Technical recommendations for the use of carotid duplex ultrasound for the assessment of extracranial blood flow

Kate N. Thomas,1,2 Nia C. S. Lewis,3 Brigid G. Hill,1 and Philip N. Ainslie3

1Department of Surgical Sciences, University of Otago, Dunedin, New Zealand; 2School of Physical Education, Sport and Exercise Sciences, University of Otago, Dunedin, New Zealand; and 3Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia Okanagan, Kelowna, Canada

Submitted 18 May 2015; accepted in final form 8 July 2015

Thomas KN, Lewis NCS, Hill BG, Ainslie PN. Technical recommendations for the use of carotid duplex ultrasound for the assessment of extracranial blood flow. Am J Physiol Regul Integr Comp Physiol 309: R707–R720, 2015. First published July 8, 2015; doi:10.1152/ajpregu.00211.2015.—Duplex ultrasound is an evolving technology that allows the assessment of volumetric blood flow in the carotid and vertebral arteries during a range of interventions along the spectrum of health and chronic disease. Duplex ultrasound can provide high-resolution diameter and velocity information in real-time and is noninvasive with minimal risks or contraindications. However, this ultrasound approach is a specialized technique requiring intensive training and stringent control of multiple complex settings; results are highly operator-dependent, and analysis approaches are inconsistent. Importantly, therefore, methodological differences can invalidate comparisons between different imaging modalities and studies; such methodological errors have potential to discredit study findings completely. The task of this review is to provide the first comprehensive, user-friendly technical guideline for the application of duplex ultrasound in measuring extracranial blood flow in human research. An update on recent developments in the use of edge-detection software for offline analysis is highlighted, and suggestions for future directions in this field are provided. These recommendations are presented in an attempt to standardize measurements across research groups and, hence, ultimately to improve the accuracy and reproducibility of measuring extracranial blood flow both within subjects and between groups.

carotid artery; ultrasound; cerebral blood flow measurement; cerebral hemodynamics; transcranial Doppler

DUPEX ULTRASOUND IS BEING used with increasing regularity in integrative human physiology research to determine blood flow and blood vessel diameter changes in the extracranial carotid and vertebral arteries in response to experimental interventions. This is especially important in light of a growing body of evidence, indicating these elastic arteries are important in contributing to the regulation of cerebral blood flow (74). Historically, it was accepted that measuring intracranial blood flow velocity using transcranial Doppler (TCD) ultrasound (for example, of the middle cerebral artery) was a reliable and valid index of blood flow, as the diameters of such vessels (and the proximal feeding arteries) were understood to be constant during physiological challenges, including alterations in the arterial partial pressure of carbon dioxide and oxygen, and orthostatic maneuvers (24, 35, 59, 70, 75). Contrary to this long-held notion, recent data indicate that the extracranial arteries may, indeed, dilate or constrict in response to perturbations in arterial blood gas concentrations (73) and blood pressure (11, 38), and with varying exercise intensity (56). Similarly, there is some evidence to indicate that intracranial vessels, as assessed by transcranial color-coded ultrasound and magnetic resonance imaging (MRI), also change diameter in response to hypoxia (75) as well as to hypocapnia and hypercapnia (13, 72); this notion was recently editorialized (2). The implications of changes in the diameter of the middle cerebral artery during modest elevations in carbon dioxide are that reactivity might be underestimated by up to 58% (14). Magnetic resonance angiography (MRA) techniques to determine diameter, together with TCD measures of blood velocity, have recently been shown to estimate intracranial blood flow with good precision and accuracy (78). The innovative use of these modalities together has potential for improving the capacity to assess flow itself rather than surrogates (e.g., velocity alone). However, MRA is expensive and currently provides only a static and supine measure of diameter. It should be noted, however, that the validity of TCD as a measure of cerebral blood flow does not necessarily hinge on the changes in velocity and/or diameters of the feeding vessels; as yet, there is no direct evidence to indicate that changes in flow within the intracranial vessels directly mirrors changes in flow in the extracranial vessels. These assumptions need to be tested under a range of different experimental conditions of interest. If there

Address for reprint requests and other correspondence: K. N. Thomas, Dept. of Surgical Sciences, Dunedin School of Medicine, PO Box 913, Dunedin 9054, New Zealand (e-mail: kate.thomas@otago.ac.nz).

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are divergent responses between measures of flow and/or velocity in the extracranial vessels and velocity in the intracranial vessels, this could mean either 1) measures of intracranial velocity do not reflect flow, or 2) the intracranial vasculature is responding differently to the extracranial vasculature. Both outcomes are possible until direct evidence can support one or the other hypothesis; as such, parsimonious interpretation of data is needed.

