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● *Original Contribution*

THE LOW–FLOW-MEDIATED ARTERIAL CONSTRICTION IN THE UPPER LIMBS OF HEALTHY HUMAN SUBJECTS ARE ARTERY SPECIFIC AND HANDEDNESS INDEPENDENT

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Abstract—Low–flow-mediated constriction (LFMC) has been used to assess resting endothelial function in peripheral conduit arteries. The literature describes discrepancies in the behaviour of radial versus brachial artery in response to low-flow state, the reasons for which were not addressed in a systematic and scientific way. Moreover, the influence of handedness on observed LFMC responses has not been investigated. The present study aimed at systematic measurement and comparison of the LFMC responses in radial and brachial arteries of both dominant and non-dominant arms of healthy human volunteers. We also investigated the physiological factors associated with differential LFMC response of radial versus brachial artery in the same group of subjects. Longitudinal B mode ultrasonographic cine loops of radial and brachial arteries were acquired at baseline and after producing distal circulatory arrest. Cine loops were screen grabbed and analyzed later using automated edge detection algorithms to measure end-diastolic diameters. Distal circulatory arrest was produced over the proximal forearm (for the brachial artery) and over the wrist (for the radial artery) at 250 mm Hg for 5 min after baseline measurements. Results suggested that arterial location ($p = 0.0001$) and baseline diameter ($p < 0.0021$) emerged as independent predictors of LFMC response. Differences in the LFMC responses are handedness independent and could be attributed to the arterial location along with the differences in their baseline diameters. (E-mail: dinu.chandran@aiims.edu)

Key Words: **Low–flow-mediated constriction, Endothelial function, Brachial artery, Radial artery, Dominant, Non-dominant, Ultrasonography.**

INTRODUCTION

Cardiovascular diseases (CVDs) are presently the leading cause of morbidity and mortality in the human population (Prabhakaran et al. 2016). Endothelial dysfunction has been described as one of the early pathophysiological events in the development and progression of atherosclerosis that culminates in major CVDs (Davignon and Ganz 2004). Non-invasive methods for assessment of endothelial function are helpful for the early detection, risk stratification and evaluation of therapeutic strategies and lifestyle interventions on reversing the pathophysiology of various CVDs. Flow-mediated dilation (FMD) by ultrasonography is the gold standard method to non-invasively assess vascular endothelial function of peripheral conduit arteries. FMD is a simple and non-invasive measure of the capacity of the endothelium to cause smooth muscle cell relaxation and vasodilation in response to increase in luminal shear stress (Celermajer et al. 1992). Although FMD gives information about the "recruitability" of endothelial vasomotor function (*i.e.,* the modulation in the production and release of mediators to produce vasodilation by endothelium) (Yeboah et al. 2007), it does not derive information about the response of endothelium at resting levels of shear stress (Gori et al. 2010). Moreover, recent studies also indicate that an abnormal FMD should not be interpreted as irrefutable evidence of endothelial dysfunction (Gori et al. 2011). Compared with FMD, low–flow-mediated constriction (LFMC) involves quantification of the decrease in conduit artery diameter in response to low luminal blood flow (Gori et al. 2008). Vasoconstriction during the low-flow state, known as LFMC, has been recently reported as a promising non-invasive tool for assessing the functioning of the endothelium in the resting state (Gori et al. 2012).

LFMC has been shown to be affected by the presence of various cardiovascular risk factors and CVDs.

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However, there has been stark contrast in the pattern observed in radial versus brachial artery LFMC (Levenson et al. 1987; Anderson et al. 1989; Gori et al. 2010; Weissgerber et al. 2010). Additionally, it has been reported that the prevalence and magnitude of LFMC differs markedly between radial and brachial arteries of healthy human adults, the physiological basis of which is currently unknown (Levenson et al. 1987; Filitti et al. 1991; Mullen et al. 2001; Spieker et al. 2003; Parker et al. 2006; Gori et al. 2008). These observations are important because ultrasonographic assessment of brachial and radial arteries for assessment of LFMC responses are usually done at contiguous positions in the arterial tree separated only by 6−8 cm. While most of these previous reports have been done in isolation either in the brachial or radial artery, one previous study has measured and compared the LFMC responses in the brachial and radial arteries of the same group of subjects (Weissgerber et al. 2010). Additionally, most of the studies in the past do not mention the arm used for assessment of LFMC with reference to handedness of the subject, which could possibly have some effect on the vascular responsiveness to the low-flow state. Most people have a tendency to use one hand more over the other to perform various activities. The hand used more often is called the dominant hand, and this salient characteristic is defined as hand dominance. However, humans who can typically use their two hands equally to perform the same task are said to have ambidextrous handedness. Mixed handedness is a characteristic in which a person favors one hand for some tasks and the other hand for other tasks (Oldfield 1971; Veale 2014).

The discrepancies in LFMC responses observed in radial and brachial arteries might be attributed to inherent differences in structural and functional properties of vessels and/or differences across studies concerning the status of dominance of the arm used to study LFMC. Interestingly, no study has been conducted to date to compare LFMC response in radial and brachial arteries of dominant versus non-dominant arms. Therefore, the present study aimed at systematic measurement and comparison of the LFMC responses in radial and brachial arteries of both dominant and non-dominant arms of healthy human volunteers. We also investigated the physiological factors associated with differential LFMC response of radial versus brachial arteries in the same group of subjects.

