Brachial Artery “Low-Flow Mediated Constriction” Is Associated with Myocardial Perfusion Defect Severity and Mediated by an Altered Flow Pattern during Occlusion

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Keywords

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Abstract

Introduction: The relationship between low flow-mediated constriction (LFMC), a new proposed measure of endothelial function, with cardiovascular disease severity and its hypothesized stimulus, that is, low flow, has not been comprehensively evaluated. The study evaluated association between change in brachial artery diameter during constriction with severity of myocardial perfusion defect (PD) and alterations in different components of flow profile. **Methods:** Brachial artery responses to occlusion were assessed in 91 patients and 30 healthy subjects. Change in antegrade and retrograde blood flow velocities (delta antegrade blood flow velocity and retrograde blood flow velocity), antegrade shear rate and retrograde shear rate (delta ASR and RSR, respectively), and oscillatory shear index (delta) during forearm occlusion at 50 mm Hg above systolic pressure, from baseline was calculated. Myocardial perfusion was evaluated in patients using exercise single positron emission com-

puted tomography and % myocardial PD was calculated from summed stress score. **Results:** LFMC emerged as independent predictor of defect severity after correcting for age and gender ($p = 0.014$). Sixty-seven patients (73.6%) and 15 healthy subjects (50%) showed constriction during occlusion. In stepwise backward regression analysis, RSR contributed 35.5% and ASR contributed 20.1% of the total 63.9% variability in artery diameter during occlusion. **Conclusion:** The results suggest that LFMC is independently associated with myocardial perfusion severity and is “mediated” by an altered flow profile during occlusion. © 2021 S. Karger AG, Basel

Introduction

The term “low flow-mediated constriction” (LFMC) was coined in 2008 by Gori et al. [1] to describe the reduction in the artery diameter during occlusion and has been considered as a new method for noninvasive assessment of endothelial function. The earliest published description of changes in peripheral arterial diameter during occlusion dates back to 1987 in a study by Levenson et al. [2], in which changes in the brachial artery

diameter in response to occlusion were assessed in normotensives and hypertensives. The study reported a nonsignificant reduction in brachial artery diameter during occlusion, while the net blood flow and velocity reduced significantly.

Recently, LFMC has been investigated in the brachial and the radial arteries as a measure of endothelial function. In the radial artery, a greater constriction during occlusion has been reported for healthy subjects while patients show a reduction in the constrictive ability [1]. On the other hand, in the brachial artery, a greater constriction during occlusion has been seen in patients with cardiovascular diseases or risk factors associated with cardiovascular disease (CVD), while healthy subjects show minimal constriction. Unlike flow-mediated dilation (FMD), which is an established marker of endothelial dysfunction and has been shown to correlate with coronary endothelial dysfunction [3], there are limited data on LFMC and CVD severity or coronary ischemia. In 1 study, a significant positive association was reported between radial artery LFMC with SYnergy between PCI with TAXus and CABG (SYNTAX) score in patients of coronary artery disease [4]; however, there are no reports regarding the same for the brachial artery, which is the common site for assessment of FMD.

Additionally, there is an incomplete understanding of the mechanisms underlying LFMC. The term “Low flow mediated constriction” was given to indicate the reduction in artery diameter during a “low flow” state as opposed to the “high flow” mediated dilation. A working hypothesis for LFMC is that the “Low flow state” is possibly associated with a shift in balance toward vasoconstrictors, like endothelin-1 (ET-1) and prostaglandins, due to a reduction in the shear stimulus to the endothelium [1, 5]. However, in a study by Weissgerber et al. [6] reduction in shear rate during occlusion was not found to be associated with radial artery diameter changes in a population of pregnant and nonpregnant women. Interestingly, in a previous study, an increase in retrograde flow (flow from the periphery toward the heart) and decrease in antero-grade flow (flow from the heart toward the periphery) was observed during supra-systolic occlusion [7]. The increase in retrograde flow could be the result of increase in distal vascular resistance leading to impedance mismatch and therefore increase in reflected waves from the site of supra-systolic occlusion [8]. Retrograde oscillatory flow profile has been associated with endothelial dysfunction in cell culture studies [9–12]. Furthermore, an acute increase in retrograde flow using sub-diastolic oc-

clusion has been reported to cause endothelial dysfunction, as assessed by a reduction in FMD [13]. To the best of our knowledge, there are no data available on the assessment of LFMC and its possible association with both antero-grade and retrograde flow in either healthy subjects or patients with CVD.

