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# Association between Preoperative Vascular Function and Postoperative Arteriovenous Fistula Development

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## ABSTRACT

Arteriovenous fistula (AVF) maturation failure is the primary cause of dialysis vascular access dysfunction. To evaluate whether preoperative vascular functional properties predict postoperative AVF measurements, patients enrolled in the Hemodialysis Fistula Maturation Study underwent up to five preoperative vascular function tests (VFTs): flow-mediated dilation (FMD), nitroglycerin-mediated dilation (NMD), carotid-femoral pulse wave velocity, carotid-radial pulse wave velocity, and venous occlusion plethysmography. We used mixed effects multiple regression analyses to relate each preoperative VFT to ultrasound measurements of AVF blood flow rate and venous diameter at 1 day, 2 weeks, and 6 weeks after AVF placement. After controlling for AVF location, preoperative ultrasound measurements, and demographic factors (age, sex, race, and dialysis status), greater NMD associated with greater 6-week AVF blood flow rate and AVF diameter (per absolute 10% difference in NMD: change in blood flow rate =14.0%; 95% confidence interval [95% CI], 3.7% to 25.3%; $P<0.01$; change in diameter =0.45 mm; 95% CI, 0.25 to 0.65 mm; $P<0.001$). Greater FMD also associated with greater increases in 6-week AVF blood flow rate and AVF diameter (per absolute 10% difference in FMD: change in blood flow rate =11.6%; 95% CI, 0.6% to 23.9%; $P=0.04$; change in diameter =0.31 mm; 95% CI, 0.05 to 0.57 mm; $P=0.02$). None of the remaining VFT parameters exhibited consistent statistically significant relationships with both postoperative AVF blood flow rate and diameter. In conclusion, preoperative NMD and FMD positively associated with changes in 6-week AVF blood flow rate and diameter, suggesting that native functional arterial properties affect AVF development.

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Although the arteriovenous fistula (AVF) is considered the preferred type of chronic hemodialysis vascular access,[1] a substantial proportion of new AVFs fails to mature sufficiently to be used for dialysis.[2–4] AVFs are created by a direct anastomosis between a native artery and vein, and successful AVF maturation requires substantial increase in the blood flow rate and diameter of the inflow artery and draining vein to support the high blood flow that is needed in the extracorporeal dialysis circuit.[5] Others have reported a rapid increase in the AVF blood flow rate and diameter that occurs as early as 1 day after AVF creation and continues to increase progressively over the subsequent few weeks.[6–9] Preoperative

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arterial and venous functional properties may modulate the magnitude of these postoperative changes.

The ability of arteries and veins to dilate in response to physiologic stimuli can be assessed noninvasively with several vascular function tests (VFTs). Flow-mediated dilation (FMD) and nitroglycerin-mediated dilation (NMD) assess the capacity of the brachial artery to dilate. FMD measures the increase in brachial artery diameter in response to a sudden increase in blood flow and partly depends on the ability of the endothelium to release the endogenous vasodilator nitric oxide.[10] In contrast, NMD measures the intrinsic ability of the arterial media to respond directly to an exogenous nitric oxide donor and is, therefore, independent of a functional endothelium.[10] Pulse wave velocity (PWV) measures the velocity of arterial blood flow, and it is directly related to arterial stiffness and inversely related to the ability of the artery to dilate in response to increased pressure.[11,12] Carotid-femoral PWV largely provides a measure of stiffness of the aorta, an elastic artery, whereas carotid-radial PWV relates to stiffness of the muscular arteries in the upper extremity. Finally, venous occlusion plethysmography (VOP) measures venous capacitance (CAP; *i.e.*, the ability of the vein to dilate to accommodate the increased blood volume). Preoperative measures of VOP were shown in a small study to predict AVF maturation.[13] It is, therefore, plausible that each of these VFTs predicts the physiologic responses of the AVF (*e.g.*, change in the blood flow rate and luminal diameter) after its creation, but there is a dearth of information in this context. Relationships of preoperative VFTs with postoperative AVF blood flow and diameter could provide insight into the mechanisms underlying these aspects of AVF maturation.