MATERIALS AND METHODS

Subjects

In this study, 20 healthy young adults of both sexes (23.50 ± 2.06 y, 7 men] were recruited. Potential recruits were made aware of the study by an in-campus advertisement on notice boards inviting volunteers to participate in the study. Self-reported histories of diseases and their cardinal manifestations as well as past medical or surgical treatments were relied upon to exclude patients who had a history of cardiovascular disorders, renal disease, hypertension or diabetes mellitus. Subjects using tobacco in chewable or inhalable form for any duration or having a history of any substance abuse on the basis of the subject's own disclosure were excluded from the study. Subjects included in the study were neither performing any exercise training nor were they athletes participating in sports where one arm is preferentially used. Subjects were asked to report to the laboratory after overnight fasting (8−12 h) at 9:00 to 9:30 am in the morning. Subjects were instructed to abstain from caffeinated beverages for at least 12 h before the tests, any dietary supplements, consumption of any over-the-counter medications known to affect vascular functions including nonsteroidal anti-inflammatory drugs and vitamin C for at least 72 h before the test and from vigorous physical exertion for at least 24 h before the assessment. Female subjects were studied during the early follicular phase on the basis of self-reported menstrual history. All recordings were undertaken in the morning to control for circadian influences. The total time required for recording per subject has been estimated to be around 1.5 h. All subjects were explained the testing protocol, and a written informed consent was obtained before starting the study, which was conducted in the department of physiology at All India Institute of Medical Sciences, New Delhi, India. The institute ethics committee for research on human subjects approved the study Ref. No. IECPG-17/ 16.02.2017, RT-28/ 22.03.2017.

Assessment of hand dominance

The Edinburgh Handedness Questionnaire/Inventory, a 10-item measurement scale developed by R.C. Oldfield in 1971 for determining objectively the dominance of right or left hand, was used to assess the hand dominance. The questionnaire contains a list of instructions to be carried out by the individual being assessed, which are rated by direct observation of the individual's behaviour or by self-report of everyday behaviour (Oldfield 1971).

Ultrasonographic Imaging

Measurement of LFMC in the subjects was done non-invasively using vascular ultrasonography. Recordings were conducted in a temperature-controlled (23−27°C), noiseless laboratory environment. The Edinburgh Handedness Questionnaire/Inventory was used to assess arm dominance (Oldfield 1971). On arrival in the laboratory, subjects were instructed to have supine rest

for 15 min before baseline recordings were taken. Arterial imaging was performed using B mode ultrasonography at baseline and during the period of distal circulatory arrest. A sphygmomanometer with attached aneroid barometer was used for recording the arterial blood pressure and for producing distal arterial occlusion at the level of the proximal forearm to induce a low-flow state in the brachial artery and at the distal forearm and wrist to induce a low-flow state in the radial artery by inflating the cuff (39 × 11.3 cm) to a pressure of 250 mm Hg for 5 min (Fig. 1). Resting blood pressure was measured in the arm manually by sphygmomanometry (until two consecutive measurements at 5-min intervals did not differ by more than 10 mm Hg; average of two corresponding consecutive values was considered as the blood pressure).

Since no existing guidelines are available for LFMC, imaging protocol conformed to the existing guidelines for measurement of FMD in humans (Corretti et al. 2002; Thijssen et al. 2019). To measure the arterial diameter, continuous 2-D gray-scaled longitudinal ultrasound images of the brachial and radial arteries were captured in B-mode using Vivid-e (GE Healthcare, Chicago, Illinois, United States) Ultrasonograph equipped with software for 2-D imaging, color and spectral Doppler and an internal electrocardiogram (ECG) monitor. A high-frequency linear array transducer (10 MHz) was used to visualize the radial artery at the lateral aspect of forearm 10 cm above the wrist joint and brachial artery 2−3 cm above the ante-cubital fossa (Fig. 1). A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall is selected, as shown in Figure 2. The edges are best visualized when the angle of insonation is perpendicular. Thus, clear visualization of both the near (anterior) and far (posterior) wall lumen-intima boundaries indicated that the imaging plane is bisecting the vessel in the longitudinal direction, and diameters measured from these images likely reflect the true diameter (Corretti et al. 2002). Gain, acoustic output, transmission focus position, zoom factor, edge enhancement, depth settings and dynamic range were adjusted to enable clear visualization of the near (anterior) and far (posterior) walls. Imaging was done at a frame rate of 40 Hz for better temporal resolution to identify the end diastolic frames. To ensure that diameters were measured during the same phase of cardiac cycle in compliance with the existing guidelines (Corretti et al. 2002), lead II ECG signal was simultaneously acquired to synchronize the measurement of arterial diameters with the end-diastolic phase of the cardiac cycle in brachial and radial arteries, which corresponds to the onset of R wave in the ECG signal.

The experimental protocol consisted of three phases, namely, baseline (lasting for 1 min), phase of occlusion (lasting for 5 min) and phase of recovery (lasting for 30 min) following the release of occlusion. The sequence in which the four arteries (radial artery of dominant arm, radial artery of non-dominant arm, brachial artery of dominant arm, brachial artery of non-dominant arm) were assessed was randomly assigned by a random number table generated online. Each subsequent artery was assessed after 30 min of recovery period following the release of occlusion to ensure the restoration of parameters to their baseline state.

Transfer of ultrasound data into analysis workstation and conversion into Audio Video Interleave (AVI) format

The pre-recorded B mode ultrasound cine loops during baseline and last 30 s of occlusion were converted to AVI format through a frame grabber (Epiphan DVI2 USB 3.0 by Epiphan Systems Inc, Palo Alto, California, USA) connected at one end to the display output of ultrasound machine and on the other end to one of the universal serial bus (USB) interfaces of the analysis

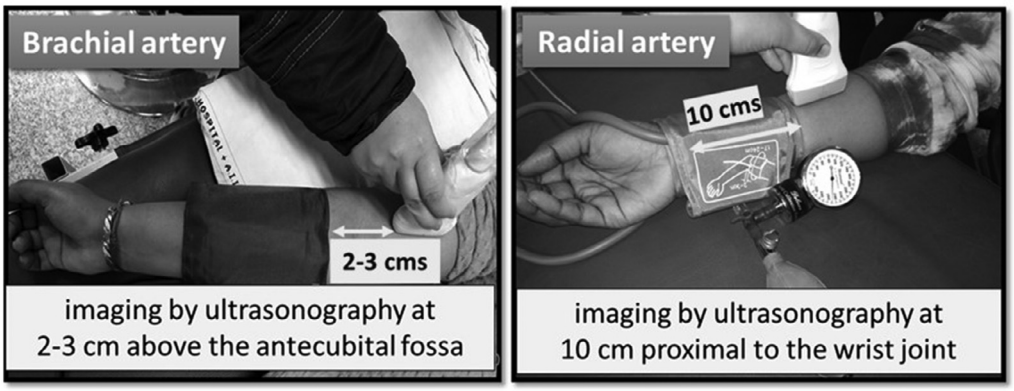

Fig. 1. The anatomy required for obtaining B-mode ultrasound images of brachial and radial artery. The figure also shows the position of the occluding cuffs used to produce distal circulatory arrest.

