Evaluation of transcutaneous Doppler ultrasonography for the measurement of blood flow in the femoral artery of pigs

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Objective—To compare measurements of blood flow in the common femoral artery of pigs by use of a Doppler ultrasonography (DDU) and a reference ultrasonic transit-time flow (TTF) method and to examine the impact of Doppler spectral waveform measurement techniques on volumetric estimates.

Animals—5 healthy female pigs.

Procedures—Femoral arterial blood flow was measured simultaneously in anesthetized pigs by use of a TTF probe (left femoral artery) and transcutaneous DDU (right femoral artery). A range of flow states was induced pharmacologically by using xylazine, bradykinin, dobutamine, and isoﬂurane. Volumetric blood flow was calculated from DDU waveforms, using the product of the flow velocity integral (FVI), the cross-sectional vessel area, and heart rate. Three calculations of FVI were obtained by manually tracing the Doppler spectral envelopes at the outer envelope, the modal, and the inner envelope of the spectral dispersion pattern. Data analysis included calculation of Pearson correlation coefficients and Bland-Altman limits of agreement.

Results—Blood flow measured by DDU was more closely correlated with TTF measurements when the modal or inner envelope tracing method was used (r, 0.76 and 0.78; limits of agreement, –100 to 54.2 and –48.5 to 77.0 mL/min, respectively). Limits of agreement for the outer envelope tracing method were –238.5 to 64 mL/min.

Conclusions and Clinical Relevance—Transcutaneous DDU is a reliable noninvasive technique for measuring blood flow in the femoral artery of pigs over a range of flow states. Tracing the inner envelope of the Doppler spectral dispersion pattern provided the best estimate of blood flow in this study. (Am J Vet Res 2003;64:43–50)

Peripheral blood flow is necessary to support tissue metabolism and flow-dependent functions in organs of the body. The ability of blood vessels to dilate and constrict in response to various neurologic, hormonal, and local factors is essential to the regulation of tissue perfusion. Blood flow to tissues is related to blood pressure as well as the size of larger (conduit) arteries and smaller (resistance) arterioles that control flow through tissue beds. Accurate measurement of arterial blood flow is essential in studies of peripheral vascular function and the evaluation of patients with peripheral arterial disease, and it is currently receiving attention as a noninvasive means of assessing coronary artery reactivity. Reactive femoral artery and brachial artery flow is also used as a marker of endothelial dysfunction in human patients with atherosclerosis and heart failure. Possible applications in veterinary medicine exist in the fields of anesthesiology or cardiology or for use in assessing the vascular response of vasoactive therapeutic compounds.

Pigs are commonly used for the study of vascular responses and cardiovascular disease. Pigs fed diets high in fat and cholesterol will develop coronary artery disease and other vascular disorders similar to those found in people. The response of porcine blood vessels to various interventions or treatments, including exercise, have been a focus of considerable research, and accurate measurement of changes in femoral artery flow in pigs could provide a noninvasive means of assessing the effects of exercise on endothelial function.

Qualitative Doppler ultrasonographic techniques are widely used clinically in patients with vascular disease to identify abnormal patterns of blood flow. Some quantitative Doppler indices have also been developed for use in identification of vascular disease, such as the pulsatility index and peak forward blood flow velocity, but calculation of volumetric blood flow has typically been measured by invasive techniques or in research settings. Methods used to measure volumetric blood flow include dye indicator or thermodilution methods, electromagnetic flow meters, and injection of radioactive microspheres. Recently, it has been documented that ultrasonic transit-time flow (TTF) probes are highly accurate in small and large mammals and are an accepted means of flow measurement. The TTF probes measure the difference in transit times of ultrasound waves as they travel between the upstream and downstream transducers of a probe, and the concept is not based on the Doppler principle. Returning signals reflect flow from across the entire vessel area, permit-
ting calculation of blood flow. The TTF probes are implanted surgically around the vessel for short- or long-term studies, requiring anesthesia for placement. Anesthetic drugs can affect blood pressure and vascular responses, and animals must be euthanized after completion of long-term studies with implanted probes. Some probes are only effective for a limited time after implantation.17

A noninvasive method for quantifying blood flow that has accuracy similar to that for the TTF method would be useful, especially for long-term studies. Doppler ultrasonography can be used to noninvasively measure blood flow velocity, and blood flow rate can also be derived when the cross-sectional area of the vessel is known. The cross-sectional area of a vessel can be measured by 2-dimensional (2-D) ultrasonograph, and a combination of 2-D and Doppler ultrasonography (duplex Doppler ultrasonography [DDU]) allows both measurements to be obtained by use of the same probe. In a number of studies, investigators have compared results for DDU with in vitro measurements of blood flow20 and in vivo measurements obtained by use of electromagnetic flow probes.20,21,22

The objective was to evaluate the effect of spectral waveform measurement technique on the correlation between measurements obtained by use of TTF methods in peripheral vessels. Furthermore, effects of envelope tracing techniques of spectral Doppler signals on flow calculations have not been evaluated, particularly in the setting of spectral dispersion (broadening of the spectral envelopes).