The Hemodialysis Fistula Maturation (HFM) Study is a prospective multicenter cohort study of AVF maturation.[14] Study participants underwent standardized preoperative ultrasounds of the upper extremity arteries and veins and VFTs within 45 days before AVF surgical creation. In this report, postoperative AVF ultrasounds performed at 1 day, 2 weeks, and 6 weeks were used to evaluate the relationships of preoperative functional properties of arteries and veins with subsequent AVF characteristics. We hypothesized that higher FMD and NMD, lower PWV, and higher venous CAP would be associated with greater AVF blood flow rate and diameter after AVF creation.

## RESULTS

Table 1 summarizes baseline demographic and clinical characteristics of the study cohort. The preoperative ultrasound vascular measurements (medians and 15th to 85th percentiles) were as follows: inflow artery diameter of 3.9 mm (2.4–5.0 mm), minimum vein diameter of 3.0 mm (1.7–4.1 mm), and brachial artery flow rate of 62.7 ml/min (37.0–107.3 ml/min). Excluding patients who experienced AVF thrombosis or intervention, the 6-week postoperative ultrasound AVF measurements were as follows: inflow artery diameter of 4.8 mm (3.4–5.9 mm), average vein diameter of 6.2 mm (4.8–8.1 mm), and AVF blood flow of 917 ml/min (426–1628 ml/min). Table 2 summarizes the results of the five preoperative VFTs included in this study, including the 85th and 15th percentiles.

**Table 1.** Baseline demographic and clinical characteristics of the study cohort (*n*=602)

| Baseline Factor | *N* (%) or Median (15th to 85th Percentiles) |
|---|---|
| Age, yr | 56.4 (40.8–68.5) |
| Women | 180 (30) |
| Black[a] | 264 (44) |
| On hemodialysis | 383 (64) |
| Diabetes | 353 (59) |
| Peripheral artery disease | 91 (15) |
| Coronary artery disease | 156 (26) |
| Upper arm fistula | 459 (76) |
| Body mass index, kg/m$^2$ | 29.3 (22.8–38.2) |
| Vascular calcification[b] | 265 (44) |

[a]Self-reported race was missing for eight subjects, all with upper arm AVFs.
[b]Vascular calcification (on preoperative ultrasound) score ≥1 on a 0–2 scale.

Figure 1 depicts the relationships between the ultrasound outcomes and each VFT predictor variable after adjustment for AVF location, the preoperative ultrasound assessment, and several demographic case mix factors as covariates. Each relationship is normalized to provide estimates of adjusted comparisons in the ultrasound outcomes between the 85th and 15th percentiles of the respective VFT variables to simplify comparison of the strength of the relationships with the ultrasound outcomes across the five VFT factors. The comparisons of the ultrasound outcomes between the 85th and 15th VFT percentiles are expressed as adjusted ratios of the geometric mean levels of AVF blood flow rate and adjusted algebraic differences in mean AVF vein diameter. We used differences for absolute diameters but ratios for blood flow rates, because AVF diameters were symmetrically distributed, whereas AVF flows were positively skewed in the study cohort. Each comparison was made using a mixed effects regression analysis with a random clinical center effect and fixed effects for the VFT predictor variable and the covariates noted above.

The preoperative brachial NMD was positively associated with both the postoperative AVF diameter (Figure 1, left panel) and blood flow rate (Figure 1, right panel). These associations reached statistical significance by the 2-week ultrasound assessment and continued to strengthen at the 6-week ultrasound assessment. Brachial FMD exhibited similar, although slightly less pronounced, relationships with 6-week AVF diameter and blood flow rate. None of the other three VFTs (CAP slope, carotid-femoral PWV, or carotid-radial PWV) exhibited consistent statistically significant relationships with postoperative AVF diameter or blood flow rate, although the 2-week AVF vein diameter exhibited a weak but nominally

