Use of a flow-mediated vasodilation technique to assess endothelial function in dogs

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Objective—To develop and assess the reproducibility of a protocol to noninvasively test endothelial function in dogs on the basis of the flow-mediated vasodilation (FMD) procedure used in humans.

Animals—5 healthy spayed female dogs.

Procedures—Luminal arterial diameter and blood flow velocity in the brachial and femoral arteries were measured with ultrasonography. The within-dog reproducibility of these ultrasonographic measurements was tested. An occlusion period of 1, 3, or 5 minutes with an inflatable cuff was used to create the FMD response. Measurements made at 15, 30, and 60 seconds following release of the occlusion were compared with measurements made immediately prior to each occlusion to assess the FMD response.

Results—Within-dog reproducibility of measurements revealed moderate to high correlations. Change from baseline in luminal arterial diameter was most substantial when measured at 30 seconds following release of occlusion, whereas blood flow velocity changes were maximal when measured at 15 seconds following release. The brachial imaging site provided a larger number of significant FMD responses than the femoral site. The 3-minute occlusion period provided equal or better responses than the 5-minute occlusion period.

Conclusions and Clinical Relevance—Ultrasonographic measurement of the FMD responses was a feasible and reproducible technique and significant changes from baseline were detected. The FMD responses in dogs were most substantial when performed at the brachial artery with blood flow velocity and luminal arterial diameter changes from baseline measured at 15 and 30 seconds, respectively, following release of a 3-minute occlusion period. (Am J Vet Res 2006;67:1533–1540)

The vascular endothelium is a monolayer of cells that coats the inside of the closed circulatory system of all vertebrates. Once thought to be an inert lining of the vascular lumen, the endothelium is now widely viewed as an organ in and of itself with a variety of complex structural, signaling, and metabolic functions.1 The normally functioning endothelium is a selective barrier between the circulating blood and every living cell in the body.2 Endothelial cells play a critical role in proper vasomotion, cellular growth, coagulation, and inflammation in local vascular beds, and systemically.2 Their location throughout the body, as well as their inherent ability to act independently makes endothelial cells ideal for these regulatory functions.4 A healthy endothelium maintains normal blood flow through the vascular system by regulating a baseline vasodilatory, antithrombotic, and anti-inflammatory state. The properly functioning endothelium will also limit excessive vascular smooth-muscle growth and coordinate the growth of newly forming vessels.5 Endothelial dysfunction complicates cardiovascular and other life-threatening diseases in humans and can have negative local and systemic effects.6 In various disease states, the homeostatic balance normally maintained by the endothelium may be shifted toward vasoconstriction, coagulation, inflammation, and deleterious thickening or convolution of vessels.4 In humans, this can result in a life-threatening disease process when it involves the coronary or cerebral circulation.5

Nitric oxide is a critical regulatory molecule involved in many of the endothelial-dependent functions that maintain normal circulatory homeostasis.10,11 Baseline and inducible vasodilation in response to local stimuli, such as tissue hypoxia and increased shear stress from increased blood flow, is an important NO-mediated, endothelial-dependent function.12 In a paracrine fashion, NO stimulates relaxation of the smooth muscle cells in the surrounding vascular wall.14 In part, endothelial dysfunction results from decreased synthesis of NO or increased oxidation of NO to nitrite and nitrate end products.15 This decrease in NO bioavailability associated with endothelial dysfunction is known to play a role in cardiovascular and other diseases in humans.5,16,19

Depressed endothelial-dependent vasodilation has been observed in dogs with experimental heart failure induced by rapid ventricular pacing.20,22 These studies used invasive monitoring techniques that are impractical for clinical use. Alternatively, serum nitrite and nitrate, end products of NO metabolism, have been measured in dogs with heart disease but are nonspecific indicators of endothelial function.20,24 A noninvasive technique to specifically assess endothelial function in dogs would be beneficial in the study of endothelial function in cardiovascular and other serious diseases commonly afflicting companion animals. For example,
the measurement of endothelial function could help elucidate the pathophysiologic properties of heart failure in dogs, as well as provide an end point for the study of various medical or nutritional treatments for cardiac disease in dogs.

