Measuring peripheral resistance and conduit arterial structure in humans using Doppler ultrasound

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Naylor, Louise H., Cara J. Weisbrod, Gerry O’Driscoll, and Daniel J. Green. Measuring peripheral resistance and conduit arterial structure in humans using Doppler ultrasound. J Appl Physiol 98: 2311–2315, 2005. First published February 3, 2005; doi:10.1152/japplphysiol.01047.2004.—The purpose of this study was to establish valid indexes of conduit and resistance vessel structure in humans by using edge detection and wall tracking of high-resolution B-mode arterial ultrasound images, combined with synchronized Doppler waveform envelope analysis, to calculate conduit artery blood flow and diameter continuously across the cardiac cycle. Nine subjects aged 36.7 (9.2) yr underwent, on separate days, assessment of brachial artery blood flow and diameter response to 5-, 10-, and 15-min periods of forearm ischemia in the presence and absence of combined sublingual glyceryl trinitrate (GTN) administration. The peak brachial artery diameter was observed in response to the combination of ischemic exercise and GTN administration, one in combination with GTN. The peak brachial artery diameter was observed in response to the combination of ischemic exercise and GTN; a significant difference existed between resting brachial artery diameter and peak brachial artery diameter, indicating that resting diameter may be a poor measure of conduit vessel structure in vivo. Peak brachial artery flow was also observed in response to a combination of forearm ischemia exercise and GTN administration; the response being greater than that induced by periods of ischemia, GTN, or ischemic exercise alone. These data indicate that noninvasive indexes of conduit and resistance vessel structure can be simultaneously determined in vivo in response to a single, brief, stimulus and that caution should be applied in using resting arterial diameter as a surrogate measure of conduit artery structure in vivo.

blood flow; arterial diameter; high-resolution ultrasound

RESISTANCE VESSEL STRUCTURE in humans has traditionally been derived from measurement of maximal hyperemic responses to limb ischemia (3, 17, 28). This approach, usually involving plethysmographic blood flow measurement (14), is based on the assumption that peak reactive hyperemia in response to a maximal vasodilator stimulus is limited by maximal resistance vessel cross-sectional area (CSA) in the ischemic region. For example, the hyperemic response to a period of 10 min of upper arm occlusion has been used to establish forearm resistance vessel structural enlargement in athletes relative to matched controls (8, 25), that exercise training increases resistance vessel structure (6, 23, 26), that inactivity has the opposite effect (24), and that changes in wall-to-lumen ratio occur in hypertension (3, 28).

Recently, Doppler ultrasound methodology has been utilized to directly visualize brachial artery diameter and measure flow through conduit arteries in humans (7, 11, 12, 19–21). This method possesses the advantages of being noninvasive, provides absolute blood flow measures, and, most importantly, greatly improves temporal resolution (5). However, the classic plethysmographic studies (17), which typically identified peak reactive hyperemic responses over 5- to 10-s periods within the first 30 s after occluding cuff deflation, have not been repeated utilizing the improved temporal resolution of the Doppler ultrasound approach. It is therefore unclear what magnitude of real maximal flow is achievable and which ischemic stimulus is most appropriate to induce peak reactive hyperemia as an index of resistance vessel structure using the Doppler ultrasound approach.

The adoption of ultrasound technology has encouraged investigators to report measures of arterial diameter as indexes of conduit vessel structure. Several cross-sectional studies have reported enlargement of conduit vessels in athletes relative to matched controls (2, 13, 22, 33), and two recent studies reported enhanced femoral artery diameter after exercise training (2, 16). All of these studies reported resting arterial diameter as an index of conduit structure, with no data relating to arterial diameter in response to stimuli. Resting arterial diameter is dependent on sympathetic nervous system tone, circulating hormone modulation, and local paracrine effects, and it may therefore be a relatively poor index of vascular structure. However, no studies have investigated the optimal stimulus to induce maximal arterial dilatation in vivo.

In the present study we applied a software analysis system that utilizes automated edge detection and wall tracking of high-resolution B-mode arterial ultrasound images, combined with synchronized Doppler waveform envelope analysis at 20–30 Hz, to provide continuous measures of conduit artery diameter and flow across the cardiac cycle. Forearm cuff ischemia, ischemic exercise, and administration of glyceryl trinitrate (GTN), a potent pharmacological vasodilator that possesses the advantage of being rapidly active by sublingual administration, were used to identify the stimuli required to induce peak forearm reactive hyperemic blood flow and brachial artery dilatation in vivo.