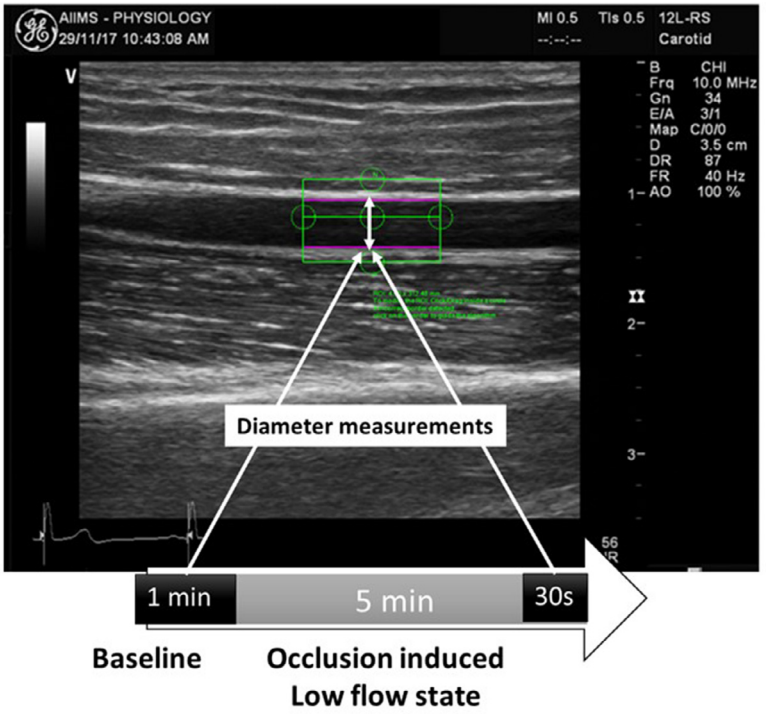

Fig. 2. A representative ultrasound image along with the recording protocol. The green rectangle indicates the region of interest marked for automated edge detection and diameter measurements using Brachial Analyzer. The pink lines mark automatically detected lumen-intima interface identified by the software. End-diastolic diameters were measured during 1 min of baseline recording and the last 30 s of the phase of occlusion.

workstation running automated edge-detection software Brachial Analyzer (Medical Imaging Applications, LLC, Iowa, USA).

Analysis of video loops and measurement of arterial diameter

The captured AVI files were subsequently analyzed using automated edge-detection software Brachial Analyzer (Medical Imaging Applications, LLC, Iowa, USA), which included the following:

(i) Calibration for diameter measurements was done manually by dragging a line between two points on the vertical dimension scale that appear on the right boarder of ultrasound images. Actual distance between the two selected points in millimeters can be derived from this vertical scale. Based on this value in millimeters which is fed into the interface corresponding to the pixel to pixel distance marked by the line drawn over the scale, the software generates the calibration value for diameter measurements;

(ii) Defining the region of interest (ROI) on a selected frame of every individual study by manually selecting a segment of artery within a rectangular box (Fig. 2) where the lumen-intima interfaces of the near and far walls are clearly visualized. This acts as a training frame for the software to fix the ROIs for all subsequent frames;

(iii) Brachial Analyzer uses an automated graph-search method for near and far wall border detection and subsequent vessel diameter measurement. The border detection approach is guaranteed to yield an optimal solution with respect to the underlying border-detection cost function. The method automatically adjusts its cost function parameters to properties of the analyzed vessel in one frame of the sequence that is analyzed under operator's supervision. After independent detection of the near- and far-wall borders in each image frame of the sequence, automated quality control steps are incorporated to support maintaining in-frame diameter measurement accuracy, temporal consistency and overall reproducibility. Details of the automated edge detection methods employed by the software are mentioned elsewhere (Sonka et al. 2002);

(iv) The extracted frame to frame diameters depicting consecutive cardiac cycles plotted were visually inspected for aberrancy in edge detection to ensure that diameter measurements were always calculated from the intima-lumen interface at the near and far vessel walls and invalid diameter values were subsequently rejected. End-diastolic diameter values were automatically extracted using ECG R-wave triggers (considering the

frame which corresponds to the onset of R wave in simultaneously recorded ECG signal as the end-diastolic frame) and were exported directly *via* software to an Excel (Microsoft, Redmond, Washington, U.S.) data sheet for subsequent statistical analysis.

STATISTICAL ANALYSIS

Data of each parameter were tested for normality of distribution using standard normality tests. Data with normal distribution were expressed as mean ± standard deviation and non-Gaussian data were expressed as median with interquartile range. Paired or unpaired Student's *t* test (or their non-parametric equivalents, Wilcoxon signed rank test and the Mann-Whitney test, respectively) were used to compare the mean differences. The prevalence of LFMC in brachial and radial arteries was compared using Fisher's exact test. Correlation analysis between variables was done using Pearson or Spearman test, as appropriate. The acceptable limit of alpha error was kept at 5% for all the analysis. Multiple regression analysis was performed to identify the independent predictors of LFMC response in the pooled data of brachial and radial arteries. GraphPad Prism (Version 8.2; GraphPad Software Inc, San Diego, California, USA) and MedCalc for Windows, version 15.0 (MedCalc Software, Ostend, Belgium) was used for statistical analysis of the data.

RESULTS

Demographic characteristics, baseline blood pressure and laterality of hand dominance of the subjects and descriptive statistics of the various study parameters are shown in Table 1. None of the subjects showed ambidexterity or mixed handedness.

LFMC responses in radial artery

Significant reduction ($p < 0.0001$) in diameter (mm) of the radial artery was observed from baseline to low-flow state in both dominant (Fig. 3) and non-dominant arms (Fig. 4).