Various procedures to monitor endothelial function exist for humans, but no noninvasive test of endothelial function has been widely accepted for use in veterinary medicine. Flow-mediated vasodilation involves the ultrasonographic measurement of endothelial-dependent vasoreactivity in a peripheral artery of an arm or leg. An increase in the local blood flow (ie, reactive hyperemia) to the limb is created after release of a 5-minute arterial occlusion, created by inflation of a sphygmomanometric (blood pressure) cuff to suprasystolic pressures. The increased blood flow and resulting shear stress on the luminal endothelial surface are the triggers for NO release and subsequent vasodilation of the conduit arteries of the limb. This series of events is recorded with ultrasonographic measurements of the change from baseline in arterial diameter and blood flow velocity through these dilated conduit arteries. Flow-mediated vasodilation is impaired in humans with cardiovascular disease, and a decreased FMD response has been correlated with numerous cardiovascular risk factors. Flow-mediated vasodilation improves in human patients receiving certain cardiovascular treatments, including preventative medication, exercise, and dietary and lifestyle changes.

The purpose of the study reported here was to develop a protocol to noninvasively test endothelial function in dogs on the basis of the FMD procedure used in humans. The specific objective of this study was to determine whether ultrasonography could be used to reproducibly measure the arterial diameter and blood flow velocity at baseline in dogs; compare brachial and femoral imaging sites for measurement of arterial diameter and blood flow velocity in dogs; compare a 1-, 3-, and 5-minute occlusion period in creating a change in arterial diameter and blood flow velocity in dogs; and compare times after cuff deflation (15, 30, or 60 seconds) to document a change in the arterial diameter and blood flow velocity measurements in dogs. Once this noninvasive technique to measure endothelial function is developed in healthy dogs, future studies can evaluate whether changes in FMD occur in dogs with cardiovascular diseases, such as dilated cardiomyopathy, chronic valvular disease, or congenital heart diseases. Furthermore, if dogs with cardiovascular disease have altered FMD, then FMD measurements could be used as a risk assessment tool or as a technique to document the clinical benefit from drug, nutritional, or lifestyle interventions.

Materials and Methods

Animals—Healthy dogs with no history of cardiovascular disease or other major health problems were enrolled in this study. Faculty, staff, or students at Tufts University’s Cummings School of Veterinary Medicine owned all dogs. The Tufts University Institutional Animal Care and Use Committee approved the study, and all owners signed an informed consent form. Dogs between 1 to 10 years of age, of either sex and neuter status, and weighing > 15 kg were eligible for the study. Dogs with a body condition score > 7 of 9 and those receiving any oral medication, other than heartworm preventative or glucosamine, were excluded. Dogs did not appear to have cardiovascular or other systemic disease on the basis of no abnormal findings on a complete history, physical examination, measurements of arterial blood pressure (Doppler technique), serum biochemical analysis, PCV determination, urine dipstick analysis, and determination of total solids concentration, BUN concentration, and blood glucose concentration.

Arterial diameter and blood flow measurement protocol—On the basis of the established guidelines for testing FMD in the brachial artery of humans and results of a recent study evaluating ultrasonographic measurements of blood flow in the femoral artery of pigs, the brachial artery along the medial aspect of the brachium and the proximal aspect of the femoral artery in the inguinal area were chosen as imaging sites. An ultrasound machine with a variable-frequency (5- to 8-MHz) curvilinear transducer was used to image the arteries in these 2 areas. The ultrasound machine had received regular preventive maintenance, including calibration. However, no calibration was performed during the study. In our preliminary studies, dogs were imaged on several days to locate specific anatomic landmarks along the brachial and femoral arteries that could be repeatedly found by different investigators and would allow for consistent imaging of the diameter and velocity measurements at the same location in all dogs (data not shown).

Dogs were placed in dorsal recumbency on a padded table with their body in a 10° to 20° oblique position to the right toward the ultrasonographer. Electrocardiographic electrodes were placed on the plantar and palmar aspects of the metatarsal and metacarpal pads on both hind feet and on the left front foot and were connected to the ultrasound machine with ECG lead wires. A 5- to 10-cm² area of hair was clipped over the imaging sites, which were located by superficial palpation of the arterial pulse. Dogs were allowed to acclimatize in this position before measurements were obtained.