**Table 2.** Values for preoperative VFTs for the fully imputed data

| VFT | *N* of Patients | Mean | SD | Median | 15th to 85th Percentiles |
|---|---|---|---|---|---|
| Brachial artery FMD, % | 549 | 4.8 | 5.0 | 3.9 | 0.34–9.81 |
| Brachial artery NMD, % | 460 | 7.2 | 6.3 | 5.8 | 1.25–13.97 |
| Carotid-femoral PWV, m/s | 448 | 10.7 | 3.2 | 10.0 | 7.6–13.7 |
| Carotid-radial PWV, m/s | 449 | 8.8 | 1.7 | 8.8 | 7.1–10.6 |
| CAP slope by VOP, % | 569 | 0.53 | 0.36 | 0.47 | 0.29–0.70 |

Summary statistics are on the basis of observed data only without imputation of missing values.

significant direct association with the CAP slope and an inverse association with carotid-femoral PWV. Very similar results were obtained when each of these analyses was restricted to the subcohort of patients with nonmissing preoperative VFT measurements (data not shown).

Tables 3 and 4 provide additional details on the relationships between baseline VFTs and the postoperative AVF diameter and blood flow measurements at 6 weeks. Each 10% higher absolute level of brachial NMD was associated with a 14.0% (95% confidence interval [95% CI], 3.7% to 25.3%) higher 6-week AVF blood flow rate and a 0.45 mm (95% CI, 0.25 to 0.65 mm) greater AVF diameter. Similarly, for each 10% higher absolute level of brachial FMD, there was an 11.6% (95% CI, 0.6% to 23.9%) higher AVF blood flow rate and a 0.31 mm (95% CI, 0.05 to 0.57 mm) greater 6-week AVF diameter. Qualitatively similar relationships were obtained when the analyses were restricted to subjects with