The current study aimed to evaluate the association between LFMC and CVD severity. Furthermore, the study also aimed to examine the “stimulus-response” characteristics of LFMC by evaluating the association between brachial artery constriction during occlusion with the alterations in the retrograde, antero-grade, and oscillatory flow profiles in healthy subjects and patients with ischemic heart disease (IHD).

Materials and Methods

A total of 122 consecutive patients clinically diagnosed with recent (≤ 6 months) history suggestive of IHD were included in the study. Patients with at least 2 of the following features: (i) clinical symptoms suggestive of IHD; (ii) hospitalization with records showing dynamic ECG of ST-elevation, ST-depression, or abnormal Q wave; (iii) elevation of cardiac biomarkers, were recruited for the study. All patients were diagnosed by a single experienced cardiologist before recruitment. Out of these, 107 stable patients underwent myocardial perfusion imaging using exercise Technetium-99 single positron emission computed tomography (Tc⁹⁹-SPECT), and 89 patients (83.2%) showed perfusion abnormalities at rest or stress, and 18 showed a normal scan during both rest and stress. Vascular function assessment could be performed for 75 out of the 89 patients with perfusion defects (PDs) and 16 of the 18 patients with normal scans. Of the 75 patients with PD who completed the vascular assessment, 34.6% ($n = 26$) had hypertension, 34.6% ($n = 26$) had diabetes mellitus, and 37.4% ($n = 28$) had a history of smoking. Out of the 16 patients with normal scans, 43.7% ($n = 7$) had hypertension, 37.5% ($n = 6$) had diabetes mellitus, and 18.7% ($n = 3$) had a history of smoking. All patients had given up smoking after the event. Patients on medications affecting vascular function were asked to withhold the same for 24 h before vascular assessment. Additionally, 30 healthy subjects were recruited for assessment of vascular parameters. All healthy subjects were non-hypertensive ($<140/90$ mm Hg), nondiabetic (fasting blood sugar <126 mg/dL), nonsmokers with no known history of any chronic cardiovascular or renal disease or on any medications affecting vascular function, and with a normal resting 12-lead ECG. Subjects with any recent infection within 1 week of the investigations were excluded from the study.

The study was approved by the institute Ethics Committee for research on human subjects, and the investigations conformed to the Declaration of Helsinki. A written, informed consent was obtained from all patients and subjects before starting the study. All investigations were performed between 8:00 and 10:00 a.m., and subjects were instructed to report in the morning after a period of overnight fasting. All subjects were also instructed to refrain from caffeinated beverages and heavy exercise a day before the study.

Fig. 1. Representative PW doppler records of 2 subjects showing brachial blood flow velocity at rest (**a**) and during occlusion (**b**). The vertical bars indicate velocity scale in cm/s and the horizontal bar indicates time where the distance between the 2 major divisions is 1 s. The shaded area above the horizontal axis shows the time-averaged ABFV while the unfilled circle at the peak of waveform shows the ABFV_{max} for 1 cardiac cycle. Similarly, the shaded

area below the horizontal axis represents the time-averaged RBFV while the unfilled circle at the peak of waveform shows the RBFV_{max} for 1 cardiac cycle. Consecutive 8–10 such cycles were averaged to calculate the respective time-averaged and maximum velocities. ABFV, antegrade blood flow velocity; RBFV, retrograde blood flow velocity; ABFV_{max}, maximum ABFV; RBFV_{max}, maximum RBFV; PW, pulsed-wave.