Consequently, the scope for ultrasound in basic science and clinical research applications is expanding. For example, cerebral blood flow and reactivity are modestly predictive of stroke risk (27, 51) and cognitive decline (26, 40); therefore, more accurate volumetric assessment may provide new clinical application. Since the advent of automatic edge-detection wall-tracking software for use with high-resolution ultrasound systems, it is possible to continuously track changes in diameter and blood velocity during dynamic situations. Thus, the incorporation of measures of both extracranial and intracranial blood flow, diameters, and/or velocities can provide new insight into the mechanisms of cerebral blood flow regulation. Given the increasing popularity in numerous research fields of using duplex ultrasound to assess extracranial blood flow in the estimation of global and regional cerebral blood flow, our intent is to provide practical recommendations on technical and methodological aspects of performing carotid and vertebral artery ultrasound that will improve the accuracy and reproducibility of measuring extracranial blood flow both within subjects and between groups. An important preface to this article is that a basic understanding of ultrasound technology is required for full comprehension of the following methodological guidelines. We recommend reading dedicated ultrasound technology texts (9, 21) for more detail; also see section Limitations of Ultrasound.

Methodological Recommendations

Subject preparation and care. Vascular tone and function in peripheral arteries can be acutely influenced by diet (36, 69), alcohol (30), caffeine (47), prior exercise (15, 53, 66), medications (29, 63), time of day (34, 44), and menstrual cycle phase (1); therefore, depending on the experimental question, we recommend following the conservative instructions for preparation of subjects prior to peripheral artery assessments when it comes to carotid artery assessments also ([65], see Fig. 1). Similar guidelines with regard to diet and exercise have been described for determination of arterial stiffness by carotid-femoral pulse wave velocity (71). In brief, this includes avoiding alcohol, caffeine, and strenuous exercise for ≥12 h prior to testing; subjects should ideally be fasted for ≥6 h; and experiments should be conducted at the same time of day, and during the same menstrual cycle phase for premenopausal women. If premenopausal females are being compared with males, then assessments should be made ideally between days 1 and 7 of the menstrual cycle. However, it should be noted that differences in the length and phase of the menstrual cycle are common, and the only way to ensure that females are in the low-hormone phase is via blood samples and related hormone analyses. Clear history of menstrual cycle length is likely an acceptable approach, and if in doubt, both estrogen and progesterone are low during the early follicular phase (days 1–4); by day 7, estrogen may be starting to increase, so experiments should be avoided if an irregular cycle is noted. As many medications have both direct and indirect vascular effects, if possible, subjects should refrain from taking all medications for at least four half-lives of the drug prior to extracranial blood flow measures (12, 22, 28, 63). In pathological groups, although cessation of many cardiovascular medications (i.e., beta blockers, ACE-inhibitors, nitrates, calcium channel blockers, etc.) may not be possible, their potential to confound the results should be considered and documented. Because of the potential of nonsteroidal anti-inflammatory medications to markedly influence cerebral blood flow (19, 42), these pharmacological agents should be discontinued for 2–3 days if possible.

For evaluation at rest, to allow for stabilization of blood volume and blood pressure, the subject should rest in a quiet temperature-controlled environment for a period of at least 15 min before assessment. To perform the assessment, the subject should be in a comfortable supine position on a bed with the neck fully exposed. The optimal window of insonation can vary, but in the majority of cases instructing the subject to turn their head ~30° toward the contralateral side will elongate the neck sufficiently, with the transducer placed either anterior or posterior to the sternocleidomastoid muscle. A low pillow or, in some cases, no pillow may also aid with positioning the neck. Avoid applying excessive pressure on the neck with the transducer. Although during the physiological trials (e.g., exercise, orthostatic stress, sympathetic activation) the same approaches can be used, the challenges of getting reliable images are much greater. In our experience, the following three points should be considered. First, much greater practice is needed when movement (e.g., deeper and/or more rapid breathing and any body movement) is expected. We would suggest that additional familiarization for both the sonographer and participant is required. The participant can be familiarized to breathe as regularly as possible and, importantly, avoid tensing of accessory muscles of the neck, which hinder ultrasound quality. Second, when changes in breathing, blood pressure, and/or intrathoracic pressure are marked, the duration of data collection should be extended to ensure a reliable steady state can be assessed without signal artifact (for further details, see: Diameter and Flow Assessment Protocol). Finally, investigators should be prepared to discard data when the quality is suboptimal for automatic edge-detection analysis due to demanding experimental conditions; the high-quality images required for analysis is, thus, both an advantage and a paradoxical limitation of the current technology.