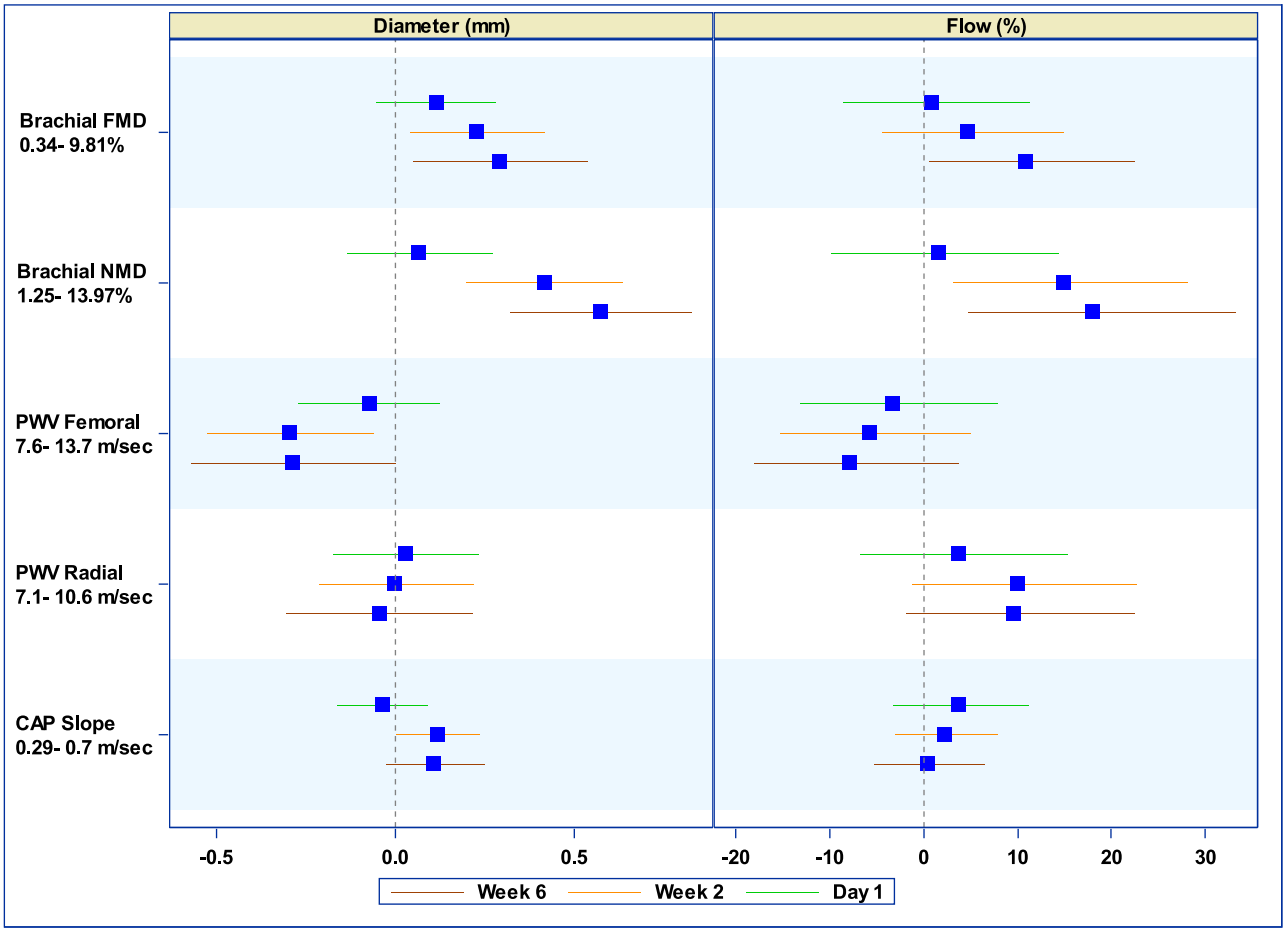


**Figure 1.** Postoperative AVF diameter and blood flow are associated with the preoperative FMD and NMD. Ratios of mean AVF blood flow rates and differences in mean AVF vein diameters (millimeters) between the 85th and 15th percentiles of preoperative VFT results using mixed effects regression models. Results are adjusted for AVF location, preoperative ultrasound measures (inflow artery diameter, minimum vein diameter, and brachial artery blood flow), and baseline demographics (age, sex, race, and dialysis status). Expressing the results as comparisons between the 85th and 15th percentiles for each VFT factor allows the strength of the displayed relationships to be compared between the different VFT factors. Results are displayed for day 1 (top line; green), week 2 (middle line; orange), and week 6 (bottom line; brown) ultrasounds. The blue squares represent the estimated ratios of mean blood flow rates or mean differences in diameters for VFTs at the 85th and 15th percentiles, and the colored lines are the 95% CIs. Multiple imputation was performed to impute missing vascular function and ultrasound measurements.

**Table 3.** Association of 6-week AVF flow rate with preoperative predictor VFT variables controlled for AVF location, preoperative ultrasound features, and baseline demographics

| Predictor Variable | Difference in Blood Flow, % | 95% CI | *P* Value |
|---|---|---|---|
| Brachial FMD per 10% increase | 11.6 | 0.6 to 23.9 | 0.04 |
| Brachial NMD per 10% increase | 14.0 | 3.7 to 25.3 | <0.01 |
| Carotid-femoral PWV per 4-m/s increase | −5.2 | −12.2 to 2.5 | 0.18 |
| Carotid-radial PWV per 4-m/s increase | 11.2 | −2.0 to 26.1 | 0.10 |
| VOP per 1% increase in CAP slope | 1.2 | −12.2 to 16.6 | 0.87 |

Preoperative ultrasound features were preoperative inflow artery diameter, minimum vein diameter, and brachial artery flow. Baseline demographics were age, sex, 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 used to impute missing vascular function and ultrasound measurements.

nonmissing VFT measurements (Supplemental Appendices 1–3, Supplemental Tables 1–4). Figure 2 depicts the shapes of the relationships of brachial NMD with the 6-week AVF blood flow rate and diameter.