The objective of the study reported here was to compare blood flow measurements obtained by use of DDU with measurements obtained by use of TTF methods in the common femoral artery of pigs. A second objective was to evaluate the effect of spectral waveform measurement technique on the correlation between measurements obtained by DDU and TTF methods.

Materials and Methods

Animals—Five healthy female pigs that weighed between 37 and 46 kg were used in the study. The Institutional Animal Care and Use Committee of the University of Missouri-Columbia approved all procedures.

Anesthesia—Pigs were sedated by IM injection of ketamine hydrochloride (35 mg/kg) and xylazine hydrochloride (2.25 mg/kg). Isoflurane (4 to 5% in 100% oxygen) was administered via facemask to allow endotracheal intubation. General anesthesia was maintained by administration of isoflurane (1 to 2% in oxygen). Ventilation was assisted as necessary to maintain PaCO2 between 35 and 40 mm Hg. During the study, saline (0.9% NaCl) solution was administered IV at a rate of 10 mL/kg/h via a catheter inserted in an ear vein of each pig. Heart rate (HR) was monitored on an ECG by use of standard limb leads. Anesthetized pigs were placed in dorsal recumbency. The limbs were maintained in a fixed degree of extension throughout the study.

Surgical insertion of instruments—The right carotid artery was surgically exposed by use of a midline cervical incision. A 3-F catheter introducer system with a hemostatic valve was inserted into the artery. A 3-F multipurpose cardiovascular catheter was advanced through the introducer into the carotid artery. Fluoroscopic guidance was used to advance the catheter into the aorta to the origin of the external iliac arteries. An angiogram was performed by use of manual injection of 3 mL of iodinated contrast agent to verify that the catheter tip was accurately placed approximately 1 cm cranial to the trifurcation of the distal portion of the abdominal aorta. The catheter was secured and coupled to a 3-port connector.

A second incision was made in the left inguinal region over the common femoral artery near the termination of the external iliac artery. The femoral artery was gently dissected from surrounding fat and connective tissue. Care was taken to avoid injuring the adventitia of the femoral artery. A perivascular TTF probe was placed 1 cm cranial to the origin of the saphenous branch of the common femoral artery. Acoustic coupling gel was applied to fill any space between the vessel and TTF probe to facilitate adequate acoustic conduction.

An ECG, arterial blood pressure (ABP), and blood flow in the femoral artery were recorded continuously during the study. Analogue signals from these 3 sources were routed to a personal computer equipped with a data-acquisition system. The ABP in the distal portion of the aorta was measured via the arterial catheter connected to a disposable pressure transducer and interfaced to a bioamplifier. The transducer was calibrated against a mercury manometer at the onset of the study. A TTF meter was connected to the TTF probe and interfaced to the computer acquisition system to calculate blood flow in the left femoral artery. The TTF probe was calibrated at baseline (prior to any treatments) in saline solution. Calibration was repeated prior to each recording period. Signals were digitized online by use of proprietary software and stored for subsequent analysis.

Transcutaneous DDU recordings—Blood flow in the right femoral artery was measured noninvasively by use of DDU methods, as described elsewhere.29 A commercial ultrasonographic imaging system equipped with a duplex linear-array transducer was used at an operating frequency of 10 MHz and recording depth of 2 cm. The transducer was manually positioned on the skin; ultrasonic coupling gel was used to ensure adequate contact. The transducer was oriented parallel...
to the common femoral artery and angled to allow imaging of the femoral artery and the more distal saphenous branch. Ultrasonographic gain, compression, gray scale, and reject were adjusted to achieve optimal resolution of the femoral arterial walls and lumen. Care was taken to ensure that the probe position was stable and consistent throughout the study.

Pulsed-wave Doppler mode was activated by use of a lateral crystal in the transducer array, and a 1-mm sample volume was steered to the center of the vessel. This sample volume was chosen because it allowed precise placement within the lumen. Preliminary studies conducted by our laboratory group had indicated that a larger sample volume (one that filled the vessel lumen) did not produce differences in velocity results. The sample volume was centered in the same location as the TTF probe on the contralateral artery (1 cm cranial to the origin of the saphenous branch of the femoral artery). This position was chosen to provide a repeatable and standardized location and to minimize turbulence from flow into branch vessels. An angle of incidence of ≤ 60° to the direction of the blood flow was adopted in all instances. A consistent angle was maintained throughout each study. Spectral Doppler signals were optimized by use of duplex imaging and the audio-spectral characteristics of returning Doppler signals. The velocity scale was adjusted to prevent appearance of the signal above and below the baseline (ie, aliasing) of the spectral display. The lowest possible wall filter was chosen so that slower moving blood flow would also be included in calculation of the volumetric flow. Automated angle correction was activated for processing returning Doppler signals. Two-dimensional images and Doppler spectra (100 mm/s) were recorded on videotape for subsequent off-line analysis.