For the brachial artery, the imaging site was found by placing the ultrasound transducer proximal to the elbow joint on the medial aspect of the brachium and locating the artery in cross section with 2-dimensional grayscale and color-flow Doppler modes. By scanning the brachial artery proximally toward the axilla, a deep muscular branch was found just proximal to the collateral ulnar artery. Two-dimensional arterial diameter and pulsed-wave Doppler blood flow velocity measurements were obtained immediately proximal to this deep muscular branch. The femoral artery was imaged proximally at the level of the intersection of the medial aspect of the thigh and the ventral aspect of the abdominal body wall. Light pressure was applied to the transducer at both sites to avoid arterial and, in the case of the femoral site, venous occlusion. The ultrasonographic gain and gray scale were adjusted at both imaging sites to obtain optimal contrast between the lumen and the wall of the vessels. In some instances, the intimal layer could be viewed between the vessel lumen and the surrounding smooth muscle layer of the wall. Cross-sectional vessel diameter measurements were obtained with the transducer perpendicular to the limb and, thus, perpendicular to the vessel (ie, transverse-axis view) with measurement calipers placed parallel to the ultrasound beam (Figure 1). In this image orientation, a circular appearance to the luminal arterial profile was also used to determine a cross-sectional image that was perpendicular to the longitudinal axis of the vessel. Longitudinal vessel diameter and pulsed-wave Doppler measurements were obtained at the same sites, following rotation and angulation of the transducer so that the ultrasound beam was parallel to the vessel (ie, longitudinal-axis view) with an angle of insonation ≤ 60° (Figure 2). In this image orientation,
a midsagittal section through the artery was determined by viewing the intimal wall layer, when possible, or by scanning the vessel for the maximal diameter.

Reproducibility of baseline ultrasonographic measurements—Arterial diameter and blood flow velocity measurements were made during reproducibility studies on the brachial and femoral arteries. Each dog was studied on 2 days and by 2 investigators on each day. Each investigator, on each day, performed 2 separate baseline reproducibility examinations. Between each reproducibility examination (or transducer placement), the ultrasound transducer was removed from the dog and the imaging sites were again found by use of the aforementioned landmarks. Each reproducibility examination involved the measurement of each of the arterial diameter and blood flow velocity indices from 3 consecutive cardiac cycles. On the basis of concurrent ECGs, internal (luminal) arterial diameters were measured at the beginning of the QRS complex (ie, at end diastole; Figure 1) and at, or immediately after, the T wave when the vessel diameter appeared to be at its maximal dimension following cardiac systole. For each site during reproducibility testing, internal arterial diameter measurements were obtained from transverse- and longitudinal-axis ultrasonographic views of the brachial and femoral arterial imaging sites. In addition, 3 blood flow velocity measurements were made from the pulsed-wave Doppler tracing obtained from the longitudinal-axis view (Figure 2). These included a peak early systolic velocity (ie, peak systolic velocity), the subsequent first negative velocity (ie, minimum velocity), and the second peak positive velocity after the negative deflection (ie, peak diastolic velocity). In dogs that had sinus arrhythmia at the time of the study, the first cardiac cycle for the 3 consecutive velocity measurement sets was obtained when heart rate began to increase following the sinus pause.

FMD protocol—Following reproducibility testing, vessel occlusion was performed to evaluate FMD. A blood pressure cuff attached to a sphygmomanometer was placed around the antebrachrom just distal to the elbow joint and secured in this location with tape. For hind limb occlusion, a vascular tourniquet cuff attached to a sphygmomanometer was placed around the thigh just proximal to the stifle joint and secured medially and laterally with a single long nylon fastening strap wrapped over the dog's back to prevent the cuff from slipping distally. Occlusion of arterial blood supply to the distal portion of the limbs was confirmed by loss of pulse signal from an ultrasonic Doppler flow detector with the probe over the dorsal pedal artery for the hind limb and superficial palmar arterial arch for the forelimb. A pressure of at least 50 mm Hg greater than the pressure required to eliminate the Doppler signal was used for each occlusion during the protocol. Cuff pressures of approximately 220 and 300 mm Hg were required for the forelimb and hind limb, respectively. Cuff pressures that were considerably higher than systemic arterial pressure were required to occlude blood flow in the femoral artery for each dog.