METHODS

Subjects

Nine healthy volunteers (33% women) aged 36.7 (9.2) yr with mean height 172.8 (2.7) cm and weight 75.3 (4.0) kg, were recruited from the community. All subjects were screened for cardiac abnormalities and cardiovascular disease before entering the study. Subjects who smoked or were on cardiovascular medications were excluded.

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The study procedures were approved by the Ethics Committee of Royal Perth Hospital and adhered to the Declaration of Helsinki, and all subjects gave informed consent.

Experimental Design

Each subject underwent nine separate testing sessions, conducted at the same time on separate days. Each session consisted of vascular assessment, using high-resolution B-mode ultrasound imaging, of resting brachial artery diameter and of flow and diameter and flow after various durations of forearm ischemia, or ischemic exercise, with or without GTN administration. The order of interventions was randomized for each subject. All measures were performed after 12-h abstinence from alcohol and strenuous physical activity.

Experimental Procedures

Vascular measurements. Patients rested supine with the nondominant arm extended and immobilized with foam supports at an angle of ~80° from the torso. Heart rate was continuously monitored with a three-lead electrocardiograph, and mean arterial pressure was determined from an automated sphygmomanometer (Dinamap 8100, Critikon, Tampa, FL) on the contralateral arm. A rapid inflation and deflation pneumatic cuff was positioned on the imaged arm immediately distal to the olecranon process to provide a stimulus to forearm ischemia. A 10-MHz multifrequency linear array probe, attached to a high-resolution ultrasound machine (Aspen, Acuson, Mountain View, CA), was used to image the brachial artery in the distal third of the upper arm. Ultrasound parameters were set to optimize longitudinal, B-mode images of the lumen-arterial wall interface. When an optimal B-mode image was obtained, the probe was held stable in a stereotactic clamp, and the probe position relative to the radiiale was recorded for repeat sessions. Continuous Doppler velocity assessment was also obtained using the Aspen, and it was collected using the lowest possible insonation angle (always <60°), which did not vary during each study.

REACTIVE HYPEREMIA STUDIES. Resting brachial artery diameter, and diameters after forearm ischemia, or ischemic exercise, were assessed at separate visits. On each occasion, a rapid inflation and deflation pneumatic cuff, placed around the forearm immediately distal to the humeral epicondyles, was used to provide the ischemic stimulus. After an initial 20-min rest period, baseline scans assessing resting vessel diameter and flow were recorded over 2 min. The forearm cuff was then inflated to >200 mmHg for either 5, 10, or 15 min (order randomized). Diameter and flow recordings resumed 30 s before cuff deflation and continued for 10 min thereafter. Peak brachial artery diameter and flow, and the time taken to reach these peaks after the release of the occlusion, were recorded. On one further testing occasion, the 5-min period of forearm ischemia consisted of 1 min ischemia, followed by a 3-min isotonic handgrip exercise bout, and a final 1 min of ischemic. The ischemic handgrip exercise emulated previous studies (1) and involved one contraction every 3 s of a 3-kg load.

GTN AND REACTIVE HYPEREMIA STUDIES. Subject preparation was as described above. Baseline scans were followed by sublingual administration of a 400-μg spray dose of GTN and a 10-min continuous recording period. The time taken for peak dilatation after GTN administration was determined for each subject (mean 319, SD 26 s). On separate subsequent visits, 5-, 10-, and 15-min periods of forearm ischemia were superimposed on the GTN stimulus, with GTN administered during the occlusion period at a time preceding cuff deflation such that the deflation corresponded with the previously determined peak GTN diameter for that individual. In one additional session, GTN and 5 min of limb ischemia were also combined with a 3-min period of ischemic handgrip exercise, as described above.