Ultrasound Orientation Conventions

The transducer should always be oriented with the head of the transducer (usually marked on the side of the casing) pointing toward the operator’s thumb when scanning in transverse, or toward the patient’s head when scanning in a sagittal plane. The head of the transducer is represented on screen by a marker, which varies between systems (see the small “T” in the top left of the image in Fig. 2). This orientation is a universal convention and should always be used.
Identification of Carotid Vessels

A schematic diagram of the extracranial carotid arteries is shown in Fig. 3. Beginning in brightness mode (B-mode, two-dimensional, gray scale), with a generous application of gel to the transducer, identify the common carotid artery (CCA) at the base of the neck in a transverse plane (see Fig. 2). The internal jugular vein can be seen lateral to the CCA and the thyroid can be seen medial to the CCA. If there is any question about the identity of the CCA vs. the internal jugular vein, press down with the transducer to compress the internal jugular vein; the CCA will not compress with light pressure. From the base of the neck, course the transducer cranially toward the bulb and bifurcation of the CCA (Fig. 4). At the level of the bulb, rotate the transducer clockwise into a sagittal plane. This point is usually 1–3 cm below the angle of the jaw (9). In the sagittal plane, two vessels—the internal (ICA) and external (ECA) carotid artery (Fig. 5)—can be seen originating from the bulb. Note these vessels will likely not both be visible in one plane unless they are within the scan plane of the transducer. In most experimental physiological studies, the diameter and flow in the CCA, ICA, and/or vertebral are of interest. It is essential to confidently differentiate between the ICA and ECA as the downstream vascular beds of these arteries behave differently. The ICA and ECA can be differentiated using the following five steps (50).

1. When scanning in a sagittal plane, the ECA tends to be positioned anterior-medially, while the ICA tends to be more posterior-lateral. This is true in 95% of cases (49). A slight pivot of the transducer (ranging from about 10 to 40°) between anterior-medial and posterior-lateral positions should allow alternate visualization of each vessel in longitudinal section. In some cases, both ICA and ECA can be seen in one plane, appearing as a “tuning fork” (Fig. 5).

2. The diameter of the ECA is usually smaller than that of the ICA at the bifurcation (Fig. 5); the average ICA diameter is
The diameters become similar beyond the bifurcation.

3. The ECA has eight extracranial branches in the neck, some of which are usually visible in the proximal section; these branches supply the thyroid, face, and scalp. The ICA has no extracranial branches (50) (Fig. 5). Use color Doppler if necessary to help visualize ECA branches (Fig. 6).

4. The spectral Doppler waveform of the ECA is a high resistance waveform, with a sharper upstroke, narrow systolic peak, rapid decline during diastole toward the baseline, and lower end-diastolic velocity. The waveform of ICA is a low resistance waveform with a more gradual upstroke, broader systolic peak, and continuous but tapering forward flow throughout the cardiac cycle, and a higher end-diastolic velocity (Fig. 7 and Table 1).

5. A temporal tap (i.e., firm manual tapping in the region of the superficial temporal artery) can be used to further confirm the identity of the ECA; tapping produces a distortion of the spectral Doppler waveform of ECA. However, the tapping can sometimes produce perturbations to the waveform of ICA also (54); therefore, both vessels under consideration must be examined during the temporal tap maneuver. If the perturbations are found in only one vessel, then this is the ECA; if found in both ECA and ICA then this is not a reliable tool for identifying vessels.

The steps above should be followed in the order 1–5, or until the sonographer is certain of the identity of the vessels. Once the identity of the vessels has been confirmed, the experimental protocol can continue.

Screening for Pathology

The subclinical process of atherosclerosis begins in infancy (61), and the carotid arteries, in particular, the bulb and proximal ICA, are sites with heavy atherosclerotic load in aging individuals. The presence of atherosclerotic plaques may be observed during this examination (for example, Fig. 8, on the shallower wall). If disease is present, we recommend referral to a clinical vascular laboratory for a detailed carotid artery assessment, as diagnosis of such disease is beyond the scope of this article. Discovery of atherosclerosis in the carotid arteries may be an exclusion criterion for participation in the experiment, so screening should be performed before any intervention begins. This screening is of particular importance for cerebrovascular physiology experiments; if extracranial stenosis is present, then the potential of intracranial velocities via TCD to reflect cerebral blood flow or function (e.g., reactivity or autoregulation) will be limited (52).

Vertebral Artery

The vertebral artery (VA) is visible on ultrasound between its origin from the subclavian artery and approximately the transverse process of C4. With a longitudinal view of the distal CCA visible, pivot the transducer laterally (i.e., toward the ipsilateral ear). The VA will be located deeper and lateral to the CCA and internal jugular vein, with only sections of it visible between the vertebral processes (Fig. 9). Color and/or spectral Doppler should be used to ascertain
that flow is in the antegrade direction; retrograde flow is not uncommon and can indicate proximal disease.

**Technical Recommendations**

To perform carotid investigations, a linear transducer with medium to high frequency should be used to increase axial resolution, i.e., 7 MHz or greater. However, as diameter changes being assessed may be modest and, therefore, near the limit of axial resolution of the system, the highest frequency possible should be used (e.g., axial resolution for standard transducers of 7 and 12 MHz is 0.220 mm and 0.128 mm, respectively). Once an initial scan has been performed for screening purposes and to correctly identify the vessels, the following adjustments can be made, in the order below (see Fig. 1), to optimize the system for diameter and velocity measurements.