Arterial diameter and blood flow velocity measurements were made at the femoral and brachial artery sites at baseline and following release of a 1-, 3-, and 5-minute occlusion period. Systolic and diastolic arterial diameters were measured at baseline, as described from the transverse-axis view, and measurements were repeated after each occlusion period at 15-, 30-, and 60-second time points following cuff deflation. Pulsed-wave Doppler tracings were used to measure blood flow velocities at baseline, as described from the longitudinal-axis view, and then after release of each occlusion period at 15, 30, and 60 seconds following cuff deflation. Pulsed-wave Doppler velocity measurements could not be performed simultaneously as the result of the different transducer orientations and the short time frame for recording FMD changes, separate occlusions were required for these 2 measurements. The procedure was done on alternating limbs (forelimb and hind limb), and the occlusions were performed in a randomized order for each dog, with randomization order generated by a computer program. A 10-minute washout period was used before repeating the procedure on a limb. Baseline and 60-second postocclusion release measurements were made at the time of imaging by use of electronic calipers on the ultrasound machine. Fifteen- and 30-second postocclusion release time-point images were electronically stored, and measurements were subsequently made by the investigator who performed the original study.

Figure 1—Example of a 2-dimensional grayscale ultrasonographic image of the reverse-axis view used to measure internal arterial diameter with calipers on the near and far walls. This image is of the femoral arterial imaging site, and the measurement was obtained during end diastolic flow, aligned to the beginning of the QRS complex (arrowhead below ECG). Depth scale in centimeters. A = Femoral artery. V = Femoral vein. BPM = Beats per minute.

Figure 2—Examples of pulsed-wave Doppler velocity tracings obtained from the longitudinal-axis view of the artery used to measure blood flow velocity. These images are from the brachial arterial imaging site of the same dog before (A) and 15 seconds after (B) release of a 3-minute arterial occlusion period. The first velocity tracing (A) was obtained after a pause in the rhythm that was created by sinus arrhythmia. Notice that there is considerable beat-to-beat variation in peak systolic velocity (PSV), minimum velocity (MV), and peak diastolic velocity (PDV), which were consistently observed in dogs with sinus arrhythmia. In the second Doppler velocity tracing (B), notice the loss of the negative wave that results in a zero MV value at 15 seconds following cuff deflation. Also, notice the laminar blood flow pattern in panel A, compared with the turbulent flow pattern in panel B. BPM = Beats per minute.
Statistical analysis—For the reproducibility study, we estimated ICCs° computed from a random effects ANOVA.° The following sources of variability were examined for each diameter and velocity measurement index: between the 3 measurements made by an investigator during each transducer placement (ie, within-placement variability), between 2 transducer placements (ie, interplacement variability) made by the same investigator on the same day, between measurements made on different days by the same investigator (ie, day-to-day variability), and between investigators on the same measurement within a transducer placement (ie, interinvestigator variability). An ICC ≥ 0.80 indicated a high correlation between measurements, whereas an ICC from 0.50 to 0.79 indicated a moderate correlation, and an ICC from 0.20 to 0.49 indicated only a slight correlation.° For the FMD protocol, within-dog changes in arterial diameter and blood flow velocity following release of occlusion were estimated separately for each outcome by use of a random effects ANOVA. From these models, we determined the significance of the mean of within-dog changes from baseline and whether these within-dog changes differed determined the significance of the mean of within-dog changes significant.

Results

Animals—Five dogs of various breeds (2 Labrador Retrievers, 2 Golden Retrievers, and 1 mixed-breed dog) participated in this study. Mean age was 5.4 ± 3.3 years, and all dogs were females that had been spayed. Median body weight was 31.4 kg (range, 21.9 to 34.6 kg), and median body condition score was 6.5 (range, 4.5 to 6.5) on a 1 to 9 scale.

Reproducibility—Intraclass correlations for the arterial diameter and blood flow velocity measurements were mostly moderate to high for the 4 sources of variability tested (Table 1). In general, the femoral arterial imaging site provided for higher correlation between like measurements, compared with the brachial artery imaging site. Also, correlations were higher between measurements of internal arterial diameter, compared with measurements of blood flow velocity. Measuring arterial diameter from the transverse-axis view at the femoral imaging site allowed for highly correlated measurements during systole and diastole for all sources of variability tested (ie, within-placement variability, interplacement variability, day-to-day variability, and interinvestigator variability), except during diastole for day-to-day variability, which had a moderate correlation. In general, lower correlations were observed between day-to-day like measurements, although day-specific analyses (data not shown) revealed higher interinvestigator correlations on the second day that each dog was imaged, compared with the first day.