Brachial artery diameter and blood flow analysis. Posttest assessment of brachial artery diameter was performed by using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias (32). Briefly, the video signal was taken directly from the ultrasound machine and, using an IMAQ-PCI-1407 card, was encoded and stored as a digital DICOM file on the personal computer. Subsequent software analysis of this data was performed at 20–30 Hz using an icon-based graphical programming language and toolkit (LabVIEW 6.02, National Instruments, Austin, TX). Our laboratory has shown that reproducibility of measurements using this semiautomated software is significantly better than manual methods, reduces observer error significantly, and possesses an intraobserver coefficient of variation of 6.7%. Further details of this analysis software and its detailed validation are available in recent publications (7, 32).

Blood flow, calculated as the product of CSA and Doppler flow velocity, was derived from synchronized B-mode ultrasonography and Doppler velocity measures, using the suite of software packages (LabVIEW 6.02, National Instruments). Vessel CSA was calculated from the continuous (~30 Hz) software-derived arterial diameter measures by using the following equation: CSA = π r^2. Once the study has been acquired as a DICOM file, data were displayed as plots of the arterial diameters and velocities against time. These plots were used to calculate and display blood flow as a continuous variable across the cardiac cycle at ~30 Hz. Operator-controlled cursors were then used to select and zoom on the section of data to be analyzed. Our laboratory’s recent study indicated that this method of blood flow assessment is closely correlated with actual flow through a “phantom” arterial flow system (7).

Data Analysis

Peak blood flow was recorded as the highest area under the blood flow curve data across a 10-s period after each stimulus, this epoch being selected to provide valid comparison to previous studies of peak hyperemia that employed plethysmography. Peak brachial artery diameter was recorded as the peak diameter measured from data collected at 30 Hz, after each intervention (7, 32). Statistical analyses were performed using SPSS 11.0 (SPSS, Chicago, IL) software. All data are reported as mean (SD), and statistical significance was assumed at P < 0.05. One-way ANOVA and post hoc paired t-tests were used to assess significance of difference. Two-way ANOVA was used to examine the impact of the addition of exercise to the ischemic responses. Pearson product-moment correlations coefficients were used to assess degree of association between various measures.

RESULTS

Resting systolic blood pressure, diastolic blood pressure, and heart rate were 125 (6) mmHg, 71 (8) mmHg, and 67 (12) beats/min, respectively. There were no significant differences in hemodynamics before, or in response to, any of the interventions.

Comparison of Blood Flow Responses to Ischemia, Ischemic Exercise, and GTN

Descriptive analysis of blood flow responses to ischemia and ischemic exercise, and the combination of these interventions with GTN, revealed that peak brachial artery flow was obtained by using a combination of GTN, 5-min ischemia, plus ischemic exercise (Fig. 1A). The lowest brachial artery flow response was evoked by administration of GTN alone, which was associated with significantly lower flows than all other interventions (all P < 0.01). Increasing periods of ischemia alone were associated with stepwise increases in reactive hyperemic flows (Fig. 1A), whereas flow responses to GTN + 10-min ischemia and GTN + 15-min ischemia induced no greater flow response than the combination of GTN + 5-min ischemia.
Two-way ANOVA revealed that, whereas the addition of GTN to the ischemic stimuli (5, 10, and 15 min) did not significantly increase flow responses, the effect of adding exercise to the 5-min ischemic response, in the presence or absence of GTN, was significant ($P < 0.05$).

Analysis was also performed to determine the time taken to reach peak blood flow after each intervention. There were no significant differences in the time taken to reach peak flows for 5 min [12 (3) s], 10 min [15(3) s] or 15 min [21 (3) s] of ischemia or GTN + 5 min [9 (3) s], GTN + 10 min [12 (3) s] or GTN + 15 min [17 (12) s] of ischemia. For all interventions, peak flows were therefore recorded in the first 20 s after cuff release for all interventions involving an ischemic stimulus. The time to peak flow recorded after administration of GTN alone was 239 (63) s, which significantly exceeded all other interventions (all $P < 0.001$).