## DISCUSSION

Given that all five preoperative VFTs used in this study assessed vascular functional properties that are of potential relevance to AVF maturation, we hypothesized that each of them would be associated with postoperative changes in AVF blood flow rate and diameter. Consistent with our hypothesis, we observed statistically significant associations of NMD and FMD with both the 6-week postoperative AVF flow rate and diameter, with slightly stronger relationships observed for NMD compared with FMD. Although we had hypothesized that stiffness of the arterial conduit used to create the AVF, as assessed by carotid-radial PWV, would restrict arterial outward remodeling,[15] this study failed to show such a relationship. Although carotid-femoral PWV exhibited a trend for an inverse relationship with 6-week AVF diameter, neither carotid-femoral PWV nor carotid-radial PWV exhibited statistically significant relationships with AVF blood flow. Finally, although we observed a weak inverse relationship of VOP with vein diameter at 2 weeks, we did not observe a consistent relationship of VOP with AVF blood flow or diameter across the three consecutive postoperative assessments.

FMD and NMD measure the ability of arteries to dilate in response to physiologic and direct biochemical stimuli, respectively. Thus, one might expect patients with higher FMD and NMD values to have higher postoperative AVF blood flow rates and diameters. FMD measures endothelium–dependent arterial responsiveness to hyperemia. After release of the BP cuff, blood flow increases in response to local vasodilators released during ischemia, and this increased blood flow results in increased shear stress on the arterial wall, which in turn, stimulates the release of nitric oxide from the endothelium and subsequent dilation of healthy arteries. FMD was originally considered to result almost exclusively from shear stress–induced endothelial production of nitric oxide.[16] Because the endothelium is the primary source of nitric oxide in the vasculature, FMD was considered a marker of endothelial function, with attenuated FMD reflecting a diseased endothelium. More recent data, however, suggest that FMD reflects not only nitric oxide production[17,18] but also, the arterial response to other vasodilatory factors, such as prostaglandins, adenine, and endothelium–derived hyperpolarizing factor.[19–21]

In contrast to FMD, NMD measures arterial dilation after exogenous administration of nitroglycerin, an exogenous nitric oxide donor. Thus, NMD assesses endothelium-independent vasodilation and reflects both physical properties of the arterial wall and arterial smooth muscle function. One would, therefore, expect greater vasodilatory responses to exogenous nitric oxide to translate into higher postoperative AVF blood flow rate and diameter, and this was, in fact, observed in this study (Figures 1 and 2, Tables 3 and 4).

PWV assesses arterial stiffness. Because stiffer vessels dilate less, one would expect negative associations of changes in postoperative AVF flow rate and diameter with preoperative

**Table 4.** Association of 6-week AVF diameter with preoperative predictor VFT variables controlled for AVF location, preoperative ultrasound features, and baseline demographics

| Predictor Variable | Difference in Diameter, mm | 95% CI | *P* Value |
|---|---|---|---|
| Brachial FMD per 10% increase | 0.31 | 0.05 to 0.57 | 0.02 |
| Brachial NMD per 10% increase | 0.45 | 0.25 to 0.65 | <0.001 |
| Carotid-femoral PWV per 4-m/s increase | −0.19 | −0.37 to −0.00 | 0.05 |
| Carotid-radial PWV per 4-m/s increase | −0.05 | −0.35 to 0.25 | 0.76 |
| VOP per 1% increase in CAP slope | 0.27 | −0.06 to 0.61 | 0.11 |

Multiple imputation was applied to impute missing vascular function and ultrasound measurements.
Preoperative ultrasound features were preoperative inflow artery diameter, minimum vein diameter, and brachial artery flow. Baseline demographics were age, sex, race, and dialysis status. Multiple imputation was used to impute missing vascular function and ultrasound measurements.