Myocardial Perfusion Imaging

Images were acquired on GE Infinia Hawkeye dual-head Gamma Camera mounted using low energy all-purpose collimators with parallel holes. The patients were positioned in feet first supine position with arms extended over the head. The imaging was performed with electrocardiography synchronization in auto-tracking mode with 8 frames per cardiac cycle. The SPECT acquisition was performed in step and shoot mode with noncircular body contoured orbit with a total of 60 projections (30 projections for each detector) and images acquired over 180° from right anterior oblique to left posterior oblique view in L-mode with counter-clockwise rotation. Time for each projection was 20 s leading to total scan duration of approximately 14 min. A zoom factor of 1.3 was used and images were acquired in 64 × 64 matrix with the photopick centered at 140 keV for Tc-99m with 20% window. Both stress and rest images were acquired with same acquisition parameters. All studies were processed with commercially available Emory Cardiac Tool box (ECTbox; Emory University, Atlanta, GA, USA) software on a Xeleris nuclear medicine workstation (GE Medical Systems; Waukesha, WI, USA). The SPECT images were reconstructed with FBP technique using Butterworth filter with critical frequency of 0.4 and order 10 for stress images and a critical frequency of 0.52 and order 5 for rest images.

A 20-segment heart model was used for localization of the PDs. Both rest and exercise stress images were acquired. Defect size and severity were considered in each of the 20 segments on the stress and rest images and was scored from 0 to 4, with 0 being normal, 1 mild, 2 moderate, 3 severe, and 4 absent radiotracer uptake. The summed stress score (SSS) was calculated as the sum of the stress scores of all the segments. Similarly, the summed rest score (SRS) was calculated as the sum of the rest scores of all the segments. The summed difference score (SDS) was calculated as the difference between the summed stress and the summed rest scores and is re-

flective of ischemia. Scoring for all patients was done by a single experienced nuclear medicine consultant who was blinded to the results of the vascular function assessment. The % PD was calculated using the formula:

$$\% \text{ perfusion defect} = \frac{\text{SSS}}{80} \times 100.$$

where 80 is the maximum possible defect.

Baseline Diameter and Blood Flow Velocity Measurements

A 10 MHz linear array ultrasound probe (MindRay Medical International Ltd., M7) was used to image the brachial artery. Longitudinal B-mode images of the brachial artery were acquired 2–3 cm above the anti-cubital fossa. Simultaneous lead-2 ECG was acquired for R-wave gating of the diameter. Video loops of at least 8–10 consecutive cardiac cycles were recorded and saved for offline analysis. This was followed by recording of the brachial artery blood flow in Pulsed-wave (PW) doppler mode. Duplex mode was used to simultaneously record the brachial artery diameter and blood flow velocity waveform for shear rate calculation with the insonation angle at 60°. Automated edge detection and flow analysis software (medical imaging applications LLC, Brachial Diameter and Flow Analyzer module) was used for brachial artery diameter and blood flow velocity analysis, respectively.

Brachial artery diameter was obtained by extracting the end-diastolic diameter from the beat-to-beat diameter waveform. For flow analysis, time-averaged mean antegrade and retrograde blood flow velocities (ABFV_m and RBFV_m, respectively) were calculated separately using the area under the curve of the antegrade and retrograde flow waveform, respectively (Fig. 1a). Additionally, the maximum value of antegrade and retrograde blood flow velocities for each cardiac cycle was cal-

culated from the crest and trough of the flow velocity waveform for each cycle, respectively ($ABFV_{max}$ and $RBFV_{max}$, respectively) (Fig. 1a). All values were calculated for 8–10 consecutive cardiac cycles and then averaged to obtain the baseline diameter and flow velocity.

Time-averaged mean antegrade shear rate (ASR_m) and retrograde shear rate (RSR_m) and maximum antegrade shear rate (ASR_{max}) and retrograde shear rate (RSR_{max}) were calculated using the following formula:

$$\text{Shear rate} = 4 \times \text{velocity/diameter}$$

where $ABFV_m$, $RBFV_m$, $ABFV_{max}$, and $RBFV_{max}$ were used to calculate the respective shear rates.