Comparison of Diameter Responses to Ischemia, Ischemic Exercise, and GTN

Descriptive analysis of brachial artery diameter responses revealed that peak brachial artery diameter was also observed as a result of the combination of GTN, 5-min ischemia, and ischemic exercise (Fig. 1B). The addition of GTN resulted in greater diameter responses to all ischemic and ischemic exercise stimuli. Resting brachial artery diameter [4.05 (0.60) mm] was significantly lower than that observed in response to all stimuli, including the peak diameter observed to GTN administration [4.94 (0.63) mm; $P < 0.01$]. The correlation between absolute diameter values at rest and in response to GTN and ischemic exercise achieved significance (r = 0.75, $P < 0.05$), but no relationship was observed between resting diameter and the change in diameter in response to GTN (r = −0.3, P = not significant) or the percent change in diameter after GTN (r = −0.2, $P = NS$). Two-way ANOVA revealed that, although the addition of GTN to the ischemic stimuli (5, 10 and 15 min) did not significantly increase diameter responses, the effect of adding exercise to the 5-min ischemic response, in the presence or absence of GTN, was significant ($P < 0.001$).

The time taken to reach peak diameter after the 5-min ischemia was 61.11 (35.13) s, 10-min ischemia was 60.66 (30.09) s, and 15-min ischemia was 63.44 (28.02) s. Peak diameters were therefore recorded in the 60–65 s after cuff release for all interventions involving an ischemic stimulus. There were no significant differences in the time taken to reach peak diameter between these stimuli. In response to GTN administration alone, peak diameter was observed 319 (18) s after sublingual administration, significantly exceeding all other interventions (all $P < 0.001$).

DISCUSSION

This study has demonstrated that peak reactive hyperemic brachial artery blood flow and diameter responses are induced in response to a combination of GTN administration, forearm ischemia, plus ischemic exercise. The flow and diameter responses to this stimulus were greater than those observed in response to a range of periods of ischemia alone, GTN administration alone, ischemia in combination with GTN, or ischemic exercise alone. Finally, resting arterial diameter was significantly different from peak diameter, and the correlations between resting and peak measures were relatively modest. These results suggest that caution should be applied in using resting arterial diameter as a surrogate measure of conduit artery structure in vivo.

Forearm ischemia is a stimulus that has been used to induce reactive hyperemia as a measure of resistance vessel structure for many decades, on the basis of the principle that a stimulus that evokes peak hyperemia provides an indirect assessment of maximal CSA of the resistance vessel bed in question (17, 30). Patterson and Whelan (17) demonstrated that 10- and 15-min periods of forearm ischemia induced large hyperemic responses that could be used as indexes of maximal vasodilator capacity of forearm resistance vessels in humans, and Takeshita and Mark (28) indicated that ischemic exercise did not further increase the hyperemic response observed to a period of 10-min forearm ischemia. A similar ischemic stimulus was adopted by Folkow et al. (3, 4) as a means of assessing the relationships that exist between hypertension and structural vascular remodeling, and studies of the impact of chronic exercise (8, 25), exercise training (6, 23, 26), and physical inactivity (24) on the vasculature have utilized peak reactive hyperemia as an index of resistance vessel structural

Fig. 1. Brachial artery flow (A) and diameter (B) responses to periods of forearm ischemia or ischemic exercise alone, or in combination with glyceryl trinitrate (GTN) administration. Peak brachial artery flow and diameter were both observed as a result of combined GTN, 5-min ischemia, and ischemic exercise. Two-way ANOVA revealed a significant main effect for the addition of exercise to the ischemic responses. Noninvasive Doppler ultrasonography allows indices of both conduit and resistance vessel structure to be simultaneously determined in response to a single, brief laboratory visit.
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adaptation to these stimuli. All of the above studies utilized forearm plethysmography, a traditional method of blood flow assessment (31), to determine peak blood flow responses after occluding cuff deflation. This approach, although valid, is highly influenced by motion artifact and possesses very poor temporal resolution; typically one measure of blood flow is derived every 7–10 s. When measuring a phenomenon as transient as peak reactive hyperemia, it is likely that the derivation of a single measure using plethysmography involves substantial error and that the “real” peak flow may be underestimated. One aim of the present experiment was to determine which combination of noninvasive stimuli induced peak hyperemia through the brachial artery using Doppler ultrasound assessment. Our observation that peak brachial artery flows result from combined limb ischemia, GTN administration, and ischemic exercise possesses logical integrity given that these interventions represent a combination of potent physiological and pharmacological stimuli.