Table 1. Demographic characteristics, baseline blood pressure and laterality of arm dominance of the subjects and descriptive statistics of the various study parameters

S. No	Subject Characteristic (n = 20)	Value
1	Mean age (in y)	23.50 ± 2.06
2	Sex (male:female)	7:13
3	Blood pressure (mm Hg)	Systolic = 118 ± 2 Diastolic = 80 ± 4
4	Dominance (right:left)	19: 1

The values of age and blood pressure are expressed as mean ± standard deviation. The values of sex and hand dominance are expressed as ratios.

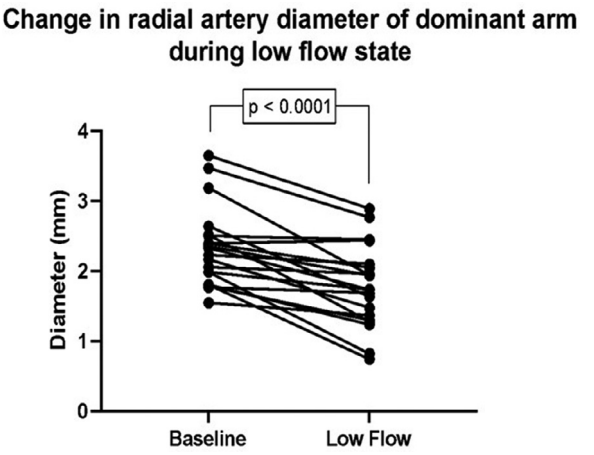

Fig. 3. Graphical representation of change in radial artery diameter of dominant arm during low-flow state.

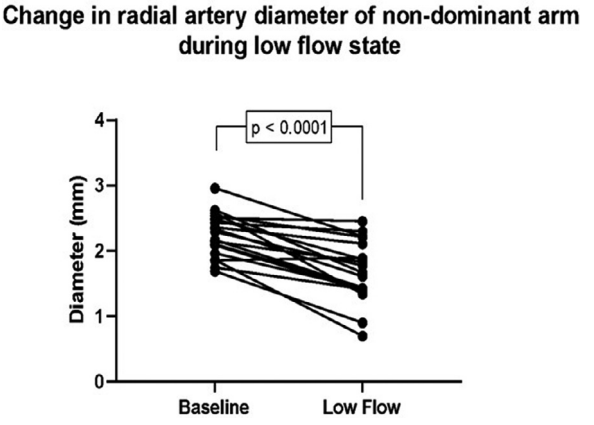

Fig. 4. Graphical representation of change in radial artery diameter of non-dominant arm during low-flow state.

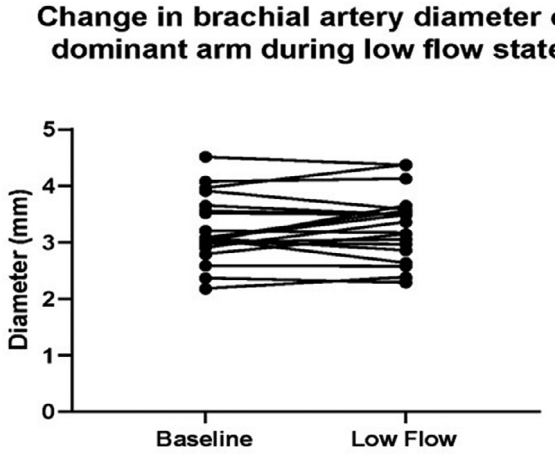

Fig. 5. Graphical representation of change in brachial artery diameter of dominant arm during low-flow state.

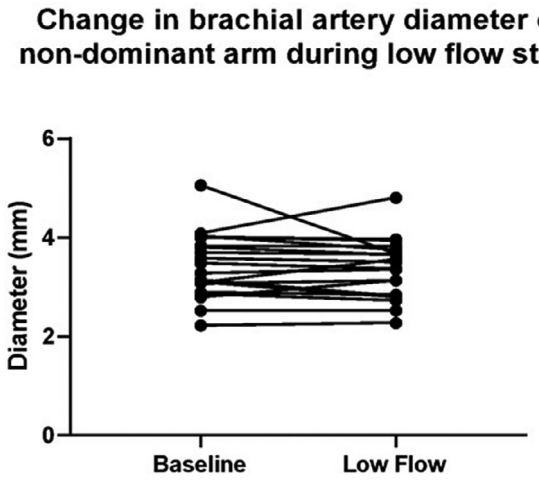

Fig. 6. Graphical representation of change in brachial artery diameter of non-dominant arm during low-flow state.

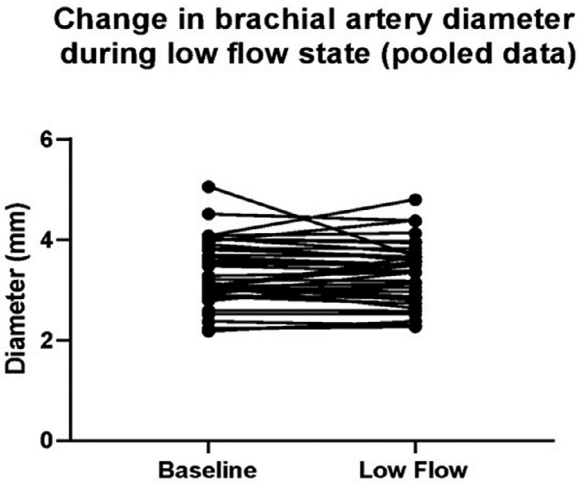

Fig. 8. Graphical representation of change in Brachial artery diameter (pooled data) during low-flow state.

LFMC responses in brachial artery

Diameter (mm) of brachial artery was comparable between baseline and low-flow state in both dominant (Fig. 5) and non-dominant arms (Fig. 6).

Prevalence of LFMC in radial versus brachial artery

When the data of dominant and non-dominant arms were pooled, similar findings were observed. A significant reduction ($p < 0.0001$) in the diameter (mm) of the radial artery was seen during low-flow state, whereas the diameter (mm) of the brachial artery was comparable between baseline and the low-flow state (Fig. 7) and (Fig. 8), respectively.