Oscillatory shear index (OSI) at baseline was calculated using the formula:

$$OSI = \frac{RSR_m}{(ASR_m + RSR_m)}.$$

Low Flow-Mediated Constriction

After acquisition of baseline diameter and flow data, the forearm was occluded by inflating a blood pressure cuff to 50 mm Hg above the systolic pressure of the subject for 5 min. During the last 1 min of occlusion, diameter in B-mode was acquired for 50 s and diameter and blood flow velocity in duplex mode PW doppler was acquired for last 10 s. After the release of occlusion by rapid deflation of the blood pressure cuff, an initial 1 min recording was done in PW mode to record the peak flow followed by brachial artery diameter measurement for 3 min. All images were recorded and stored for offline analysis.

Analysis was done using medical imaging applications automated edge detection software. The beat-to-beat diameter was extracted for the 50 s period. The lowest end-diastolic diameter during the 50 s period was identified. The end-diastolic diameter values just before and after the lowest diameter were assessed to ensure that the values were consistent. 4–5 diameter values around the lowest diameter were then averaged to obtain the diameter during the low flow state for calculation of LFMC using the following formulas:

Delta LMFC = lowest diameter during occlusion – diameter at baseline.

$$\%LFMC = \frac{\text{lowest diameter during occlusion} - \text{diameter at baseline}}{\text{diameter at baseline}} \times 100.$$

PW doppler waveform loop acquired during the last 10 s of 5 min occlusion, was used to calculate the flow velocities attained during the low flow state. $ABFV$ and $RBFV$ were analyzed separately as for the baseline and the respective shear rates during low flow state was calculated (Fig. 1b). The change in antegrade, retrograde, and net shear rate during low flow state was calculated for the mean and maximum shear rates ($\Delta_{LF} SR_m$ and SR_{max}) using the following formula:

$$\Delta_{LF} SR = \text{shear rate during occlusion} - \text{shear rate baseline.}$$

where the respective ASR_m , RSR_m , ASR_{max} , RSR_{max} , and net shear rate were used for the calculations.

OSI during LFMC (LFOSI) was calculated from the ASR_m during occlusion ($LFASR_m$) and the RSR_m during occlusion ($LFRSR_m$) using the formula:

$$LFOSI = LFRSR_m / (LFASR_m + LFRSR_m).$$

Change in OSI during LFMC was calculated as:

$$\text{Delta OSI} = LFOSI - \text{OSI at baseline.}$$

Flow-Mediated Dilation

After 5-min occlusion, the cuff pressure was released and the increase in blood flow velocity was recorded for 1 min in PW mode followed by acquisition of the brachial artery diameter in B-mode for 3 min. The end-diastolic diameters of the 3 min record were assessed to identify the peak diameter using automated edge detection software. Diameter values just before and after the peak diameter were checked to ensure that the values were consistent. 4–5 diameter values around the peak diameter were averaged to obtain the diameter in response to reactive hyperemia. 1 min of release was analyzed. Peak flow was calculated as an average of the area under the curve of the duration for which the flow was the highest.

FMD was calculated by using the following formula:

Delta FMD = peak diameter after occlusion – diameter at baseline.

$$\%FMD = \frac{\text{peak diameter after occlusion} - \text{diameter at baseline}}{\text{diameter at baseline}} \times 100.$$

At high flows, retrograde flow disappears completely; therefore, the change in ASR during reactive hyperemia for the mean and maximum shear rates ($\Delta_{HF} SR_m$ and SR_{max}) using the following formula:

$$\Delta_{HF} SR = \text{shear rate after occlusion} - \text{shear rate at baseline.}$$

where the respective ASR_m and ASR_{max} were used for the calculations.

Forearm Vascular Resistance

Forearm vascular resistance (FVR) was calculated from the mean arterial pressure (MAP) and time-averaged mean blood flow (in cm/min) of the brachial artery. MAP was derived from the peripheral systolic and diastolic pressures. FVR was then calculated using the formula:

$$FVR = \frac{MAP}{\left[\left(ABFV_{AUC} - RBFV_{AUC} \right) \times 60 \right] \times \pi r^2}.$$

The MAP was assumed to remain constant as it has been reported to not change significantly during occlusion [14]. FVR during LFMC and FMD was calculated from the mean blood flow velocities and radius (r) during occlusion and after release, respectively. Δ_{LF} FVR and Δ_{HF} FVR were calculated by subtracting the baseline FVR from the FVR during LFMC and FMD, respectively.