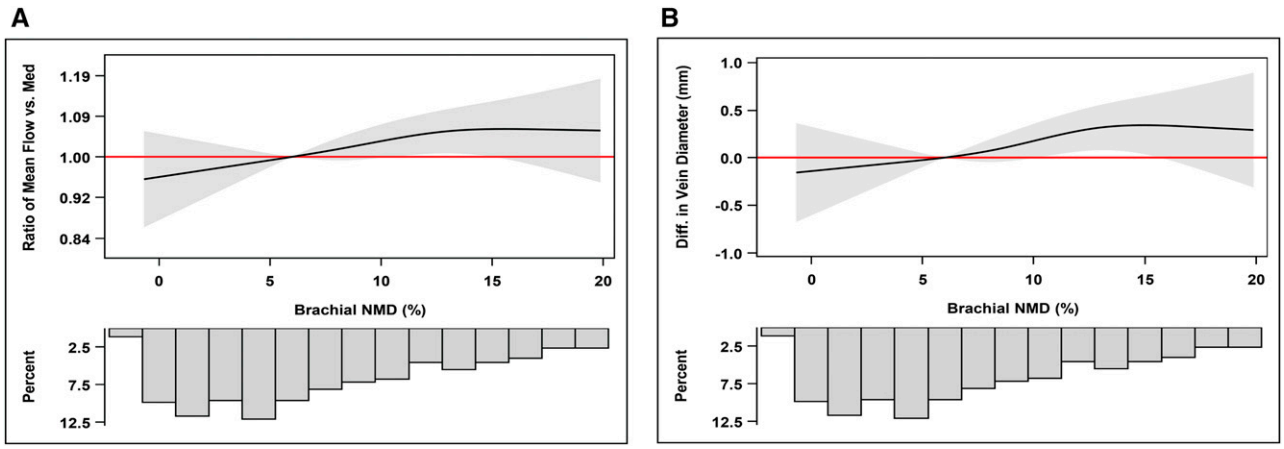


**Figure 2.** A greater preoperative NMD is associated with a greater 6-week postoperative AVF blood flow and diameter. (A) The *y* coordinates on the solid curve provide the ratio of the adjusted geometric mean 6-week AVF blood flow rate at the indicated brachial NMD level to the adjusted geometric mean blood flow rate at the median brachial NMD, which is used as the reference. (B) The *y* coordinates provide the difference between the adjusted mean 6-week AVF vein diameter at the indicated brachial NMD and the adjusted mean AVF vein diameter at the median brachial NMD reference. Analyses are adjusted for AVF location, baseline ultrasound measurements, and baseline case mix covariates. Multiple imputation was performed to impute missing vascular function and ultrasound measurements. The shaded regions indicate pointwise 95% CIs. Histograms below the curves show the distribution of NMD values in the study cohort.

PWV. Carotid-femoral PWV focuses on the stiffness of the central aorta, an elastic artery. Carotid-femoral PWV might be relevant to AVF maturation, because central aortic stiffness is an important determinant of the pulsatility and mechanical forces acting on the newly created AVF. In contrast, the carotid-radial PWV measures the stiffness of more peripheral (axillary, brachial, and radial) arteries that are muscular arteries. Notably, both PWV techniques are limited, because they represent indirect measures of arterial stiffness and are influenced by the collective stiffness of several different arteries that may differ in their stiffness.

Whereas NMD, FMD, and PWV each measure the functional properties of arteries, VOP assesses the ability of veins to dilate in response to physical forces, namely blood engorgement by cuff occlusion of the arm. A previous study of 17 patients found a positive association of AVF maturation with preoperative CAP assessed by VOP.[13] VOP has been the gold standard for measuring limb blood flow for many years.[22] However, it is not highly reliable for measuring the compliance of large veins for several reasons. First, it estimates the overall compliance of the entire venous system in the limb rather than only the compliance of the large veins, which are the vessels of interest in AVF maturation. Second, it does not directly measure the change in venous volume but rather, the sum of changes in both intravascular and extravascular volumes in the arm.[23] Third, the relationship between cuff pressure and actual venous pressure may differ in patients with increased resting venous pressure.[24,25] These limitations of VOP are balanced by its noninvasive nature, relative simplicity, and long track record. To more accurately investigate the relationship between the mechanical properties of the vein used for AVF creation and postoperative AVF remodeling, direct measurement of the vein of interest would be necessary. For example, the elasticity imaging technique using high–resolution ultrasound speckle tracking, which has been tested on a patient with CKD in a proof of concept study,[26] might be used for this purpose.