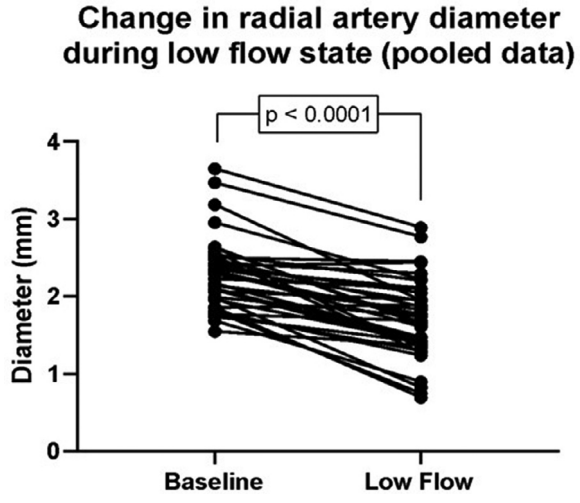

Fig. 7. Graphical representation of change in brachial artery diameter (pooled data) during low-flow state.

Moreover, the prevalence of LFMC was found to be higher in the radial artery (95%) compared with the brachial artery (57.5%; $p = 0.0001$) (Table 2).

We investigated whether the differential LFMC response of radial and brachial arteries can be attributed to inter-subject variability in responses. It was seen that brachial artery response clustered into two categories: (i) subjects whose arteries showed constriction during low-flow state and (ii) subjects whose arteries did not show constriction, as shown in Figure 9. However, in the case of the radial artery, although most of the subjets showed constriction, there was a variation in the magnitude of the responses, as shown in the frequency histogram in Figure 10. We hypothesized that, had inter-subject variability influenced the differential LFMC responses, subjects who did not show constriction during low-flow state in the brachial artery should have shown lesser magnitude of constriction in the radial artery, and vice versa. But, surprisingly, comparison of radial artery ΔLFMC responses in brachial artery responders and non-responders were found to be comparable (Fig. 11), suggesting that inter-subject variability did not influence the differential LFMC responses in radial and brachial arteries.

Table 2. Prevalence of low−flow-mediated constriction response in radial and brachial arteries

LFMC response	Radial artery	Brachial artery
Present (n)	**38 (95%)**	**23 (57.5%)**
Absent (n)	**2 (5%)**	**17 (42.5%)**

The values of prevelance of LFMC are expressed as percentage (%).
LFMC, low−flow-mediated constriction.

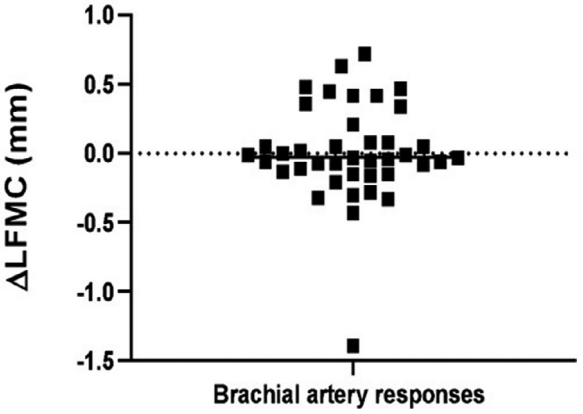

Fig. 9. Brachial artery low−flow-mediated constriction (LFMC) responses in subjects.

LFMC responses in dominant versus non-dominant arm

ΔLFMC responses of radial artery were found to be comparable between both dominant and non-dominant arms (Fig. 12). Similar findings were seen in the case of the brachial artery (Fig. 13), suggesting that arm dominance did not affect LFMC responses.

Identification of the independent predictors of LFMC responses in the pooled radial and brachial data

Further, to investigate the independent predictors of differential LFMC responses in radial and brachial arteries, multiple regression analysis was done in the pooled data of both brachial and radial arteries, considering ΔLFMC as the dependent variable and the following parameters as independent variables (Table 3):

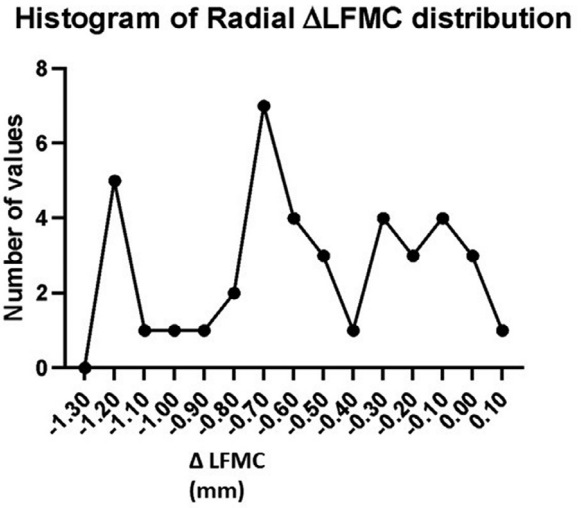

Fig. 10. Frequency histogram of radial artery ΔLFMC distribution.

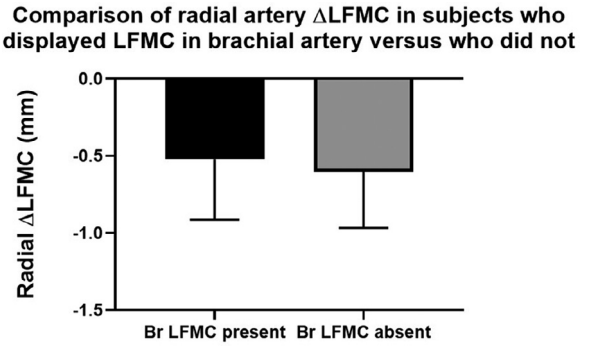

Fig. 11. Comparison of radial artery ΔLFMC responses in subjects who displayed LFMC in brachial arteries versus subjects who did not.

1. Age
2. Sex
3. Dominance of arm
4. Artery (brachial/radial)
5. Baseline diameter

Artery (radial or brachial) ($p = 0.0001$) and baseline diameter ($p = 0.0021$) emerged as independent predictors of ΔLFMC in the regression model that could explain 42% of variance ($R^2 = 0.4281$).