Statistical Analysis

The data distribution was tested using Shapiro-Wilk, Anderson-Darling, Kolmogorov-Smirnov, and D'Agostino-Pearson test. Data were considered to be normally distributed if at least

Table 1. Descriptive data of the study population

Parameter	PD (n = 75)	NPD (n = 16)	HC (n = 30)
Peripheral SBP, mm Hg	128 (118–148)	124 (108–155)	120 (116–130)
Peripheral DBP, mm Hg	80 (70–86)	77 (61–83)	80 (72–84)
Peripheral PP, mm Hg	52 (41–64)*	50 (38–74)	42 (39–50)
Heart rate, bpm	76.3±12.2*	74.4±11.5 [#]	64.2±8.53
Fasting sugar, mg/dL	118.0 (94.0–145.0)*	99.0 (95.0–124.0) [#]	87.0 (80.0–94.2)
Total cholesterol, mg/dL	128.0 (110.5–148.5)*	154.0 (112.8–171.0) [#]	185.0 (167.5–210.0)
LDL, mg/dL	76.5 (65.0–92.0)*	84.0 (70.0–118.3) [#]	117.7 (103.6–142.0)
HDL, mg/dL	34.0 (31.0–40.0)*	39.0 (31.0–57.0) [#]	39.5 (34.0–41.0)
VLDL, mg/dL	15.0 (13.0–18.5)*	12.5 (10.0–25.2) [#]	29.0 (24.9–31.0)
Triglycerides, mg/dL	112.5 (90.8–155.0)*	110.5 (79.0–125.0) [#]	148.0 (127.0–158.3)
Ejection fraction, %	48.0 (38.5–59.0)	62.0 (58.0–65.0) [†]	–
Delta LFMC, mm	–0.16±0.20*	–0.15±0.39	–0.03±0.23
% LFMC	–3.82±4.75*	–3.09±8.93	–0.64±6.11
Delta FMD, mm	0.24 (0.12–0.44)	0.18 (0.06–0.43)	0.28 (0.14–0.48)
% FMD	5.60 (2.63–11.1)	5.30 (1.45–9.72)	7.28 (3.40–12.2)

Parametric data are expressed as mean ± SD and nonparametric as median (IQR). Significance was considered at $p < 0.05$. PD, perfusion defect group; NPD, no perfusion defect group; HC, healthy control group; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LFMC, low flow-mediated constriction; FMD, flow-mediated dilation; SD, standard deviation; IQR, interquartile range. *Indicates significant difference between PD and HC. [#]Indicates significant difference between NPD and HC. [†]Indicates significant difference between PD and NPD.

2 or more tests were positive for normal distribution of which 1 was either Shapiro-Wilk or Anderson-Darling. Gaussian data are expressed as mean ± standard deviation, and non-Gaussian data are expressed as median (interquartile range). Group differences were tested using 1-way ANOVA with Tukey's post hoc correction for multiple comparisons or Kruskal-Wallis test with Dunn's post hoc correction for multiple comparisons. Change in absolute diameter and flow from baseline was assessed using repeated measures ANOVA. Pearson's test was used to test correlation between normally distributed continuous variables, while Spearman's test was used for nonparametric data. Best-fit models for LFMC were obtained using stepwise backward elimination method. All analysis was done using GraphPad Prism version 8.3.0 (GraphPad Software LLC.) and Minitab version 19.2020.1.0 (Minitab LLC).

Results

The age for the 3 groups, patients with PD group, patients with normal scans (no perfusion defects [NPD] group) and healthy controls (HC group) was comparable (55.1 ± 9.3 vs. 53.2 ± 10.4 vs. 51.3 ± 9.3 years, respectively). The descriptive data for the study population are described in Table 1. The absolute values of diameter and flow in all 3 groups are detailed in online supplementary Table 1 (see www.karger.com/doi/10.1159/000519558 for all online suppl. material).