This is the first study, to our knowledge, to investigate the use of various stimuli to induce peak arterial dilation as a measure of conduit vessel structure. With the advent of ultrasound-based technology, studies have begun reporting resting arterial diameter as an index of conduit structure (2, 13, 16, 22, 33). However, resting diameter is influenced by numerous competitive vasodilator and constrictor influences and we applied the same reasoning recognized as essential in the assessment of resistance vessel structure: that to facilitate comparisons of a physiological capacity, it is preferable to provide a maximal stimulus, so as to diminish the impact of functional differences between subjects at rest (28). The peak diameter response observed in the present study was in response to GTN administration combined with forearm ischemia and ischemic exercise. Resting arterial diameter was significantly lower than that observed in response to all stimuli, and the correlations between resting diameter and peak diameter change were not compelling. This suggests that the use of resting arterial diameter as an index of conduit vessel structure may not be an optimal approach in humans and that the use of a peak stimulus, by removing potentially confounding influences, may provide a better surrogate for conduit vessel structure in vivo.

There are several important limitations of the present study. Although we utilized a combination of potent physiological and pharmacological stimuli, it is possible that a different combination of ischemia or vasodilator drugs might have induced greater arterial diameter and blood flow changes. For example, dipyridamole has been used to maximally dilate coronary vessels in the assessment of flow reserve (27), and prazosin is commonly used to evaluate the effects of shear stress on vascular remodeling in skeletal muscle (18). It seems unlikely, however, that the GTN response we observed was less than maximal, given that the arterial diameter response was not greatly enhanced by further blood flow and shear stress stimuli. Furthermore, we administered a double dose (800 μg) of GTN to three of the subjects in the present study, following predosing with paracetamol. The brachial artery diameter response rose by 23.9 ± 5.4% over baseline in response to this dose, compared with 22.3 ± 3.7% to 400 μg. By comparison, the peak dilator response, to combined GTN, ischemia, and ischemic exercise was 27.0 ± 5.4% in these subjects. We therefore do not believe that the use of a higher dose of GTN, with associated potential for adverse effects, is justified or necessary to obtain a peak brachial artery response. Similarly, our anecdotal experience was that the 3-kg, 3-min handgrip ischemic exercise load was near maximal; longer or greater loads were reported as painful by the subjects who trialed them. Another limitation of this study is that we used a relatively small sample, but the results were unambiguous, and our study of nine subjects possessed 80% power to detect a 0.5-mm difference in brachial artery diameter at the 5% probability level. We do not believe that a larger group would qualitatively change the results. Our findings regarding the stimuli required for maximal change in diameter and hyperemia are also specific to the upper limb vasculature; different durations of ischemia or combinations of drug dose may be required to induce peak responses in other vascular beds such as the lower limbs. Finally, three of our subjects were premenopausal women and previous studies indicate that estrogen levels influence vasodilator responses (10). However, all subjects responded maximally to the combination of GTN, ischemia, and ischemic exercise, regardless of their gender, and it is unlikely that estrogen would induce selective improvement in the response to any one of the stimuli within a given individual.

Although GTN possessed the advantages of being rapidly acting via a noninvasive administration route, some caution is also appropriate in the recommendation of the use of a pharmacological agent for induction of maximal diameter and reactive hyperemic responses in humans. However, GTN is a safe drug at the dose we utilized and is widely used both clinically and experimentally (9, 15, 29). The most common adverse event observed is headache, which is nonetheless rare and can be managed effectively with paracetamol. Contraindications to administration of this nitrate include hypotension, orthostatic intolerance, and the use of phosphodiesterase inhibitors and other vasodilators due to the potential for hypotension. In circumstances where GTN administration is not advisable, the use of an ischemic exercise stimulus may provide the best alternative for inducing peak hyperemia and dilator responses as surrogate indices of resistance and conduit vessel structure.

In summary, the present study was designed to identify optimal in vivo measures of conduit and resistance vessel structure by determining the stimuli required to induce peak change in brachial artery diameter and flow in vivo. Fortuitously, peak brachial artery diameter and flow were observed to the same stimulus; the combination of GTN administration, forearm ischemia, and ischemic exercise. This study demonstrates that noninvasive assessment of both conduit and resistance vessel structure can be simultaneously undertaken in a single, brief laboratory visit.

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GRANTS

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