**B-mode.** B-mode is the representation in gray scale of echo-producing structures underlying the transducer.

**DEPTH.** The depth of the live B-mode screen should be set so that the vessel of interest is at a depth of about two-thirds of the display. This approach ensures that movement will not result in the vessel shifting off-screen but also means that there is no wasted insonation of tissue beyond the area of interest, thus allowing for higher frame rates (21), and maximizes the pixels allocated to the region of interest. The best reflectivity of tissue interfaces, such as the vessel wall, occurs when the incident ultrasound beam is at 90° to the interface. To achieve a perpendicular alignment and, therefore, an improved image, the operator can “heel-toe” the transducer (tilt from end to end) or assess the vessel from a different acoustic window.

**Fig. 4.** Transverse (TS) view of the carotid bifurcation into external carotid artery (ECA) and internal carotid artery (ICA).

**Fig. 5.** Saggital view of the common carotid artery (CCA) and the carotid bifurcation into external carotid artery (ECA) and internal carotid artery (ICA).
FOCUS. The focus should be adjusted to the point of interest, which ensures the focal zone encompasses the vessel and, therefore, provides the best lateral resolution at this level (21).

GAIN. The B-mode image should be optimized to clearly delineate the vessel wall from the lumen, particularly, if using automatic edge-detection wall-tracking software. This optimization can be achieved in several steps. First, increase the overall gain so as to increase echogenicity of the tissue throughout the image to the point where the vessel lumen remains echo-free. Then selectively adjust the time gain compensation (TGC) sliders, so that gain is increased at depths corresponding to tissue surrounding the vessel and the vessel walls, but decrease the gain at the depth corresponding to the vessel lumen (for example, Fig. 5). Decrease dynamic range (compression) to increase the contrast of the image; this reduces the range of grays used and results in a more black-and-white image. Increase noise rejection (filter) to eliminate clutter or acoustic noise from weak echoes; this should be particularly useful in removing weak echoes from within the vessel lumen. Further settings like edge enhancement, persistence, and varying gray maps may be available on a high-tech system, and can be useful, but for the majority of research-level machines, adjusting the settings described above will suffice. It may be prudent to perform basic adjustments, save them as a system preset (if this capability is present), and then use this preset to study all subjects with individual adjustments to only depth, focus, gain, and TGC to provide some standardization across tests.

**Color Doppler.** Color Doppler uses color maps superimposed over the B-mode image to indicate blood flow velocity and direction; usually shades of blue are allocated to movement in one direction (e.g., away from the transducer), and shades of red to movement in the opposite direction (e.g., toward the transducer). The brighter the hue of each color, (i.e., yellow vs. red, or light blue vs. dark blue) the higher the velocity. It is often useful to utilize color Doppler initially for several reasons: 1) to aid in identifying branches of the ECA, and 2) to locate the fastest flowing stream of blood in a vessel.

**COLOR BOX PLACEMENT.** Place the color box over the vessel of interest. The box should be tall enough to overlap the vessel diameter, and the width of the box should be reduced to ~3–4 cm to preserve the frame rate. Steer the box so that there is an adequate angle (<90°) between the beam direction and the vessel otherwise the Doppler shift will not be coded in color (See Fig. 6; the color box has been steered 20° to the left (i.e., approximately 70° to the vessel) to optimize color fill within the vessels).

**COLOR SCALE.** Set the color velocity scale (often labeled as pulse repetition frequency, PRF) to detect flow without spuriously superimposing color over tissue outside the vessel walls. This can be achieved by turning the scale down to the point of aliasing (bright mosaic color pattern), then turn it up one or two levels, to achieve adequate color sensitivity. A color scale between 20 and 30 cm/s should be adequate in most carotid artery assessments. The part of the vessel with the brightest color hue represents the fastest flowing stream. If the vessel is straight, this is likely in the center; however, if the vessel is curved, this is likely toward the outer curvature of the wall. If the scale is set too high, there will be poor sensitivity to low Doppler shifts, i.e., little or no color will be seen.

**COLOR GAIN.** Increase color gain until there is good color fill within the vessel but no bleeding out into surrounding tissue.

**Pulsed-wave Doppler.** Pulsed-wave Doppler (PWD, spectral Doppler) is used to calculate the velocity of moving reflectors (e.g., red blood cells) based on the received Doppler frequency shift.