CONCLUSION

Differences in the LFMC responses of radial and brachial arteries are handedness independent and could be attributed to the arterial location along with the differences in their baseline diameters.

DISCUSSION

The present study aimed at systematic measurement and comparison of the LFMC responses in radial and brachial arteries of both dominant and non-dominant arms of healthy human volunteers. We subsequently investigated the factors that could explain the observed differences in the LFMC responses of radial versus brachial artery.

It was observed that the arterial diameter decreased significantly in response to distal occlusion only in the radial artery and not in the brachial artery of both dominant and non-dominant arms as well as in the pooled data, as depicted in Figures 7 and 8, respectively. This is in concordance with most of the previous reports (Levenson et al. 1987; Filitti et al. 1991; Mullen et al. 2001; Spieker et al. 2003; Parker et al. 2006; Gori et al. 2008; Gori et al. 2011; Gori et al. 2012) on LFMC in radial and brachial arteries. Thijssen et al. (2008) reported dilation of the brachial artery when a low-flow state was induced in healthy human subjects. Moreover, the prevalence of

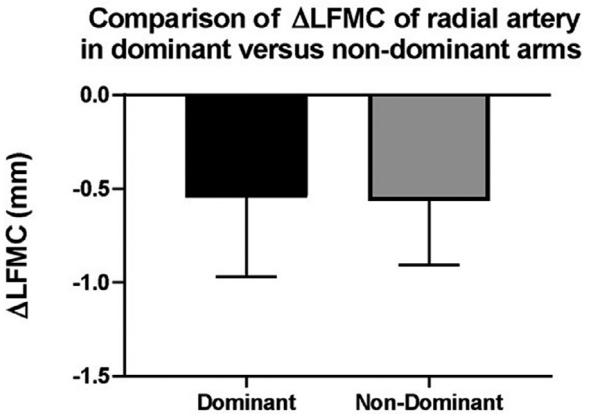

Fig. 12. Comparison of radial artery ΔLFMC responses in dominant versus non-dominant arms.

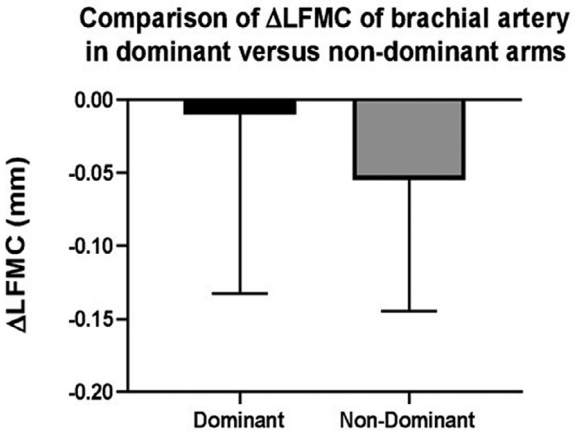

Fig. 13. Comparison of brachial artery ΔLFMC responses in dominant versus non-dominant arms.

LFMC was significantly lower in the brachial artery (57.5%) in comparison to that of the radial artery (95%); $p = 0.0001$ in accordance with the results of previous studies, which separately assessed either brachial or radial arteries (Levenson et al. 1987; Filitti et al. 1991; Mullen et al. 2001; Spieker et al. 2003; Parker et al. 2006; Gori et al. 2008).

We additionally investigated the role of arm dominance to see any activity-dependent change in vascular reactivity or endothelium-dependent responses to low-flow state. It was observed that ΔLFMC was found to be comparable between dominant and non-dominant arms of both radial ($p = 0.8924$) as well as brachial arteries ($p = 0.4018$), as depicted in Figures 12 and 13, respectively, which has not been investigated yet. Thus, based on the evidence presented in the present study, it may be inferred that differences in the usage of arm depending on the side of dominance does not influence the vasoconstrictory response to low-flow state. Similar observations have been reported by Thijssen et al. (2014) for brachial artery FMD in healthy human subjects.

We also investigated the contribution of inter-subject variations in the inherent capacity of arteries to constrict in response to low-flow state to the differences apparent in radial versus brachial artery LFMC responses. While LFMC responses of the brachial artery displayed two clusters of "constrictors" and "non-constrictors," as shown in Figure 9, the radial artery, with a remarkably high prevalence of LFMC (95%), showed variations in the magnitude of the vasoconstrictory responses, as shown in the frequency histogram in Figure 10. We hypothesized that if inter-subject variability were contributing to the differential LFMC responses in radial and brachial arteries, subjects who showed constriction during low-flow state in brachial artery (constrictors) should have shown a higher magnitude of constriction in the radial artery, and vice versa. But in contradiction to our hypothesis, radial artery ΔLFMC responses in brachial artery "constrictors" and "non-constrictors" were found to be comparable (Fig. 11), suggesting that the differential LFMC responses in radial and brachial arteries are possibly independent of the inter-subject variations in vasoconstrictory response.

Furthermore, to investigate the independent predictors of differential LFMC responses in radial and brachial arteries, a multiple regression analysis was done in the pooled data of both brachial and radial arteries, considering ΔLFMC as the dependent variable and the age, sex, dominance of arm, artery (brachial/radial) and baseline diameter as independent variables. Arterial location ($p = 0.0001$) and baseline diameter ($p = 0.0021$) emerged as independent predictors of ΔLFMC response in the regression model, which could explain 42% of the variance in the dependent variable ($R^2 = 0.4281$) (Table 3).

