Occlusion cuff position is an important determinant of the time course and magnitude of human brachial artery flow-mediated dilation

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ABSTRACT
Non-invasive ultrasound techniques to assess flow-mediated vasodilation (FMD) are frequently used to assess arterial endothelial vasodilator function. However, the range of normal values varies considerably, possibly due to differences in methodological factors. We sought to determine the effect of occlusion cuff position on the time course and magnitude of brachial artery blood flow and flow-mediated dilation. Twelve healthy subjects underwent measurements of forearm blood flow using venous occlusion plethysmography (VOP) before and after 5 min of susprasystolic cuff inflation, using two randomly assigned occlusion cuff positions (upper arm and forearm). An additional 16 subjects underwent two brachial ultrasound studies, using the two cuff positions, to assess the extent and time course of changes in brachial artery diameter and blood flow. Maximum increase in blood flow (peak reactive hyperaemia), measured by VOP, occurred immediately upon each cuff deflation, but was greater after upper arm compared with forearm arterial occlusion (33.1 ± 3.1 versus 22.8 ± 2.2 ml/min per forearm tissue, P = 0.001). Maximal brachial artery FMD was significantly greater following upper arm occlusion (9.0 ± 1.2%, mean ± S.E.M.) compared with forearm occlusion (5.9 ± 0.7%, P = 0.01). The time course of the change in brachial artery diameter was affected differently in response to each protocol. The time to peak dilation following upper arm occlusion was delayed by 22 s compared with forearm occlusion. Occlusion cuff position is thus a powerful determinant of peak reactive hyperaemia, volume repaid and the extent and time course of brachial artery FMD. Positioning the cuff on the upper arm produces a greater FMD. These results highlight the need for comparisons between FMD studies to be made with care.

INTRODUCTION
Non-invasive ultrasound assessment of flow-mediated vasodilation (FMD) is frequently used to test the integrity of endothelium-dependent vasodilation. Initial studies assessing FMD in humans utilized surrogate measures of brachial artery diameter rather than direct ultrasonic images [1,2]. Further to these reports, a technique was developed that used ultrasound to obtain direct images of the brachial artery, allowing for accurate measurement of vessel dimensions in response to changes in blood flow velocity [3]. Current methodology for the non-invasive assessment of conduit artery FMD in humans is based on this study. The principle of FMD is based on the ability of endothelial cells to detect changes in shear stress. When blood flow through an arterial segment is occluded, hypoxia ensues causing vasodilation of downstream resistance vessels and a subsequent increase in blood flow upon occlusion cuff release. This augmentation of blood flow increases the shear stress exerted on the endothelium...
[4], initiating release of NO and relaxation of vascular smooth muscle cells [5], which results in conduit vessel dilation.

To date, there have been a multitude of reports employing this technique, some of which have investigated the effects of age and gender [6]. In other studies, individuals with coronary artery disease [7], risk factors for atherosclerosis [3,8], smokers [9,10], hypercholesterolaemia [11], heart failure [12], hyperhomocyst(e)inemia [13], insulin-dependent (Type I) diabetes mellitus [14] and low birth weight [15] have been assessed in relation to FMD. Changes in endothelial function during pregnancy [16], with hormone replacement therapy in post-menopausal women [17] and in males on oestrogen therapy [18] have also been reported. Additionally there have been a number of acute and chronic interventional studies in which the effects of exercise therapy in heart failure [19], cholesterol lowering [20] and dietary fat intake [21] have been examined.

From the number of studies published it is evident that there is a wide variation in ‘normal’ values of FMD. FMD in healthy middle-aged subjects with no cardiovascular risk factors are reported to range from 5.1% [18] to 21% [21]. What is reported to represent ‘normal’ in one healthy cohort is indicative of impaired FMD in another. Such differences are due, in part, to technical and methodological variations, including arterial occlusion times, positioning of the occlusion cuff and the time point of vessel measurement following cuff release.