FMD—Brachial artery diameter changed significantly from baseline for all occlusion times (ie, 1, 3, and 5 minutes) when measured at 30 seconds after cuff deflation (Table 2). The femoral artery diameter also had significant within-dog changes from baseline when measured 30 seconds after cuff deflation following a 3-minute occlusion. At 15 seconds after cuff deflation, the brachial artery changed significantly after a 3-minute occlusion (systolic measurement) and after a 5-minute occlusion (systolic and diastolic measurements). At 60 seconds after cuff deflation, the only significant changes in vessel diameter were for the brachial artery after a 5-minute occlusion (diastolic measurement) and for the femoral artery after a 3-minute occlusion (systolic measurement).

For blood flow velocity measured by pulsed-wave Doppler technique, most significant mean within-dog changes from baseline occurred 15 seconds after cuff deflation (Table 3). All velocity measurements (peak systolic, minimum, and peak diastolic) for the brachial and femoral arteries at 15 seconds after cuff deflation were significantly changed from baseline, with the exception of peak systolic velocity after a 1-minute occlusion in the brachial and femoral arteries. Fewer

<table>
<thead>
<tr>
<th>Table 1—Within-dog ICCs used to determine reproducibility of ultrasonographic measurements of arterial diameter and blood flow velocity at the brachial and femoral arterial imaging sites in 5 healthy dogs.</th>
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</thead>
<tbody>
<tr>
<td><strong>Imaging site</strong></td>
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<td>Brachial</td>
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<td>Femoral</td>
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<td>Brachial</td>
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<td>Femoral</td>
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</table>

*Moderate correlation (ICC ≥ 0.50).**High correlation (ICC ≥ 0.80).

PSV = Peak systolic blood flow velocity. MV = Minimum blood flow velocity (between systolic and diastolic peaks). PDV = Peak diastolic blood flow velocity. LD = Internal arterial diameter measured from the longitudinal-axis view. Sys = Diameter measurement made during systole by aligning to the largest vessel size near the T wave on the simultaneous ECG. Dias = Diameter measurement made at end diastole by aligning to the start of the QRS complex on the simultaneous ECG. TD = Internal arterial diameter measured from the transverse-axis view.
Table 3—Mean ± SE changes in arterial blood flow velocity (cm/s) as a measure of FMD at brachial and femoral arterial ultrasonographic imaging sites in 5 healthy dogs at 15, 30, and 60 seconds following release after a 1-, 3-, or 5-minute occlusion period.

<table>
<thead>
<tr>
<th>Site and velocity index</th>
<th>Occlusion time (min)</th>
<th>Baseline</th>
<th>15 seconds</th>
<th>P value*</th>
<th>30 seconds</th>
<th>P value*</th>
<th>60 seconds</th>
<th>P value*</th>
</tr>
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<tr>
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<tr>
<td>PSV</td>
<td>3</td>
<td>115.05 ± 13.28</td>
<td>139.44 ± 12.28</td>
<td>0.08</td>
<td>133.75 ± 12.28</td>
<td>0.96</td>
<td>128.12 ± 13.28</td>
<td>0.69</td>
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<tr>
<td>MV</td>
<td>3</td>
<td>-48.11 ± 6.83</td>
<td>-39.53 ± 6.83</td>
<td>0.03</td>
<td>-55.40 ± 6.83</td>
<td>0.06</td>
<td>-55.81 ± 6.83</td>
<td>0.05</td>
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<tr>
<td>PDV</td>
<td>3</td>
<td>29.15 ± 3.86</td>
<td>37.74 ± 3.86</td>
<td>0.04</td>
<td>31.65 ± 3.86</td>
<td>0.51</td>
<td>24.93 ± 3.86</td>
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<tr>
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<tr>
<td>PSV</td>
<td>3</td>
<td>111.27 ± 10.65</td>
<td>157.83 ± 10.65</td>
<td>&lt; 0.001</td>
<td>140.74 ± 10.65</td>
<td>0.01</td>
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<td>MV</td>
<td>3</td>
<td>-23.30 ± 3.08</td>
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<td>PDV</td>
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<td>28.44 ± 5.92</td>
<td>76.39 ± 5.92</td>
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<td>44.22 ± 5.92</td>
<td>0.02</td>
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<tr>
<td>PSV</td>
<td>5</td>
<td>116.33 ± 8.00</td>
<td>146.65 ± 8.00</td>
<td>&lt; 0.001</td>
<td>125.82 ± 8.00</td>
<td>0.23</td>
<td>113.35 ± 8.00</td>
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<tr>
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<td>&lt; 0.001</td>
<td>-14.57 ± 4.41</td>
<td>0.02</td>
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<td>81.12 ± 5.13</td>
<td>&lt; 0.001</td>
<td>40.29 ± 5.13</td>
<td>0.18</td>
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<tr>
<td>PSV</td>
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<td>127.48 ± 13.43</td>
<td>143.29 ± 13.43</td>
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<td>122.74 ± 13.43</td>
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<td>120.32 ± 13.43</td>
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<tr>
<td>MV</td>
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<td>-49.30 ± 6.79</td>
<td>-34.65 ± 6.79</td>
<td>0.004</td>
<td>-52.71 ± 6.79</td>
<td>0.42</td>
<td>-58.81 ± 6.79</td>
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<tr>
<td>PDV</td>
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<td>29.90 ± 2.93</td>
<td>44.29 ± 2.93</td>
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<td>32.18 ± 2.93</td>
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*Values of P < 0.05 indicate measurements that are significantly different from baseline. See Table 1 for remainder of key.
rapidly in the femoral artery (approx 5 to 10 seconds) than in the brachial artery (approx 20 seconds).