**Treatment periods**—Blood flow in the femoral artery was altered by changing ABP and systemic vascular resis-

![Figure 2](image-url)
Results

Doppler spectral waveform patterns—Representative velocity spectra obtained from the right femoral artery by use of DDU revealed the typical effects of intervention on flow characteristics in this...
artery (Fig 2). Baseline flow patterns were triphasic with prominent systolic and late diastolic antegrade waves that were separated by a smaller early diastolic retrograde flow. After intra-aortic administration of xylazine or administration of high-dose isoflurane, all components of the triphasic waveform were decreased. Bradykinin at all 3 dosages eliminated early diastolic flow reversal, increased forward flow, and led to spectral broadening. Dobutamine increased the magnitude of systolic flow and early diastolic reversal wave, especially at the higher rate (10 µg/kg/min). Highest peak velocities were observed after administration of bradykinin and dobutamine (Table 1).

Quantitative analysis—Mean ± SD values for HR, ABP, blood flow in the left femoral artery as measured by TTF; diameter of the right femoral artery as measured by DDU, flow velocity, FVIs, and calculated flow for each measurement method were determined. Mean ABP changed as expected for each treatment, increasing relative to baseline after administration of the α₂-agonist xylazine and the catecholamine dobutamine and decreasing after administration of bradykinin and high-dose isoflurane.

Blood flow of the femoral artery was measured by use of the TTF probe and the 3 tracing methods for Doppler spectra for all treatment groups (Fig 3). The inner envelope tracing method for the FVI resulted in values that most closely represented those measured by use of the TTF probe. The outer envelope tracing method overestimated flow measured by the TTF probe, especially when there was Doppler spectral broadening, as was observed after administration of bradykinin.

Blood flow of the femoral artery measured by each Doppler method revealed the expected correlation with blood flow measured by use of the TTF probe (Fig 4). The relationship between measurements obtained by use of the TTF probe and the outer envelope and modal tracing methods appeared to be nonlinear which con-
The correlation between TTF and the product of VFI × HR was nearly identical to DDU volumetric estimates (value for r was 0.76, 0.76, and 0.77 for inner envelope tracing, modal tracing, and outer envelope tracing, respectively).

Bland-Altman plots revealed that the outer envelope tracing method resulted in overestimation of the TTF-derived blood flow values (ie, relative bias) of 87.0 mL/min compared with the mean TTF values (Fig 5). This was especially evident at higher flow rates. The modal tracing method also overestimated TTF-derived values with a relative bias of 23.3 mL/min. The inner envelope tracing method resulted in the least relative bias (14.4 mL/min) and smallest SD (31.5 mL/min) relative to the mean TTF measurement of 99.0 mL/min.

Discussion

Endothelial dysfunction is evident in animals with atherosclerosis and other causes of chronic heart failure and can be identified by abnormal responses in regional blood flow.6,31-34 This finding has led to a search for clinically applicable techniques that can be used to noninvasively measure blood flow, particularly with the prospect of therapeutic interventions that may reverse this dysfunction.35-37 Transcutaneous DDU is already in wide clinical use,8 but it has not been validated against TTF techniques for use in analysis of quantitative flow in peripheral vessels. The modal (ie, intensity-weighted mean velocity) of the spectral Doppler envelope is usually traced when DDU is used for quantitative flow estimation,38,39 although the outer velocity envelope is sometimes traced, especially in the coronary circulation.40,41

The study reported here was designed to compare transcutaneous DDU methods for measuring blood flow with an established invasive method (ie, TTF method). A range of flow states was induced pharmacologically to enable us to evaluate accuracy of the DDU method with that for the TTF method. Mean ABP, size of conduit vessels, and vascular resistance in arterioles impact flow through the femoral artery.