This study has several strengths, including the large number of patients from multiple clinical centers, the inclusion of both forearm and upper arm AVFs, the utilization of multiple types of VFTs, and the assessment of both arteries and veins as well as the standardized central training and quality assurance for the VFTs and ultrasound techniques across centers.

This study has three notable limitations. First, the process of associating two AVF ultrasound outcome measures (AVF blood flow rate and diameter) in separate models with each of five VFT predictors at three time points for a total of 30 ($2\times3\times5$) 5%–level hypothesis tests is vulnerable to false positive results as a consequence of multiple comparisons. However, even after conservative Bonferroni adjustments for 30 comparisons, the *P* value for the relationship of brachial NMD with 6-week AVF diameter remained statistically significant. The observation of consistent relationships of brachial NMD and FMD with AVF diameter and blood flow rate across two of the three ultrasound assessments (2 and 6 weeks) also alleviates this concern. Second, the proportion of missing VFT measurements was relatively high (Table 2). Our use of a comprehensive model for multiple imputation and the consistency of our results between analyses incorporating imputed values for missing VFT measurements and analyses restricted to nonmissing VFT measurements suggest that the reported relationships of brachial NMD and FMD with the ultrasound outcomes are unlikely to be the result of bias because of missing data. Third, even after multiple imputation, it was necessary to restrict

regression analyses involving postoperative ultrasound measurements to ultrasound assessments planned to occur before patient death or AVF thrombosis to avoid interpretational paradoxes. However, the proportions of subjects excluded for this reason were relatively small (ranging from 2% to 8%) (Supplemental Table 1).

In summary, in this multicenter observational study of new AVFs, two preoperative VFTs, arterial NMD and FMD, were associated with the postoperative changes in AVF blood flow rates and vein diameter after controlling for AVF location, preoperative vascular diameters and blood flow rates, and baseline demographics. The relationship of NMD and FMD with postoperative AVF measurements suggests a role of preexisting functional properties of arteries in the early remodeling of AVF after its creation. This observation raises the possibility that pharmacologic interventions to improve arterial function in patients with CKD may improve AVF maturation.

## CONCISE METHODS

### Study Design

The HFM Study enrolled 602 patients identified at the time that they were scheduled for AVF creation at seven clinical centers. Details of the overall study design and its rationales have been published previously.[14] Patients were eligible if they were currently on maintenance hemodialysis or anticipated to require hemodialysis within 3 months; were scheduled for a single–stage AVF creation surgery in the upper extremity; were $<80$ years of age if they had not yet started dialysis; had a life expectancy of $\geq 9$ months; and were willing and able to comply with the study procedures. Furthermore, to qualify for the study, patients were required to complete a preoperative vascular ultrasound and testing in at least two of the following three preoperative VFT categories: arterial dilation as assessed by FMD and/or NMD, arterial stiffness as assessed by carotid-femoral and/or carotid-radial PWV, and venous CAP as assessed by VOP. The participants underwent standardized postoperative AVF ultrasound at 1 day, 2 weeks, and 6 weeks to measure AVF blood flow rate and draining vein diameter averaged over several locations along its length.[27,28]

### Brief Overview of the VFTs

The clinical site personnel were trained by the HFM Study Vascular Function Core to perform the VFTs using standardized protocols. Core staff analyzed the brachial artery images transmitted by the clinical centers to calculate the FMD and NMD (both reported as percentages of the baseline value). The individual VFT tests are summarized below, and detailed information is provided in Supplemental Appendix 1. All tests were obtained within 45 days before the AVF creation surgery. Tests were performed in the order given below on the arm to be used for the AVF creation unless a patent AVF was present in that arm.

*VOP*

The forearm volume was measured using a strain–gauge plethysmography device during application of an upper arm BP cuff at increasing but subsystolic pressures. Venous CAP slope was estimated from the volume-pressure relationship and expressed as a percentage increase in volume per millimeters of mercury.