The fact that arterial location emerged as an independent predictor could indicate that brachial and radial arteries differ inherently in their vascular responsiveness to a low-flow state in healthy human subjects. This is corroborated additionally by evidence also from subject populations, which showed discrepancies in the behavior of brachial versus radial arteries in response to low-flow state depending on the presence or absence of specific cardiovascular risk factor or disease state. It has been reported in the literature that brachial artery LFMC shows varying responses in an unhealthy population compared with healthy controls. Hypercholesterolemia has been shown to augment brachial artery LFMC (Filitti et al. 1991), which gets reversed by 3 months of treatment with the HMG CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase) inhibitor Pravastatin (Megnien et al. 1996). Similarly, brachial artery has been shown to constrict more during low-flow state in

Table 3. Identification of the independent predictors of low–flow-mediated constriction responses in the pooled radial and brachial data using multiple regression analysis

Parameter	Coefficient	*p* Value
Age	0.02314	0.3271
Sex	0.1231	0.1814
Dominance of arm	0.002125	0.9906
Artery (brachial/radial)	**0.8362**	**0.0001***
Baseline diameter	**−0.2626**	**0.0021***
Adjusted R square = 0.4281		

* Statistically significant ($p < 0.05$)

smokers in comparison to non-smokers (Stadler et al.1998), and the prevalence of brachial artery LFMC was higher amongst smokers than non-smokers (Norioka et al. 2016). Spiro et al. (2011) reported greater brachial artery LFMC in patients with unstable versus stable coronary atherosclerosis. Percutaneous intervention through the femoral artery markedly increased brachial LFMC that was accompanied by reduced brachial artery FMD conforming to diminished endothelial function. However, studies conducted by Harrison et al. (2011) and Irace at al. (2016) in patients with varying profiles of multiple cardiovascular risk factors reported an absence of any significant association between brachial artery LFMC and cardiovascular risk factors. On the contrary, Gori et al. (2008) showed significant blunting of radial artery LFMC in hypertensives and coronary artery disease patients. The same group of investigators in 2010 (Gori et al. 2010) extended their findings by showing significant blunting of radial artery LFMC in patients with coronary artery disease and congestive heart failure in comparison to age-matched healthy subjects. Gori et al. (2012) reported a significant negative correlation between radial artery LFMC and SYNTAX (Synergy Between Percutaneous Coronary Intervention With TAXUS drug-eluting stent and Cardiac Surgery) scores, which are composite scores that account for the number, position and anatomic characteristics of the coronary lesions. In short, LFMC in radial and brachial arteries show opposing patterns, with radial LFMC getting blunted and brachial LFMC getting augmented in the presence of cardiovascular risk factors and diseases.

The plausible mechanism through which baseline diameter might be influencing LFMC responses could be through its indirect influence on resting circumferential wall stress in the arteries. If we examine the resting wall tension in radial and brachial arteries in consistence with the Laplace law (Wall stress (t) = (P × r)/ h where P is distending pressure, r is the internal radius and h is the thickness of the vessel wall), brachial artery being a larger artery has the factor r/h nearly 50% higher than that in the radial artery (Nichols and O'Rourke 2011). Since the mean arterial pressures are comparable between the two arteries, the brachial artery is therefore exposed to 50% higher circumferential wall stress than the radial artery (Gravlee et al. 1989; Bazaral et al. 1990. It is possible that the differential vasoconstrictory response could thus originate from the differences in arterial wall stress with a baseline diameter being an important determinant of the same.

The strength of the present study is that it is the first systematic investigation of LFMC responses in both radial and brachial arteries in the same group of healthy subjects. The present study has compared the LFMC responses in radial and brachial arteries of dominant and non-dominant arms in healthy human subjects, which has not been studied before. Additionally, we also investigated the factors that could independently explain the observed differences in the LFMC responses of radial versus brachial artery. Limitations of the present investigation are that it has not taken into consideration the stimulus characteristics (luminal flow and shear rate changes during low-flow state) and its contribution to the differential response of radial versus brachial artery. Unlike FMD, for which the physiological stimulus has been identified as increase in shear stress during reactive hyperemia (Thijssen et al. 2011), the stimulus for LFMC that couples low-flow state with arterial constriction has not yet been identified. Although we did not observe any differences in LFMC responses between dominant and non-dominant arms, the results could have been meaningfully explained if we had recorded the forearm muscle masses, which could act as a potential confounder. The sample size of the present study was not adequate to investigate the correlations between ΔLFMC of dominant and non-dominant sides and estimate their concordance. Yet another limitation of our study is that our subject group involved only young healthy adults. This aspect, along with our small sample size, limits generalization of the study findings across older age groups, where the effect of aging on vascular characteristics and responsiveness could produce results different from what we observed in response to a low-flow state.

Future studies shall address the major gaps in current knowledge on stimulus response characteristics of LFMC by systematically studying the shear stress dependence of LFMC and investigating the changes in arterial wall mechanics during the vasomotor response to low-flow state.

SUMMARY

Endothelial dysfunction has been described as one of the early pathophysiological events in the development and progression of atherosclerosis that culminates in major CVDs. LFMC has been recently reported as a

promising non-invasive tool for assessing the functioning of endothelium in the resting state. It has been reported that the radial artery shows constriction when a low-flow state is induced while brachial artery fails to constrict under low-flow conditions in healthy human subjects. The literature describes discrepancies in the behaviour of radial versus brachial arteries in response to the low-flow state. The reason for these discrepancies has not been addressed in a systematic and scientific way. We measured and compared LFMC of radial and brachial arteries between dominant and non-dominant arms of healthy human adults to systematically investigate the radial-brachial discrepancy. 20 healthy young adults 18−27 y of age participated in the study. LFMC responses in both radial and brachial arteries were comparable between the dominant non-dominant arms. Arterial location and baseline diameter emerged as independent predictors ($p < 0.005$) of LFMC response in the regression model, which could explain 42% of the variance in LFMC, suggesting that differential LFMC responses could be attributed to the arterial location along with differences in the baseline diameters of radial versus brachial artery.

Conflict of interest disclosure—The authors declare no conflict of interest.

REFERENCES

Anderson EA, Mark AL. Flow-mediated and reflex changes in large peripheral artery tone in humans. Circulation 1989;79:93–100.

Bazaral MG, Welch M, Golding LA, Badhwar K. Comparison of brachial and radial arterial pressure monitoring in patients undergoing coronary artery bypass surgery. Anesthesiology 1990;73:38–45.

Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111–1115.

Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R, International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257–265.

Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004;109:27–32.

Filitti V, Giral P, Simon A, Merli I, Del Pino M, Levenson J. Enhanced constriction of the peripheral large artery in response to acute induction of a low-flow state in human hypercholesterolemia. Arterioscler Thromb 1991;11:161–166.

