD	460 (76%)	587 (98%)	570 (95%)	555 (92%)	413 (69%)	400 (66%)	373 (62%)	415 (69%)	399 (66%)	373 (62%)
Carotid-femoral PWV	448 (74%)	587 (98%)	570 (95%)	555 (92%)	403 (67%)	392 (65%)	366 (61%)	407 (68%)	394 (65%)	369 (61%)
Carotid-radial PWV	449 (75%)	587 (98%)	570 (95%)	555 (92%)	404 (67%)	393 (65%)	367 (61%)	408 (68%)	395 (66%)	370 (61%)
VOP	569 (95%)	587 (98%)	570 (95%)	555 (92%)	510 (85%)	490 (81%)	461 (77%)	514 (85%)	489 (81%)	462 (77%)

[1] Number and percent of patients with non-missing VFT measurements, irrespective of whether post-operative US measurements were missing

[2] Number and percent of patients included in analyses relating post-operative US measurements to pre-operative VFT measurements following multiple imputation of missing values. Entries are less than 602 because patients who died or reached thrombosis prior to the planned US assessments were excluded.

[3] Number and percent of patients who had either a missing post-operative AVF blood flow or a missing pre-operative VFT measurement prior to multiple imputation of missing measurements. AVF blood flows measured following intervention or thrombosis were set to missing prior to data analyses, and are counted as missing in this table.

[4] Number and percent of patients who had either a missing post-operative AVF vein diameter or a missing pre-operative VFT measurement prior to multiple imputation of missing measurements. AVF vein diameters measured following intervention or thrombosis were set to missing prior to data analyses, and are counted as missing in this table.

Table A2: Variables Incorporated in Multiple Imputation Model

Variable	Among variable set "forced" to predict all variables	Categorical Variable
Clinical Center	X	X
Female Sex	X	X
Black Race	X	X
Age at surgery	X	
Diabetes	X	X
History of Coronary Artery Disease [1]	X	X
History of Peripheral Artery Disease [2]	X	X
History of Coagulation Problems [3]		X
History of Cerebral Vascular Disease [4]	X	X
History of Inflammatory Disease		X
History of Congestive Heart Failure		X
History of Cardiac Arrhythmias or Conduction		X
History of Hyperlipidemia		X
Serum albumin (g/dL)		
Calcium (mg/dL)		
Phosphorus (mg/dL)		
Hemoglobin (g/dL)		
Serum creatinine (mg/dL)		
On Dialysis at Surgery	X	X
Interaction Between Hemodialysis at Surgery and Serum Creatinine		
Previous Catheter in Surgery Arm		X
Number Previous Permanent Vascular Accesses	X	
Baseline Antiplatelet Agent Use		X
Baseline Anticoagulant Use		X
Baseline Statin Use		X

Highest Education Level		X
Married / Living with Partner		X
Smoking Status Category (Current, Former &. Never)	X	X
Body Mass Index (kg/m2)	X	
Insurance Category (None, Medicaid/Medicare & Other)		X
General Physical Activity Index [5]		
Fistula Arm Physical Activity Index [6]		
Fistula Location (Upper Arm vs. Forearm)	X	X
Upper Arm Fistula Configuration [7]		X
PRE-OP Artery Calcification [8]		X
PRE-OP Artery Diameter [9]	X	
PRE-OP Min. Vein Diameter [10]	X	
PRE-OP Ave. Vein Diameter [10]		
PRE-OP Upper Arm Flow (log transformed) [11]	X	
PRE-OP Total Upper Arm Flow (log transformed) [12]		
PRE-OP Spectral Wave Form [13]		
PRE-OP Forearm Cephalic Vein Depth [14]		
PRE-OP Upper Arm Cephalic Vein Depth [14]		
Draining Vein Type (Basilic, Cephalic; Brachial)		X
Surgery Duration ≥ 3 Hours without Concomitant Procedure		X
Peri-Surgical Heparin Use		X
Peri-Surgical Protamine Use Following Heparin		X
Peri-Surgical Topical Thrombin Use		X
Peri-Surgical Topical Vasodilators		X
Anesthesia Category (Local, Regional, & General)		X
Arterial Vascular Control Method: Vascular clamps vs. Vessel Loops		X
Arteriotomy Length		
Concomitant Procedures During Surgery		X
Physician Recommendation for Post-op Ball Squeezing (always vs. sometimes/never)		X