**ANGLE OF APPROACH (DOPPLER ANGLE).** The angle of approach (Doppler angle) is the angle between the beam axis and the direction of travel of the moving reflector. Steer the ultrasound beam so that an appropriate Doppler angle will be possible (see below on Doppler angle and Fig. 10); this will depend on the vessel orientation in relation to the transducer. Usually a single line from the transducer-skin interface extending down through the image represents the central axis of the beam direction. The
Doppler angle must be adjusted so that it is always $\leq 60^\circ$ (30 to 60° is preferable); this ensures that a Doppler shift is adequately detected, and reduces the error associated with velocity measurement (21); see Trade-off Between B-mode and PWD. Angles of $>60^\circ$ should never be used; above 60°, a small error in angle cursor alignment will result in unacceptably large errors in the calculation of velocity due to the rapidly changing cosine function of angles greater than 60°. For example, for a 5° error in setting the angle correction cursor, at 0°, this results in $\approx 0.4\%$ error, whereas at 60°, the error is inflated to $\approx 18.4\%$ (see Fig. 7A: the beam and color box have been steered 20° to the left, which creates an angle of $\approx 50$–$60^\circ$ between the beam and the flow direction. This is further detailed in Fig. 10.).

**SAMPLE VOLUME PLACEMENT.** The sample volume cursor indicates the depth along the beam axis from which Doppler information is being obtained. This should be placed in the fastest flowing stream, and then color Doppler can be turned off (if it is being used). Turning color off has two purposes; it allows an optimal B-mode image of the vessel located near the PWD sample to be used in diameter analysis, and it improves the frame rate of the system (operating in two live modes vs. three). Adjust the width of the sample volume to encompass the desired portion of the vessel; this is dependent on the equation being used to calculate flow and should be confirmed with the analysis software engineers (see Limitations for further discussion). In most cases, the calculation of flow uses the time-averaged maximum velocity derived from the peak velocity envelope (see Analysis Considerations), so a sample volume that encompasses $\approx 80\%$ of the vessel lumen allows the peak velocity to be captured even if the vessel moves slightly, and significant wall/tissue movement will likely be excluded.
ANGLE CORRECTION. Angle correction should always accurately reflect the Doppler angle (described above). An angle correction cursor is usually located within the sample volume; this must be aligned with the direction of flow (see Fig. 10; note that the flow direction may be off-axis to the vessel walls if the vessel is particularly tortuous). The angle created between the angle correction cursor and the central axis of the beam is displayed on the screen, and this is the angle used in the calculation of velocity (see 60° to the right of the image in Fig. 7). An incorrect angle correction with respect to the blood flow will result in an incorrect calculation of velocity (see Fig. 10).

DOPPLER BASELINE. This is the horizontal line in the spectral display that represents 0 cm/s. This should be adjusted so that the spectral waveform is above the baseline in the display. If the baseline is too high, the spectral trace will appear to wrap around the display with the systolic peaks seen at the bottom of the display. There is not usually a retrograde velocity component in carotid vessels, especially ICA, but if the baseline is too low, the retrograde portion of the trace (if any) may be displayed at the top of the display in error. Note that sometimes the waveform may be displayed upside-down, i.e., below the baseline; there is an invert button to change this. This depends on the transducer and vessel orientation with respect to each other (i.e., the vessel and, therefore, flow within it may be curving away from the transducer, displayed as a negative Doppler shift).

DOPPLER SCALE. Set the velocity scale (PRF) represented by the y-axis in the spectral display so as to accommodate the velocity of the blood being measured and to allow for increases if this is expected with the specific experimental protocol. A spectral trace occupying approximately two-thirds of the display is often appropriate at baseline unless increases of significant magnitude are anticipated. The scale can be increased by increasing the PRF and vice versa. If using automated analysis software, adjusting the scale after data collection has commenced should be avoided, as this will necessitate recalibration.

DOPPLER GAIN. The gain of the spectral Doppler trace should be increased to the point of the trace being clear and defined, but not so high as to fill the background with “speckle” or noise. If gain is too high, a mirrored spectral trace may be seen below the baseline. The dynamic range of the spectral trace can also be reduced to improve the contrast of the trace, i.e., make it whiter compared with the black background; this is important if you are using automatic edge-detection software for analysis. As with B-mode, noise rejection can be increased to remove weak echoes in the background of the spectral trace.

Table 1. Reference data for blood velocity in extracranial arteries across healthy aging

<table>
<thead>
<tr>
<th>Distal CCA</th>
<th>Proximal ECA</th>
<th>Proximal ICA</th>
<th>VA</th>
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<tbody>
<tr>
<td>n</td>
<td>PSV, cm/s</td>
<td>EDV, cm/s</td>
<td>PSV, cm/s</td>
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Data are shown as 25th percentile to 75th percentile range; n = 1200 total. CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; VA, vertebral artery; PSV, peak systolic velocity; EDV, end-diastolic velocity. PSV tended to be higher in males than females by approximately 10% in the CCA and ECA, with no consistent sex difference in the ICA or VA. In contrast, Seidel et al. (58) reported a significantly higher flow volume in the VA in males compared with females; however, their sample size was small (n = 50), and the authors also report an angle of insonation of 62 ± 6° (suboptimal angle for accurate velocity measurement). Velocity in the left CCA and VA were, on average, higher than the right (CCA PSV by ~3% and VA PSV by ~5%); this is consistent with previous reports (57). Data were kindly provided, with permission, from Otago Vascular Diagnostics, Department of Surgical Sciences, University of Otago, New Zealand.