**Discussion**

Results of our study indicate that ultrasonographic measurement of the arterial diameter and blood flow velocity response following a period of limb occlusion is a feasible and reproducible method to evaluate endothelial function in dogs. The technique generally had good precision for within-placement, interplacement, day-to-day, and interinvestigator measurements made at baseline. Accuracy of the noninvasive ultrasonographic measurement of flow-mediated changes in vessel diameter and blood flow velocity has previously been demonstrated. Diameter changes of 0.1 mm have accurately been measured in vitro with phantom artery models and in vivo with direct invasive measurements by use of similar ultrasound equipment to that in our study. Results of a study in which similar equipment and techniques were used also revealed that Doppler ultrasonic blood flow velocity measurements provide good accuracy, compared with the more direct invasive measurement techniques.

Some general observations were made in the correlations of measurements made during the reproducibility study. For example, a higher correlation was found when arterial diameter measurements were made on the femoral artery than when made on the brachial artery. This is likely the result of the larger size of the femoral artery and the greater ease in locating the femoral imaging site. At the site where the brachial artery is imaged in humans, the baseline diameters are generally 0.40 to 0.50 cm, whereas in the dogs of our study, brachial artery measurements were approximately 0.25 to 0.30 cm in diameter and femoral artery measurements were approximately 0.45 to 0.50 cm in diameter at rest. Arterial diameter of the femoral artery measured from an ultrasonographic image of the transverse-axis view had higher correlations than longitudinal-axis view measurements. In previous studies, longitudinal images were more often used although the cross-sectional method of measuring FMD also has been validated. Higher correlations for the cross-sectional measurements in our study are likely the result of the ability to determine a true cross-sectional position in the transverse axis view, compared with a sagittal position in the longitudinal view. In addition, more interinvestigator variability on the first day of measurements was found than on the second day of measurements. This likely reflects a learning curve, either for the dogs and their level of relaxation or for the investigators’ technique. This ultrasonographic technique appeared to be well tolerated by the dogs in our study. Dogs lay quietly for the measurements, although because occlusion of the femoral artery required a higher cuff pressure than the brachial artery, dogs sometimes moved during occlusion of the femoral artery, especially during longer (ie, 5 minutes) occlusions, requiring an additional washout period and performing the occlusion a second time. Shorter occlusion times minimized the risk of dogs moving during the occlusion period.

Significant within-dog changes from baseline were seen in luminal arterial diameter measurements made from the transverse-axis view after release of vessel occlusion by use of the FMD technique. Mean percent within-dog changes from baseline were in the order of approximately 10%, similar to what is seen in healthy humans. Compared with dogs, human patients with endothelial dysfunction have a smaller vasodilatory change from baseline and, in some instances, even have paradoxical vasoconstriction during the reactive hyperemic period. Similarly altered FMD responses are expected in dogs with endothelial dysfunction, but this will require further study. Results of our study suggest that the 3-minute occlusion time was equal to or better than the 5-minute occlusion for evaluation of changes in arterial luminal diameter. The 1-minute occlusion period did not consistently provide a large enough response.