*PWV*

Carotid-femoral and carotid-radial PWVs were determined using the SphygmoCor Device (AtCor Medical), with velocity expressed as meters per second. Tonometry signals were obtained at the locations of the carotid, femoral, and radial pulses. Measured distances of each pulse from the sternal notch were used to calculate pulse wave propagation distances.

*FMD*

The brachial artery diameter was measured by ultrasound at baseline. A BP cuff was inflated on the upper arm to a suprasystolic pressure that was sustained for 5 minutes, and the brachial diameter measurement was repeated 55–65 seconds after releasing the cuff. FMD was calculated as the percentage change in arterial diameter from baseline.

*NMD*

The brachial artery diameter was measured at baseline and again, 3 minutes after administration of 0.4 mg sublingual nitroglycerin. NMD was calculated as the percentage change in arterial diameter from baseline.

### Statistical Analyses

*Missing Data*

Logistic and other challenges prevented performance of complete VFT testing in all 602 study participants. The numbers of patients with nonmissing VFT measurements ranged from 448 (74%) for carotid-femoral PWV to 569 (95%) for VOP. Some patients also had missing ultrasound measurements, in part because of attrition of the cohort caused by patient death and AVF thrombosis and in part because of intermittently missed ultrasound measurements (Supplemental Appendix 2, Supplemental Table 1). In addition, because our objective was to describe the role of vascular function in the natural history of AVF development, ultrasound measurements after AVF interventions were deleted before subsequent analyses. To minimize risk of bias caused by missing data, we performed multiple imputation to impute missing VFT measurements as well as missing ultrasound measurements for visits scheduled before AVF thrombosis or patient death. Missing ultrasound values after death and thrombosis were not imputed to avoid conceptual paradoxes; hence, the results pertaining to ultrasound outcomes at the day 1, week 2, and week 6 visits pertain to the subcohorts of survivors without thrombosis before these visits. In sensitivity analyses, the regression analyses relating the ultrasound outcomes to each preoperative VFT were repeated after restricting to patients with nonmissing VFT measurements. Supplemental Appendix 2 and Supplemental Table 2 show tabulations of the numbers of subjects affected by missing data and a detailed description of the multiple imputation procedure.

*Statistical Analyses Relating Ultrasound and VFT Measures*

We fit separate generalized linear mixed effects regression models to relate each of the five baseline vascular function variables to the average

AVF draining vein diameters or AVF blood flow rates at the 1-day, 2-week, and 6-week ultrasound examinations after AVF placement (*i.e.*, 5×2×3=30 models; corresponding to each combination of the five VFT measurements, the two ultrasound outcomes, and the three postoperative time points). Each of the 30 regression models was fit after adjusting for AVF location (forearm versus upper arm), preoperative ultrasound measurements (inflow artery diameter, minimum vein diameter, and brachial artery flow), and baseline demographics (age, sex, race, and dialysis status).

Additional models with natural cubic splines with four equally spaced knot points were used to evaluate linearity assumptions in the VFT measures and depict the shape of the relationships between the ultrasound outcomes and the VFT predictors after adjustment for the same covariates listed above. Sensitivity analyses were performed to test for interactions between VFT parameters and fistula location (upper arm versus forearm). Technical details of the regression models are provided in Supplemental Appendix 3.

All analyses were performed in SAS, version 9.4 (SAS Institute Inc., Cary, NC). *P* values and 95% CIs are reported on a comparison-wise basis without formal adjustment for the conduct of multiple analyses. Hence, results are interpreted as exploratory, recognizing the risk of type 1 error because of multiple hypothesis tests.

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Study group members are listed in ref. 14.

## DISCLOSURES

M.A. is a consultant for Cor-Medix and Gore. L.M.D. is a consultant for Proteon Therapeutics. A.K.C. is a DSMB member of TVA Medical, Inc. J.S.K. is the chair of the Data Safety and Monitoring Board for Proteon Therapeutics.

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See related editorial, "Predicting the Functionality and Form of a Dialysis Fistula," on pages 3508–3510.

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## 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.

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**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.

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

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.