Review
Trade-off Between B-mode and PWD

Duplex mode allows the simultaneous acquisition of B-mode and Doppler for determination of vessel diameter and blood velocity, respectively. Thus, depending on the analysis approach used (see below), these collective measurements enable the calculation of beat-to-beat blood flow, as well as potentially other relevant hemodynamic variables, e.g., vessel compliance (e.g., 37), stiffness (e.g., 37, 64), baroreflex sensitivity (e.g., 64), shear rate (e.g., 3, 46). However, an important trade-off exists: For optimal B-mode resolution (in particular, echogenicity of the vessel walls), the vessel should be orientated horizontally within the field of view (parallel to the transducer face and, therefore, perpendicular to the beam); this ensures maximum reflection and echo intensity in the image. However, for PWD, an angle between the incident beam and direction of blood flow should be 0–60°, as mentioned above. A perpendicular beam in Doppler mode will result in Doppler shifts that are too low, poorly formed waveforms, and/or incorrect determination of velocity (see Fig. 10A). A compromise must, therefore, be reached to satisfy the principles of both modalities. Beam steering allows this challenge to be overcome, as the beam can be steered to reduce the angle of the vessel relative to the beam for spectral Doppler analysis (see Fig. 10B). Similarly, a heel-toe maneuver of the transducer can be useful so that an angle of ≤60°C can be ensured (see Fig. 10C).

Protocols

Diameter and velocity assessment protocol. A baseline period of assessment of diameter and blood velocity of at least 1 min should be obtained before an intervention begins. During interventions, e.g., drug or exercise challenges, recordings should be made over a minimum of 10 cardiac cycles, but ideally 30 s or more. In subjects who reveal a clear respiratory arrhythmia and/or blood pressure variability, this may need to be extended to reflect a true average and stable basal measurement. If measuring diameter and velocity in the ICA, we recommend being at least 2 cm distal to the bulb, to avoid the influence of the bifurcation on the flow pattern, as this may be turbulent nearer to the bulb. For repeat assessments, note should be taken of the system settings and also the optimal insonation window so as to improve reproducibility. A photo-
graph of the transducer in place or, alternatively, marks on the skin may help to ensure this window.

Figure 1 gives a summary of recommendations from subject preparation through image acquisition to analysis steps. Moreover, reference values for velocities in the CCA, ECA, ICA, and VA across the healthy life span are presented in Table 1.

Analysis Considerations

Two methods of edge-detection systems are currently in use to measure changes in artery diameter. One method involves a computer-automated edge-detection system that automatically tracks the artery wall and blood velocity while the second approach involves the sonographer manually measuring these components.

Automated edge-detection approaches. The use of edge-detection software for offline analysis is recommended for the simultaneous acquisition of B-mode and Doppler information for determination of vessel diameter and blood velocity. Using this approach facilitates more objective and accurate diameter measurements and also permits synchronization of the ultrasound system and ECG (if available). Recording of the display during data collection using screen capture software allows extraction of a video clip of the complete study that can be analyzed at a later time frame by frame (e.g., Techsmith Camtasia, https://www.techsmith.com/camtasia.html). Currently, the most commonly used and commercially available edge-detection software are those developed by Medical Imaging Applications LLC (http://www.mia-llc.com/services/brachial.htm) and Cardiovascular Suite (http://www.squipu.eu/). Both software options have been used to assess vascular function in health and in disease (e.g., 6, 20, 41, 60); however, in the context for the assessment of brachial artery endothelial function, only the Medical Imaging Applications edge-detection software has been independently validated (41); however, Cardiovascular Suite demonstrated good agreement with the Medical Imaging Applications system (18) and with a reference radio-frequency echo tracking system (5). The cost of software and related support is between $10,000 and $20,000 (U.S. dollars). Various other custom-made software for automated edge-detection software has also been developed and reported extensively in the literature (e.g., 7, 45, 68, 76). With these custom-made software approaches, it is now possible to directly export the changes in diameter and velocity with high temporal resolution into other data acquisition software (e.g., Chart or MATLAB). At least in other arteries (e.g., the brachial artery), using edge-detection software not only provides a more robust and sensitive assessment of vessel diameter and velocity (76), it also limits subjectivity and bias during data analysis. Although some of these custom-made software modalities have been validated against controlled in vitro flow phantom models (e.g., 76), it is not clear whether all of them have been validated. Caution is, therefore, recommended in selecting software if validation studies have not been reported.