Duration of brachial artery occlusion has been shown to affect the degree of vessel dilation, with a minimum of 4.5 min of forearm arterial occlusion being necessary to elicit maximal dilator response [22]. Similarly, 5 min compared with 1 min and 3 min of upper arm arterial occlusion was necessary to cause significant brachial artery dilation [23]. Indeed, early studies using venous occlusion plethysmography (VOP) established that reactive hyperaemia and the pattern of the volume repaid [area under the curve (AUC)] to the forearm following circulatory arrest is dependent on cuff occlusion time [24]. In brachial ultrasound studies, forearm occlusion times have ranged from 4 min [16] to 4.5 min [3,15], compared with upper arm occlusion times of 4 min [25], 4.5 min [7] or 5 min [11,13,21] duration.

In addition to occlusion time, cuff position may also be a determinant of the wide variation in ‘normal’ FMD values reported. This may be a result of the differences in forearm muscle mass rendered ischaemic with each cuff placement. Both the superior and inferior ulnar collateral arteries, which supply the superficial flexor muscles of the forearm, arise from the brachial artery proximal to the elbow [26], and thus are not occluded with cuff placement on the forearm. Mannion et al. [27] found that FMD was greater (at 1 min post-cuff release) with upper arm occlusion compared with forearm occlusion (9.8% versus 6.8%), but, in a similar study, a difference in FMD response at this time point was not found [28]. In another study [23], which compared the brachial artery dilator response to upper versus lower arm occlusion cuff placement, different occlusion times (5 min for upper arm and 10 min for forearm) were used.

The aim of the present study was to determine the effect of occlusion cuff position not only on the magnitude but also on the time course of brachial artery blood flow responses and the ensuing FMD.

METHODS

All participants gave written informed consent to participate in the study, which was carried out with the approval of the Monash Medical Centre Ethics Committee and performed in accordance with the Declaration of Helsinki. Subjects were normal healthy individuals not taking any medication, were non-smokers, normotensive and had no family history of cardiovascular disease. Lipid levels were within the healthy range (total cholesterol < 5.5 mmol/l and low-density lipoprotein < 3.5 mmol/l).

Protocol

Subjects were initially screened with a medical history and blood test to determine glucose and lipid levels. Participants were asked to refrain from consuming caffeine-containing food and drink on the day of the procedure. The study was a randomized cross-over design with participants undergoing two brachial ultrasound studies or two VOP studies on their right arm. In study A the occlusion cuff was placed high around the upper arm, and in study B the cuff was placed around the forearm immediately distal to the elbow. All subjects rested quietly for 10 min before measurement of baseline variables.

VOP

Twelve subjects (5 male, 7 female), aged 30±8 years of age (mean±S.D.), underwent assessment of forearm muscular blood flow using VOP. Blood flow (expressed in ml/100 ml of forearm tissue per min) was assessed before and after 5 min of both upper arm and forearm arterial occlusion, using calibrated mercury-in-silastic strain gauges (D. E. Hokanson, Bellevue, WA, U.S.A.). The two interventions were separated by a 20-min interval.

In addition to the cuff on the upper arm or forearm, an occlusion cuff was placed around the wrist to exclude skin blood flow to the hand, in accordance with usual practice for plethysmographic studies. An eight-channel analog to digital conversion system (MacLab/8s; AD-Instruments, Castle Hill, Australia) was used to digitize data on-line. Chart v3.5/s software (ADInstruments) was used to both record and analyse the blood flow data.
Blood flow measurements were recorded at baseline before cuff inflation, and continuously for 5 min following cuff release. Maximal blood flow (peak hyperaemic response) and the time course of the response to each arterial occlusion was noted. Volume repaid to the forearm in the initial 30 s following cuff deflation was calculated from the AUC for blood flow.