Change in Brachial Artery Diameter, Flow and Shear Rate during Occlusion, and Its Association with Severity of PD

A significant decrease in diameter was observed in PD group but not in NPD and HC groups during occlusion when compared to the baseline. A significantly greater number of patients (67 [56 PD and 11 NPD] out of 91 [73.6%]), showed a constriction response during occlusion as compared to healthy subjects (15 out of 30 [50%], $p = 0.013$). Delta and % LFMC were significantly more negative (greater constriction) in PD than in the HC (Table 1). All 3 groups displayed a significant decrease in AB-FV_m, ABFV_{max}, ASR_m, and ASR_{max}, as well as in net blood flow velocity and shear rate during occlusion. The RB-FV_m, RBFV_{max}, RSR_m, and RSR_{max}, OSI, and FVR increased significantly during occlusion in comparison to baseline in all groups (online suppl. Table 1). Increase in RBFV_{max} during occlusion was significantly greater in patients with PDs than HC, while there was a trend toward a greater increase in RSR_{max} in PD than HC (online suppl. Table 2). All other blood flow velocity and shear rate parameters were comparable across groups.

For evaluation of association between LFMC and disease severity, the study population was subdivided on the basis % of myocardial PD into those with ≥25% defect ($n = 29$), between ≥10–25% defect ($n = 18$), between >0–

Fig. 2. **a** A significant overall trend between delta LPMC and % myocardial PD in 1-way ANOVA ($p = 0.018$). $p = 0.18$, 0.02 and 0.05 between normal perfusion (0) and those with >0–10 defects, between ≥10–25% defects and ≥25% defects, respectively. **b** A significant overall trend between % LMFC and % myocardial PD in 1-way ANOVA ($p = 0.022$). $p = 0.15$, 0.01 and 0.06 between normal

perfusion (0) and those with >0–10 defects, between ≥10–25% defects and ≥25% defects, respectively. **c** A significant positive correlation was observed between delta LPMC and delta FMD. Delta FMD (**d**) and %FMD (**e**) were significantly higher in nonconstrictors than constrictors. LPMC, low flow-mediated constriction; PD, perfusion defect; FMD, flow-mediated dilation.

10% defect ($n = 28$) and normal perfusion (no PD group and HC subjects) ($n = 46$) and 1-way ANOVA was performed for trend analysis followed by Tukey's multiple comparisons test where significant trends were observed. Trend analysis showed a significant difference for both delta and %LFMC (ANOVA $p = 0.018$ and 0.022 , respectively) across disease severity groups (Fig. 2a, b). Both delta and %LFMC emerged as independent predictors of defect severity after correcting for age and gender ($p = 0.013$ and 0.014 , respectively) using multivariate analysis.

Association of Change in Brachial Artery Diameter with Flow Velocity and Shear Rates (Stimulus Response Characterization)

For stimulus-response characterization, data of patients and subjects who showed a constriction response (67 patients and 15 healthy subjects) were pooled and the association between the reduction in brachial artery diameter with flow velocity and shear rates was evaluated. In univariate analysis, delta LPMC showed a sig-

nificant correlation with $\text{delta}_{\text{LF}} \text{ABFV}_{\text{max}}$ ($r = -0.24$, $p = 0.033$), $\text{delta}_{\text{LF}} \text{ASR}_{\text{max}}$ ($r = -0.39$, $p = 0.0003$), $\text{delta}_{\text{LF}} \text{RBFV}_{\text{m}}$ and RBFV_{max} ($r = -0.32$, $p = 0.004$ and $r = -0.29$, $p = 0.010$, respectively), $\text{delta}_{\text{LF}} \text{RSR}_{\text{m}}$ and RSR_{max} ($r = -0.39$, $p = 0.0003$ and $r = -0.38$, $p = 0.0005$, respectively). Similar associations were also seen for % LFMC. No correlation was seen between $\text{delta}_{\text{LF}} \text{FVR}$ and delta or % LFMC. The increase in OSI during occlusion showed a significant positive correlation with the increase in FVR ($r = 0.47$, $p < 0.0001$) and with $\text{delta}_{\text{LF}} \text{RBFV}_{\text{m}}$ ($r = 0.28$, $p = 0.014$) but not with $\text{delta}_{\text{LF}} \text{ABFV}_{\text{m}}$ ($r = -0.15$, $p = 0.2$).