Manual detection approaches. If edge-detection software is unavailable, it is recommended that data (diameter and velocity) be collected as often as possible depending on the experimental perturbation, e.g., every 3–4 s if “dynamic” changes are expected, or every 10 s if more steady-state measurements are being taken. With this approach, the sonographer carefully manually tracks systolic and diastolic changes in diameter and velocity (using the automated calipers on the ultrasound). A principal drawback of this method, compared with the automated approaches is that diameter and velocity measurements often cannot be collected simultaneously and, therefore, cannot be time-aligned. This is because many ultrasound systems are not able to record cine loops of the arterial diameter (which occurs in B-mode) and blood flow velocity (which is measured in the PWD mode) at the same time; often the B-mode image is frozen, while PWD is live or vice versa. In addition, cine loops are of limited duration (commonly ~10 s), both in B-mode or PWD. If operation of two live modes is not possible, separate B-mode and PWD cine loops must be saved. If both modes can operate simultaneously, a single cine loop can be recorded. In either case, manual measurements of diameter and velocity are required using built-in calipers, which begets another drawback: the subjectivity of placing the calipers.

The specifics of making the manual measures are as follows: 1) Following optimization of the B-mode image of the artery and blood flow velocity trace, a cine loop recording of both live modes is saved on the system (or a B-mode cine loop of the artery, followed by a PWD cine loop). The cine loop is manually scrolled through, and the diameter can be measured using the measurement calipers on consecutive cardiac cycles (end-diastole is most commonly used). The measurements need to be manually recorded. The cine loop is then restarted (or the PWD cine loop is opened), the velocity calipers are selected, and manual measurement of systolic/diastolic peaks is performed, and these measurements are recorded. Subsequent cardiac cycles are then analyzed in the same manner.

With either method, blood flow is later calculated offline from velocity and diameter. Standardly (16, 39), this is calculated as flow (Q) = (peak envelope velocity/2) × (π × [diameter/2])².

It should be noted that absolute measurements of velocity and flow cannot be determined with accuracy in the presence of complex flow patterns and possible nonuniform insonation of the vessel (see Limitations of Ultrasound). Reporting of relative/percentage changes is prudent, as they incorporate fewer systematic errors. Averaging repeated measurements may also be advantageous for smoothing out random errors.

Limitations of Ultrasound

B-mode. The presence of arterial calcification in the vessel walls can limit the penetration of ultrasound and can obscure the lumen and tissue deep to a calcified area due to the presence of an acoustic shadow (see Fig. 8). In these cases, reposition the transducer around the vessel in an effort to identify a plane where the plaque load is lesser, allowing adequate penetration of the vessel; however, on some occasions, the acoustic shadow cannot be overcome. Large-habitus subjects and especially those with muscular necks can be technically difficult to insonate optimally. Different insonation windows may improve visualization; however, on rare occasions, sufficient image quality cannot be achieved for the use of automatic software. Similarly, extremely mobile vessels can provide a technical challenge when capturing images for use with automatic software. Vessel mobility is sometimes noted in younger subjects due to highly compliant vessels, or in older subjects due to vessel tortuosity, and also during interventions involving increased ventilation (e.g., exercise).
Doppler. Several complications exist with regard to using Doppler technology to estimate velocity and, consequently, flow. A detailed critique of the nuances of ultrasonic measurements is not the intention of this document, but briefly, these limitations include 1) unknown velocity profiles (parabolic flow is assumed) (32); 2) intrinsic spectral broadening (67) related to the sample volume being of finite dimensions and, therefore, an array of angles of insonation for scatterers within the sample volume; 3) estimation of the angle of approach (this is set by the operator and assumed constant for all red blood cells in the sample), and this gets incorporated into the angle-correction process; 4) possible arterial lumen asymmetry (a circle is assumed in the calculation of cross-sectional area) (23); 5) nonuniform insonation of the blood vessel by the ultrasound beam (16) and equally nonuniform scattering and receiving of echoes (23); and 6) variations in Doppler signal processing (17, 23). Interested and more advanced users are referred to the referenced literature above for in-depth discussion of these important topics, which are beyond the scope of this article.

Doppler ultrasound quality assurance protocols are recommended by several professional bodies to validate the accuracy and performance of the system. Unfortunately, there is no universal consensus on the standards or test devices to be used (e.g., string and flow phantoms); however, Browne (10) provides a review of some of the current recommendations and commercially available Doppler test devices. In brief, these include establishing the primary outcome measure of the system and using an appropriate test device for this function; for example, for extracranial carotid and vertebral assessments, the primary function would likely be maximum velocity (62). However, test devices examining spectral broadening, flow direction, sample volume dimensions, and angle correction would all be important (10). Evaluating the Doppler system is done by selecting several of the known flow rate presets on the phantom and using the provided chart to convert flow to velocity to compare with the accuracy of the velocity determination by the ultrasound system.