Results of our study suggest that the 30-second time point after cuff deflation was better than the 15- or 60-second time points for measurement of arterial diameter. In humans, a 5-minute occlusion period is recommended and the optimal vasodilatory response occurs between 45 to 60 seconds after cuff deflation. Thus, it appears that a shorter occlusion time and an earlier measurement time are preferable in dogs, compared with humans. The brachial artery appeared to be a preferable site for measurement of luminal diameter changes from baseline, compared with the femoral artery. This may be attributable, in part, to the fact that occlusion of the femoral artery proved to be somewhat more difficult. Higher pressures were required for occlusion of the femoral artery resulting from the large muscle mass in the hind limb. Similar to the luminal arterial diameter responses, blood flow velocity measurements changed significantly after occlusion in our study and these changes were, in fact, even more pronounced than the changes observed in vessel diameter. Quantitative data of the means of within-dog changes from baseline for blood flow velocities suggest that either a 3- or 5-minute occlusion period produce significant changes, but compared with luminal diameters, earlier measurement following cuff deflation is required to record the maximal changes in velocity. It appeared that the maximal response was at 15 seconds after cuff deflation, with a return to baseline levels soon after this time point. This postischemia flow response is likely the result of NO release, which also may be influenced by other factors, effecting vasculature resistance downstream, such as pH, carbon dioxide, and hypoxia.

As with changes from baseline in luminal arterial diameter, a 1-minute occlusion period did not consistently provide significant changes in velocity measurements, even when measured at the 15-second time point. Because the 3- and 5-minute occlusions produced significant results, the 3-minute occlusion would likely be selected for future studies to increase patient comfort. In addition to the quantitative measurements, subjective but evident changes also were seen in the pulsed-wave Doppler velocity tracing in the period following cuff deflation. Turbulence is the broad velocity distribution of spectral echoes. This may result from variability in flow dynamics such that RBC velocity has a wide distribution (from negative to positive peak values). This is displayed on spectral Doppler
tracing as a velocity peak without an inner envelope or spectral window. The resulting image appears as a filled-in peak rather than an outline of the peak itself. The change from laminar to turbulent flow and the loss of the negative wave seen after cuff deflation suggest that additional analyses may be possible that might help to uncover differences in the endothelial function between healthy dogs and dogs with cardiovascular or other systemic diseases.

Our study has a number of limitations. A relatively small number of dogs were used, although high within-dog correlations suggest that, at least for healthy dogs, there is low variability. However, we included dogs that were all spayed females and were of similar body size. Future studies will be necessary to determine the effect of gender, neutering status, and size on reproducibility of luminal arterial diameter and blood flow velocity measurements. Also related to size is the fact that we included all medium- to large-sized dogs (> 21.9 kg). Smaller dogs will need to be tested to determine the size limitation on dogs in which the arterial diameter (particularly of the brachial artery) can be accurately measured. In smaller dogs, velocity measurements may be more accurate in monitoring the postocclusion release hyperemic changes, compared with arterial diameter measurements. For the purpose of our study, we excluded obese dogs. Dogs with large amounts of body fat may make the measurements more difficult, particularly during occlusion of the femoral artery because we found it to be a challenge, even in trim dogs, to get the cuff situated in the correct position and to maintain its position during cuff inflation. This was particularly difficult in dogs with heavily muscled hind limbs. Because reproducibility of luminal arterial diameter measurements was better in the femoral artery, compared with the brachial artery, alternative hind limb occlusion techniques could be investigated. In addition to technical challenges, other issues also may have contributed to variability, including sinus arrhythmia and excitement level of the dog, both of which could be seen to alter the pulsed-wave Doppler measurements (especially the peak systolic velocity). There also appeared to be a learning curve for the investigators in this technique that could have contributed to variability.

Nonetheless, this technique appeared to have good reproducibility and demonstrated significant changes in postocclusion luminal arterial diameter and blood flow velocity, compared with baseline. The brachial artery with a 3-minute occlusion period with blood flow velocity changes from baseline measured at 15 seconds and luminal arterial diameter measured at 30 seconds following release of occlusion appears to give the most substantial responses. These parameters can now be further refined and used to test endothelial function in dogs with cardiovascular disease, as well as response to intervention.

References

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