### Brachial ultrasound

Sixteen subjects (5 female, 11 male), aged 23 ± 4 years (mean ± S.D.), underwent assessment of endothelium-dependent FMD of the right brachial artery, using the technique first described by Celermajer et al. [3]. In 11 of the 16 subjects studies A and B were performed on separate days, within 1 week; the remaining five subjects underwent both studies, separated by a 20-min interval, on the same day. The studies were performed using an HDI3000 series Ultramark 9 ultrasound machine (Advanced Technologies Laboratories Pty Ltd, Bothell, WA, U.S.A.) with a 7–10 MHz linear array transducer. Subjects rested supine for the duration of the study. Heart rate, using a single lead ECG, was monitored throughout the study. Longitudinal images of the brachial artery were recorded proximal to the cubital fossa, with the distance from the medial epicondyle to the transducer noted for each subject. The transducer angle was also noted to maintain consistency between studies. Transmit focal zones and depth were set to optimize the image of the vessel wall, particularly the media–adventitia interface (‘M’ line), and the image was then magnified. These operating parameters were kept constant throughout the study.

In each study, baseline recordings of blood flow velocity (Doppler) and vessel image were made and these recordings were repeated following the inflation of the pressure cuff to suprasystolic pressure (200 mmHg) for 5 min. For each cuff position, a time course of the brachial artery diameter was assessed for 15 min post-cuff deflation. A second baseline recording of the vessel image was made 5 min later, after which the blood flow velocity time course was monitored for 10 min following a second cuff inflation.

### Image analysis

Brachial artery images were recorded on to Super-VHS videotape. Two representative frames from each time point were later digitized using a frame grabber (Scion LG-3) and saved for subsequent analysis. These frames were selected at the point corresponding to the R wave (end of diastole) of the ECG recording. Vessel diameter measurements were carried out using a modified version of the public domain NIH Image Program, originally developed at the National Institutes of Health (Bethesda, MD, U.S.A.). Two observers, blinded to the stage of the study, performed the analysis in which the distance between the anterior (intima–medial interface) and posterior walls (medial–adventitial interface) was measured automatically. Each frame was measured three times by each observer, thus six measurements were obtained at each time point (baseline, 1 s, 10 s, 20 s, 30 s, 40 s, 60 s, 70 s, 80 s, 100 s, 2 min, 3 min, 5 min, 10 min and 15 min; and baseline 2 at 20 min) post-cuff deflation and the means were calculated. The percentage change in vessel diameter from baseline was calculated in response to reactive hyperaemia at each of the time points, allowing identification of the time of maximal change in diameter.

### Flow analysis

Blood flow velocity (cm/s) was measured from the centre of the vessel using continuous-wave Doppler ultrasound, and was used subsequently with heart rate and vessel area (cm²) to calculate volumetric blood flow (ml/min). Reactive hyperaemia was calculated as the percentage change in flow from baseline.

### Analysis

The following parameters were measured at each intervention.

- VOP: forearm blood flow at baseline; peak forearm blood flow (peak hyperaemic response) after 5 min of arterial occlusion; area under the time-flow curve for the first 30 s after cuff release (volume repaid). Brachial ultrasound: resting brachial artery blood flow velocity; peak reactive hyperaemia (first measurement obtained at 10 s post deflation); resting brachial artery diameter; time course of changes in brachial artery diameter (FMD) from 10 s to 15 min following cuff release (group mean data); peak FMD, independent of time (calculated for each subject and averaged); time-to-peak FMD; FMD at 60 s after cuff release.

### Statistics

Results are expressed as means ± S.E. Student’s t test was used to compare paired data (blood flow responses, brachial artery diameter, FMD and time-to-peak FMD). AUC analysis was performed for blood flow time-course data. Statistical significance was accepted as P < 0.05.