Similarly, B-mode phantoms should also be incorporated into every laboratory’s quality control regimen. A large range of B-mode phantoms are available; the most important qualities when selecting a device are the speed of sound and attenuation coefficient, and a uniform image texture (25). For a detailed review of commonly performed B-mode quality control tests, see Ref. 25.

A further limitation is that ultrasound requires highly skilled and practiced operators to obtain acceptable quality images. In general, on the basis of our work and that of other laboratories, we recommend the following: 1) >150 scans of each vessel of interest are needed (ideally >50% of scans should be with feedback, including analysis from a skilled operator); 2) internal assessment, including determination of intraobserver variability and evidence of reproducible data (i.e., coefficient of variation <10% between days) should be done prior to independent data collection; 3) reading and training in ultrasound physics should be done; and 4) placement should be arranged, if possible, with a clinical vascular sonographer in a hospital setting. As with any technical approaches, innate learning ability and hand-eye coordination vary extensively between individuals.

Conclusions and Future Directions

Assessment of extracranial blood flow, combined with sensitive edge-detection software, provides a powerful means to assess regional cerebral blood flow. This approach avoids and overcomes many of the assumptions of TCD ultrasound (2) and MRI (33, 59), although both have important functions and distinct advantages as imaging tools. However, appropriate ultrasound technology and training, subject preparation, and detailed knowledge of the approach are required to perform an accurate assessment of extracranial blood flow. The recommendations provided herein represent the most recent advances in the ultrasonic measurement of extracranial blood flow. These recommendations are presented in an attempt to standardize measurements across research groups and, hence, ultimately improve the accuracy and reproducibility of measuring extracranial blood flow both within subjects and between groups.

From a pathophysiological perspective, there is growing evidence that cerebral hemodynamic impairment is an independent cause of cognitive dysfunction in a variety of cerebrovascular conditions (4, 43, 55). More specifically, cerebral hypoperfusion may be independently associated with cognitive decline (43), and conversely, higher resting cerebral blood flow has been linked to superior cognitive function (26, 40). A good example of the link between hypoperfusion and cognitive dysfunction can be observed in patients with severe congestive heart failure, where reduced cerebral blood flow due to hemodynamic decompensation correlates with the rising prevalence of cognitive dysfunction (48). Conversely, improvements in cerebral blood flow utilizing angiotensin-converting enzyme inhibitors or cardiac transplantation, increase cerebral blood flow and improve cognitive function in this population (8, 79).

The implications of intracerebral artery diameter changes in aging or the myriad of pathologies that influence the brain are unknown. Although speculative, dilation or constriction of the extracranial arteries may be an indicator of cerebrovascular health (endothelial function), similar to the way in which peripheral flow-mediated dilation is indicative of cardiovascular risk (31, 77). Thus, ultrasound measures of extracranial blood flow may provide effective, noninvasive and novel insight into cerebrovascular function, including the progression of cerebral hypoperfusion with various cognitive related disorders. In addition, ultrasound approaches to assess dilation of the extracranial neck vessels during carbon dioxide alterations offer another means to monitor reactivity not assessable via TCD. For example, the implications of changes in the diameter of the middle cerebral artery during modest elevations in carbon dioxide are that reactivity is likely underestimated by up to 58% (14). Since cerebrovascular reactivity has been modestly linked to stroke risk using TCD (51), more accurate volumetric assessment may provide new clinical insight. It is clear the utility of new measures of cerebrovascular function with extracranial ultrasound is still in its infancy; however, the application of these new approaches holds much potential for clinical use.

From a physiological perspective, in addition to monitoring baseline blood flow, extracranial vascular ultrasound can be used to provide complementary information on factors such as vessel compliance (e.g., 37), stiffness (e.g., 37, 64), baroreflex sensitivity (e.g., 64), shear rate (e.g., 3, 46). While baseline
cerebral blood flow is predictive of stroke risk (27) and cognitive decline (26, 40), the clinical value of these latter variables remains to be established. Additionally, application of ultrasound approaches will extend recent developments that indicate that the extracranial arteries have differential responses to changes in arterial blood gas oxygen and carbon dioxide concentrations (13, 14, 73), reductions in blood pressure (38), with varying exercise intensity (56), and following water immersion (11). Much insight into new aspects of cerebrovascular physiology will be gained, especially when combining a multimodal approach to extracranial and intracranial blood flow regulation (74).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: K.N.T. conception and design of research; K.N.T. and N.C.L. prepared figures; K.N.T. and P.N.A. drafted manuscript; K.N.T., N.C.L., B.G.H., and P.N.A. edited and revised manuscript; K.N.T., N.C.L., B.G.H., and P.N.A. approved final version of manuscript.

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