Total AVFs Created by Attending Surgeon 2007-2009		
Attending Performs Anastomosis		X
Surgeon Routine Use of Post-op US		X
Thrill Present		X
Thrill Extent if Present		X
Surgeon Frustrated During Surgery		X
Surgeon's Prediction of Success (Marginal, Likely & None)		X
DAY 1 Stenosis (None, at least 1 distal stenosis, anastomotic stenosis only)		X
DAY 1 Artery Diameter		
DAY 1 Average Vein Diameter[15]	X	
DAY 1 Minimum Vein Diameter[15]		
DAY 1 Fistula Flow (Square Root-Transformed)[16]	X	
DAY 1 Vein Depth [17]	X	
DAY 1 Total Upper Arm Arterial Flow (Square Root-Transformed)		
Vein Vascular Control Method: (Loops, Clamps, Occlusion, None)		X
Transposed Vein Fistula		X
FMD%	X	
NMD%	X	
FMD Hyperemic (Post-Cuff) Velocity		
Carotid-Femoral Pulse Wave Velocity	X	
Carotid-Radial Pulse Wave Velocity	X	
FMD Augmentation Index (%)		
VoP Capacitance Slope	X	
VoP Maximum Venous Outflow Slope	X	
Average Of 3 Diastolic Blood Pressures From Day Of VF Test		
Average Of 3 Systolic Blood Pressures From Day Of VF Test		
Max. - Min. Heart Rate From Day Of VF Test		
Average Of 3 Heart Rate Measures From Day Of VF Test		
% Medial Muscle Layer Occupied By Collagen	X	

Neointimal Hyperplasia (%)		
Luminal Narrowing (per 10%)	X	
Intimal Or Medial Calcification	X	X
Week 2 Stenosis (None, at least 1 distal stenosis, anastomotic stenosis only)		X
Week 2 Artery Diameter		
Week 2 Average Vein Diameter [15]	X	
Week 2 Minimum Vein Diameter [15]		
Week 2 Fistula Flow (Square Root-Transformed) [16]	X	
Week 2 Vein Depth [17]	X	
Week 2 Total Upper Arm Arterial Flow (Square Root-Transformed)		
Week 6 Stenosis (None, at least 1 distal stenosis, anastomotic stenosis only)		
Week 6 Artery Diameter		
Week 6 Average Vein Diameter [15]	X	
Week 6 Min. Vein Diameter [15]		
Week 6 Fistula Flow (Ml/Min) (Square Root-Transformed) [16]	X	
Week 6 Vein Depth [17]	X	
Week 6 Total Upper Arm Arterial Flow (Square Root-Transformed)		
Venogram Or Arteriogram Or Fistulogram Done Prior To Unassisted Maturation		X
Unassisted Clinical Maturation	X	X
Overall Clinical Maturation [18]	X	X

1 Coronary artery disease (myocardial infarction, angina, coronary artery bypass surgery, percutaneous coronary intervention)

2 Peripheral artery disease history (claudication, lower extremity angioplasty or bypass surgery , non-traumatic amputation)

3 Coagulation Problem (pulmonary embolism, deep vein thrombosis, known hypercoagulable state)

4 Cerebrovascular disease (stroke or TIA, prior carotid endarterectomy, carotid angioplasty) Inflammatory disease (scleroderma, vasculitis, SLE, inflammatory bowel disease)

5 Categorized as either 1) Employed, physical labor job, 2) Exercises 3+ days a week, 3) Heavy chores or force involving activity, 4) Freely arm moving rec activities or musical instrument, 5) None of the above

6 Defined by adding: +1 if the patient reported participation in activities in which there was force through the arm, shoulder, or hand (e.g., hammering); + 1 if the patient reported participation in

recreational activities in which the arm was moved freely (e.g., freesbie); + 1 if the patient was currently employed in a job requiring use of the arm in physical labor; -1 if the patient reported problems with the arm, shoulder, or hand that led to limitation in work and daily activities. The sum of these 4 numbers led to a score between -1 and +3; the top two categories with scores of +2 and +3 were combined to form the final index with a score between -1 and +2.

[7] Upper arm basilic vein transposition and antecubital artery, proximal forearm artery or upper arm basilic vein transposition and antecubital artery. Upper arm cephalic vein and antecubital/proximal forearm artery.