### RESULTS

#### VOP

Baseline blood flow was the same before each intervention (2.4 ± 0.2 versus 2.5 ± 0.5 ml/min per 100 ml). Upper arm arterial occlusion for 5 min induced a peak hyperaemic response of 33.1 ± 3.1 ml/min per 100 ml compared with 22.8 ± 2.2 ml/min per 100 ml after forearm occlusion (P = 0.001) (Figure 1, upper panel). These changes in flow were equivalent to increases of 1408% and 841% respectively. Peak reactive hyperaemia occurred immediately upon cuff release resulting in a significant difference in the volume repaid at 30 s (AUC for upper arm 9.7 ± 0.8 ml/100 ml versus forearm 7.5 ±
Figure 1 Blood flow responses to upper arm and forearm cuff positions, as assessed by VOP
Top panel: upper arm occlusion for 5 min elicited a significantly greater peak hyperaemic response compared with forearm occlusion ($P = 0.001$). Bottom panel: the blood volume repaid in the initial 30 s post-cuff release was significantly greater following upper arm cuff release compared with forearm cuff release ($P = 0.01$).

Figure 2 Time course of the reactive hyperaemic response to upper arm and forearm cuff positions, as assessed by VOP
The hyperaemic response to each intervention differed only within the first 30 s following cuff release. Upper arm occlusion (●); forearm occlusion (□).

0.9 ml/100 ml, $P = 0.01$) (Figure 1, lower panel). The time course of changes in blood flow are seen in Figure 2, showing that flow had returned to baseline levels by 120 s post-cuff deflation.

Figure 3 Peak FMD response to upper arm and forearm cuff occlusion, independent of time
The dilator response to upper arm occlusion (9.1%) was significantly greater than that to forearm occlusion (5.9%) ($P = 0.01$).

Figure 4 Time taken to achieve peak FMD after upper arm and forearm cuff deflation
The peak dilator response to upper arm occlusion was delayed by 22 s compared with forearm occlusion ($P = 0.006$).

Brachial ultrasound
Peak reactive hyperaemia occurred immediately upon cuff release and was significantly greater with upper arm compared with forearm occlusion. The percentage change in blood flow from baseline was 584 ± 66% versus 346 ± 61% for upper arm and forearm respectively ($P = 0.01$).

Baseline vessel diameters were similar before each of the four cuff inflations (4.07 ± 0.13 mm, 4.08 ± 0.14 mm, 4.19 ± 0.1 mm and 4.19 ± 0.1 mm). The mean coefficient of variation for the baseline measures was 2.7%. The peak FMD response to upper arm occlusion was 9.1 ± 1.2% compared with the peak response after forearm occlusion of 5.9 ± 0.7% ($P = 0.01$) (Figure 3). Not only was the peak vasodilator response different after each intervention, but the time course of the response was altered.

Upper arm cuff placement produced a peak FMD at 71 ± 4.8 s post-cuff release compared with 49 ± 3.1 s for forearm cuff placement ($P = 0.006$) (Figure 4). This delay...
Effect of cuff position on brachial artery flow-mediated dilation

Figure 5 Time course of brachial artery FMD
Upper arm occlusion caused a greater peak dilator response that occurred later than the peak response to forearm occlusion. Upper arm occlusion (○); forearm occlusion (■).

Table 1 Time point at which maximal flow-mediated dilation was observed following each cuff release
The results are expressed as the percentage of subjects within each time range.

<table>
<thead>
<tr>
<th>Time after cuff release</th>
<th>≤ 40 s</th>
<th>40–60 s</th>
<th>70–80 s</th>
<th>≥ 80 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper arm occlusion</td>
<td>0%</td>
<td>31%</td>
<td>56%</td>
<td>13%</td>
</tr>
<tr>
<td>Forearm occlusion</td>
<td>19%</td>
<td>75%</td>
<td>6%</td>
<td>0%</td>
</tr>
</tbody>
</table>

in peak FMD with upper arm cuff occlusion is evident in the time-course graph (Figure 5). FMD measurements compared at 60 s post-cuff deflation show a significant difference of 2.8% (7.7 ± 1.1% versus 4.9 ± 0.8%, P = 0.03) after upper arm and forearm occlusion respectively.