Gori T, Dragoni S, Lisi S, Stolfo GD, Sonnati S, Fineschi M, Parker JD. Conduit artery constriction mediated by low flow a novel noninvasive method for the assessment of vascular function. J Am Coll Cardiol 2008;51:1953–1958.

Gori T, Grotti S, Dragoni S, Lisi M, Di Stolfo G, Sonnati S, Fineschi M, Parker JD. Assessment of vascular function: Flow-mediated constriction complements the information of flow-mediated dilatation". Heart 2010;96:141–147.

Gori T, Parker JD, Münzel T. Flow-mediated constriction: Further insight into a new measure of vascular function. Eur Heart J 2011;32:784–787.

Gori T, Muxel S, Damaske A, Radmacher MC, Fasola F, Schaefer S, Schulz A, Jabs A, Parker J D, Thomas M. Endothelial function assessment: Flow-mediated dilation and constriction provide different and complementary information on the presence of coronary artery disease. Eur Heart J 2012;33:363–371.

Gravlee GP, Wong AB, Adkins TG, Case LD, Pauca AL. A comparison of radial, brachial, and aortic pressures after cardiopulmonary bypass. J Cardiothorac Anesth 1989;3:20–26.

Harrison M, Parkhurst K, Tarumi T, Lin HF, Tanaka H. Low flow-mediated constriction: Prevalence, impact and physiological determinant. Clin Physiol Funct Imaging 2011;31:394–398.

Irace C, Tripolino C, Scavelli FB, Carallo C, Gnasso A. Brachial low-flow-mediated constriction is associated with delayed brachial flow-mediated dilation. J Atheroscler Thromb 2016;23:355–363.

Levenson J, Simon A, Pithois MI. Brachial arterial changes in response to wrist occlusion in normotensive and hypertensive men. Am J Physiology 1987;253:H217–H224.

Megnien JL, Simon A, Andriani A, Segond P, Jeannin S, Levenson J. Cholesterol lowering therapy inhibits the low-flow mediated vasoconstriction of the brachial artery in hypercholesterolaemic subjects. Br J Clin Pharmacol 1996;42:187–193.

Mullen MJ, Kharbanda RK, Cross J, Donald AE, Taylor M, Vallance P, Deanfield JE, MacAllister RJ. Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo: Relevance to endothelial dysfunction in hypercholesterolemia. Circ Res 2001;88:145–151.

Nichols WW, O'Rourke MF. McDonald's blood flow in arteries: Theoretical, experimental & clinical principles. 6th edition London: Hodder Arnold; 2011.

Norioka N, Takemoto Y, Kobayashi M, Makuuchi A, Yoshikawa J, Yamazaki Y, Kamiyama Y, Shuto T, Yoshiyama M. Low-flow mediated constriction incorporated indices as indicators of cardiovascular risk in smokers. Atherosclerosis 2016;251:132–138.

Oldfield RC. The assessment and analysis of handedness: The Edinburgh inventory. Neuropsychologia 1971;9:97–113.

Parker BA, Ridout SJ, Proctor DN. Age and flow-mediated dilation: A comparison of dilatory responsiveness in the brachial and popliteal arteries. Am J Physiol Heart Circ Physiol 2006;291:3043–3049.

Prabhakaran D, Jeemon P, Roy A. Cardiovascular diseases in India: Current epidemiology and future directions. Circulation 2016;133:1605–1620.

Sonka M, Liang W, Lauer RM. Automated analysis of brachial ultrasound image sequences: Early detection of cardiovascular disease via surrogates of endothelial function". IEEE Trans Med Imaging 2002;21:1271–1279.

Spieker LE, Lüscher TF, Noll G. ETA receptors mediate vasoconstriction of large conduit arteries during reduced flow in humans. J Cardiovasc Pharmacol 2003;42:315–318.

Spiro JR, Digby JE, Ghimire G, Mason M, Mitchell AG, Ilsley C, Donald A, Dalby MCD, Kharbanda RK. Brachial artery low-flow-mediated constriction is increased early after coronary intervention and reduces during recovery after acute coronary syndrome: Characterization of a recently described index of vascular function. Eur Heart J 2011;32:856–866.

Stadler RW, Ibrahim SF, Lees RS. Measurement of the time course of peripheral vasoactivity: Results in cigarette smokers. Atherosclerosis 1998;138:197–205.

Thijssen DH, van Bemmel MM, Bullens LM, Dawson EA, Hopkins ND, Tinken TM, Black MA, Hopman MT, Cable NT, Green DJ. The impact of baseline diameter on flow-mediated dilation differs in young and older humans. Am J Physiol Heart Circ Physiol 2008;295:H1594–H1598.

Thijssen DH, Willems L, Munckhof I, Scholten R, Hopman MT, Dawson EA, Atkinson G, Cable NT, Green DJ. Impact of wall thickness on conduit artery function in humans: Is there a 'Folkow' effect?". Atherosclerosis 2011;217:415–419.

Thijssen DHJ, Atkinson CL, Ono K, Sprung VS, Spence AL, Pugh CJA, Grern DJ. Sympathetic nervous system activation, arterial shear rate, and flow-mediated dilation. J Appl Physiol 2014;116:1300–1307.

Thijssen DHJ, Bruno RM, van Mil ACCM, Holder SM, Faita F, Greyling A, Zock PL, Taddei S, Deanfield JE, Luscher T, Green DJ, Ghiadoni L. Expert consensus and evidence-based

recommendations for the assessment of flow-mediated dilation in humans". Eur Heart J 2019;40:2534–2547.
Veale JF. Edinburgh handedness inventory - short form: A revised version based on confirmatory factor analysis. Laterality 2014;19(2):164–177.
Weissgerber TL, Davies GAL, Tschakovsky ME. Low flow-mediated constriction occurs in the radial but not the brachial artery in healthy pregnant and nonpregnant women". J Applied Physiol 2010;108:1097–1105.
Yeboah J, Crouse JR, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: The cardiovascular health study. Circulation 2007;115:2390–2397.