Qualitative waveform changes observed were consistent with the physiologic effects of each vasoactive agent on mean ABP, systemic vascular resistance, and flow through the femoral artery. The α2-agonist xylazine and negative inotrope-vasodilator isoflurane decreased all components of the triphasic waveform, consistent with reduced flow observed by the TTF method (Table 1).18,42 Dobutamine increased peak systolic velocity in a dose-dependent manner, consistent with its expected effect at the dosages used as a positive inotrope with little effect on vascular resistance, although the effect on diastolic signals was less consistent.43 Injection of bradykinin abolished early diastolic flow reversal and increased systolic and diastolic forward-flow velocities, effects compatible with marked vasodilation in resistance arterioles.1 Despite systemic hypotension that was evident at higher doses, bradykinin produced a dose-dependent increase in blood flow of the femoral artery with noticeable spectral broadening. This broadening reflected a more heterogeneous distribution in the velocities of flowing
blood, such as when the flow is turbulent. Spectral broadening may be more prominent in arteries distal to stenotic segments associated with atherosclerosis.

Volumetric blood flow can be calculated by the use of the DDU method from the product of time-averaged mean velocity, cross-sectional area of the vessel, and HR. Although this technique has been validated by use of an in vitro model, a number of potential sources of error exist when this method is applied in vivo. Sources of error for the method of determining vessel area include incorrect assumptions about vessel shape (e.g., cross-sectional area may not be circular), changes in the vessel area over time as a result of variation throughout the cardiac cycle, and limited axial resolution of the pulsed-wave system. In the study reported here, we detected only small variations in vessel diameter, even at various blood flow rates and blood pressures. The influence of pulsatile variation in vessel diameter appears to be small, and time-averaged diameters are considered acceptable for flow computations. Mean blood flow velocity and, thus, volumetric flow may be influenced by other factors, such as nonuniform insonation of the blood vessel, intrinsic spectral broadening, and angle estimation. Accurate measurement of mean velocity requires uniform distribution of scatterers throughout the sample volume. The uniform insonation method assumes that the sample volume encompasses the entire cross section of the vessel, but this is difficult to achieve without contaminating the Doppler signal with frequency shifts from wall motion and adjacent structures such as accompanying veins. Theoretically, use of a sample volume that excludes low-velocity flow adjacent to the vessel wall could cause overestimation of volume flow, although preliminary studies conducted by our laboratory group with the protocol described here did not reveal differences in FVI values when larger sample volumes were used. The uniform insonation method also assumes that flow is laminar and axial; thus, this method is likely to be more reliable in straight arterial segments without stenotic lesions or bifurcations. Evaluation of the femoral arterial system by use of multiple-gated pulsed Doppler indicates that the velocity pattern in these vessels is complex with the maximum fluctuation in forward and reverse flow occurring near the central portion of the artery. If there is turbulent flow within the sample volume, the spectrum will be further broadened because of variation in the angle of motion with respect to the incident ultrasound beam, even when the actual RBC velocities within the sample volume are similar.

In the study described here, the DDU sample volume was placed in the center of the flow stream to minimize wall noise or signals from adjacent vessels. Differences between the outer, modal, and inner envelope tracing methods were more pronounced when turbulent flow patterns were displayed, such as after administration of nitroglycerin. Modal and inner envelope tracing methods of blood flow measured by use of DDU compared favorably to measures obtained by use of TTF, but significant overestimation was found with the outer envelope tracing method, especially when there was noticeable spectrum broadening. This may be relevant in coronary arteries and diseased vessels in which disturbed flow is common.

It is generally accepted that maximum velocity is usually overestimated by Doppler ultrasound systems for flow in a straight vessel. Although many sources of variation have been identified, including angle of insonation, intrinsic or geometric spectral broadening, intrinsic subject variability, and variation among ultrasound machines, little attention has been paid to the manner in which the spectral display is traced. Many machines will automatically calculate an intensity-weighted velocity that is based on the frequency spectrum. This may lead to an overestimation of mean velocity or FVI when there is turbulent flow. Analysis of results of the study reported here suggests that tracing the inner border of spectral signals may result in less bias when calculating volumetric blood flow.

Assuming that blood flow is typically distributed equally between the left and right femoral arteries, it is possible that physical placement of the TTF probe influenced flow, resulting in asymmetric distribution between the left and right femoral arteries. However, it has been suggested in other studies that properly implanted TTF probes do not influence flow or cause substantial trauma to vessels. The degree of spectral broadening varies with frequency bandwidth, transducer characteristics, and processing after recording. Although analysis of results of the study reported here suggested the inner envelope tracing method resulted in the least overestimation of flow volume, this finding should be evaluated with other DDU systems and in other vessels. The potential that systematic errors in measurement of cross-sectional area of an artery could have resulted in consistent overestimation for the outer envelope tracing method also must be considered.

On the basis of the results of this study, we conclude that the DDU method allows the detection of quantitative changes in blood flow in the common femoral artery. Use of this method may provide reliable results for investigating changes in regional blood flow in clinical settings.

References