[8] Brachial or radial artery calcification categorized as absent or mild/moderate/severe

[9] In forearm fistulas, radial artery diameter 2 cm cranial wrist; In upper arm fistulas, brachial artery 2 cm cranial to antecubital fossa if no high takeoff and radial artery diameter OR ulnar artery diameter (depending on whether fistula attached to radial or ulnar artery) if high takeoff.

[10] The minimum or average of the draining vein diameter measurements at 3 pre-specified locations depending on the vein

[11] Log transformation of average of 3 brachial artery flows if no high takeoff and of average of 3 radial flows OR average of 3 ulnar flows (depending on whether fistula attached to radial or ulnar artery) if high takeoff.

[12] Log transformation of average of 3 brachial artery flows if no high takeoff and average of 3 radial flows + average of 3 ulnar flows if high takeoff.

[13] Brachial or radial artery spectral wave form (PSV cm/sec).

[14] The average of the draining vein depth measured at 3 pre-specified locations.

[15] The minimum or average of the draining vein diameter measurements at 2, 5, 10, and 15 cm from anastomosis

[16] Square root transformation of average of 3 blood flow measurements.

[17] The average of draining vein depth at 2, 5, 10, and 15 cm from anastomosis.

[18] Includes assisted and unassisted clinical maturation.

<u>Appendix 3: Association of VFTs with postoperative AVF flow rate and diameter, with analysis restricted to patients with non-missing VFT measurements.</u>

Tables A3 and A4 present sensitivity analyses that correspond to Tables 3 and 4 of the main manuscript. Whereas missing VFT measurements were multiply imputed in the analyses presented in Tables 3 and 4, patients with missing VFT measurements were excluded from the sensitivity analyses presented in Tables A3 and A4 below. The results were similar between the two sets of tables.

Table A3: Association of 6-week AVF flow rate with individual VFT factors, controlling for AVF location, preoperative ultrasound features*, and baseline demographics **, excluding patients with missing VFT measurements.

Predictor variable	*Difference in blood flow (%)*	*95% CI*	*p-value*
Brachial FMD (per 10 % increase)	11.3	(-0.2, 24.1)	0.06
Brachial NMD (per 10 % increase)	14.3	(2.6, 27.3)	0.02
Carotid-femoral PWV (per 4 m/sec increase)	-4.9	(-12.0, 2.8)	0.21
Carotid-radial PWV (per 4 m/sec increase)	13.9	(-1.1, 31.3)	0.07
VOP (per 1% increase in CAP slope)	1.8	(-11.6, 17.3)	0.80

FMD, flow-mediated dilation; NMD, nitroglycerin-mediated dilation; VOP, venous occlusion plethysmography; CAP, venous capacitance; PWV, pulse wave velocity.
*Preoperative inflow-artery diameter, minimal vein diameter, and brachial artery flow
**Age, gender, race, and dialysis status
None of the summarized relationships exhibited statistically significant departures from linearity ($p > 0.05$ for evaluation of nonlinearity using cubic spline models).
Multiple imputation was use to impute missing AVF flow rate measurements. However, patients with missing VFT measurements were excluded from these analyses.

Table A4: Association of 6-week AVF diameter with individual VFT factors, controlling for AVF location, preoperative ultrasound features*, and baseline demographics **, excluding patients with missing VFT measurements.

Predictor variable	*Difference in diameter (mm)*	*95% CI*	*p-value*
Brachial FMD (per 10 % increase)	0.36	(0.10, 0.61)	0.006
Brachial NMD (per 10 % increase)	0.41	(0.19, 0.64)	<0.001
Carotid-femoral PWV (per 4 m/sec increase)	-0.23	(-0.41, -0.04)	0.02
Carotid-radial PWV (per 4 m/sec increase)	-0.08	(-0.42, 0.25)	0.63
VOP (per 1% increase in CAP slope)	0.31	(-0.03, 0.64)	0.07

FMD, flow-mediated dilation; NMD, nitroglycerin-mediated dilation; VOP, venous occlusion plethysmography; CAP, venous capacitance; PWV, pulse wave velocity.
*Preoperative inflow-artery diameter, minimal vein diameter, and brachial artery flow
**Age, gender, race, and dialysis status
None of the summarized relationships exhibited statistically significant departures from linearity ($p > 0.05$ for evaluation of nonlinearity using cubic spline models).
Multiple imputation was use to impute missing AVF vein diameter measurements. However, patients with missing VFT measurements were excluded from these analyses.