On grouping the peak FMD responses to upper arm occlusion it is seen that a large proportion (69%) of subjects lie outside the usual assessment time of 40–60 s (Table 1), resulting in an underestimation of the true dilator response. In the majority of subjects (75%), the peak FMD response to forearm occlusion occurred within the expected time frame but one quarter of the peak responses would have been underestimated.

DISCUSSION

The present study shows that occlusion cuff placement is a powerful determinant of FMD and blood flow responses in the human brachial artery. Peak FMD was significantly greater after 5 min of upper arm occlusion, as was peak hyperaemic blood flow and the volume repaid to the forearm at 30 s post-cuff deflation. The FMD results are in agreement with a recent study by Mannion et al. [27], and are most likely explained by a greater flow stimulus induced by upper arm occlusion. They showed comparable FMD responses of 9.8% and 6.8% following 5 min of upper arm and forearm occlusion respectively. In contrast, Uehata et al. [28] did not find different FMD responses with the two techniques (4.8% for upper arm and 5.4% for forearm), presumably because of the time at which vessel diameter measurements were made. Occlusion of arterial flow to a larger muscle/skin area is likely to have induced a greater degree of ischaemia and subsequent reactive hyperaemic response, resulting in greater vessel dilation. This stimulus–response relationship was also observed by Sinoway et al. [1] before the use of ultrasound, when surrogate measures of vessel diameter were used. They observed that the changes in brachial artery diameter were preceded by changes in blood flow, and that the changes in diameter were dependent on the magnitude of the blood flow change.

We have shown, in the present study, that, with a greater flow-inducing stimulus, not only is the magnitude of the vasodilator response greater but the peak response occurs later. Maximal dilation after upper arm occlusion occurred 71 s after deflation, 22 s later than that observed following forearm cuff release. In most studies measurements have been made 1 min after cuff deflation, which, according to the findings of the present study, would certainly underestimate the peak response to both occlusion cuff positions. By 1 min after forearm occlusion cuff release, peak diameter had already been achieved and was declining, whereas, after upper arm cuff release the peak was yet to be observed. Indeed, when such studies are performed, if the true peak response is to be detected it is critical to make serial measurements of vessel diameter ranging from 30–80 s or even 90 s.

In the present study, peak FMD would have gone undetected in 69% of subjects following upper arm occlusion, and 25% after forearm occlusion if the often-reported measurement time window of 45–60 s was adhered to. This data is supported by Arrowood et al. [29], who found that only 45% of subjects had maximal dilation occurring between 45 s and 60 s following forearm occlusion. By including measurements from 25 s to 70 s after cuff release, nearly all (91%) of the peak dilator responses were accounted for. In many of the published studies to date the actual FMD responses may have been significantly underestimated. In the overall assessment of endothelial function, the time point at which vessel dilation occurs in response to an increase in blood flow may prove to be of importance, in addition to the extent of vessel dilation alone.

Placement of the occlusion cuff upstream to the site of vessel imaging did not appear to have an adverse affect on the brachial artery response. It has been suggested that placement of the occlusion cuff downstream to the section of vessel being imaged is desirable in order to avoid the potential influence of ischaemic metabolites on arterial diameter [30]. If ischaemic metabolites were acting locally on the brachial artery at the imaging site,
then the dilator response would surely be diminished. In addition, occlusion cuff position had no effect on the reproducibility of measurements of baseline vessel diameter, which were the same before each of the four cuff inflations.

The results do not suggest that one particular cuff placement is preferable to the other, but the use of upper arm occlusion (hence greater changes of value in diameter) would be better for detecting subtle differences between groups of interest. In view of the increasing number of researchers using the technique, the present study highlights the need for differences in methodology to be recorded, allowing for consistency and for comparisons between studies to be made. It should be recognized that the time-to-peak FMD occurs later with a larger flow-inducing stimulus, such as with upper arm cuff occlusion. If upper arm occlusion is used then the conventional time window for measurement (45–60 s post-cuff deflation) is inappropriate and FMD will be underestimated in the majority of patients.

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