Further, the best-fit model for determinants of delta LPMC was derived using stepwise backward elimination from all variables of flow velocity and shear rate. $\text{Delta}_{\text{LF}} \text{ABFV}_{\text{m}}$, $\text{delta}_{\text{LF}} \text{ASR}_{\text{m}}$, $\text{delta}_{\text{LF}} \text{RBFV}_{\text{max}}$, $\text{delta}_{\text{LF}} \text{RSR}_{\text{max}}$ and $\text{delta}_{\text{LF}} \text{OSI}$ emerged as predictor variables in the best-fit model, explaining 63.9% of the total variation in delta LPMC, of which $\text{delta}_{\text{LF}} \text{ASR}_{\text{m}}$ contributed 20.1% and $\text{delta}_{\text{LF}} \text{RSR}_{\text{max}}$ contributed 35.6% (Table 2).

Table 2. Best-fit multiple regression model for delta LFMC

Source	DF	Seq SS	Contribution, %	Adj SS	Adj MS	F value	p value
Regression	5	1.87992	63.91	1.8799	0.37598	25.86	0
"Delta LF ABFV _m "	1	0.0143	0.49	1.0407	1.0407	71.57	0
"Delta LF ASR _m "	1	0.59057	20.08	1.1881	1.18813	81.71	0
"Delta LF RBFV _{max} "	1	0.10908	3.71	0.8791	0.87912	60.46	0
"Delta LF RSR _{max} "	1	1.04563	35.55	1.166	1.16596	80.19	0
"Delta OSI"	1	0.12034	4.09	0.1203	0.12034	8.28	0.005
Error	73	1.06148	36.09	1.0615	0.01454		
Total	78	2.9414	100.00				

Stepwise backward elimination with ANOVA showing the model parameters and their % contribution to the final model. DF, degrees of freedom; Seq SS, sequential sum of squares; Adj SS, adjusted sum of squares; Adj MS, adjusted mean squares; ABFV_m, mean anterograde blood flow velocity; ASR_m, mean anterograde shear rate; RBFV_{max}, maximum retrograde blood flow velocity; RSR_{max}, maximum retrograde shear rate; OSI, oscillatory shear index.

Association between LFMC and FMD

Delta and % FMD was not significantly different among groups. However, in the pooled data for all patients and subjects, delta LFMC showed a significant positive correlation with delta FMD (greater constriction during occlusion associated with lesser dilation after release) (Fig. 2c). Furthermore, the population showing constriction during occlusion had a significantly lower FMD than those that did not show any constriction ("Constrictors" vs. "Non-constrictors" delta FMD 0.22 [0.11–0.40] vs. 0.35 [0.15–0.53] mm, $p = 0.035$ and %FMD 5.17 [2.38–9.63] vs. 7.63 [4.19–13.5] %, $p = 0.039$) (Fig. 2d, e).

Association of LFMC with Clinical and Hemodynamic Variables

A significant negative correlation was observed between both delta and % LFMC with fasting blood sugar levels (delta LFMC $r = -0.24$, $p = 0.024$ and %LFMC $r = -0.23$, $p = 0.031$) and systolic blood pressure (delta LFMC $r = -0.21$, $p = 0.021$, and %LFMC $r = -0.21$, $p = 0.025$). No correlation was observed between delta or % LFMC with age or any of the other clinical and hemodynamic variables.

Discussion

LFMC has emerged as a new tool for assessment of endothelial function. Indeed, LFMC has been used as a measure of endothelial dysfunction in patients with cardiovascular risk factors and CVDs, and brachial artery has been reported to show a greater constriction in the diseased population [1, 15–17]. In the current study also,

brachial artery was found to show greater constriction in the patients with PDs than the HCs. Furthermore, the study demonstrates that constriction in the brachial artery during occlusion is greater in patients with increasing severity of myocardial PDs which was independent of age and gender. To the best of our knowledge, this is the first study to evaluate these changes in the brachial artery which is a commonly used site for assessing endothelial function by FMD. In a previous study, a significant positive association was reported between radial artery LFMC with SYNTAX score in patients of coronary artery disease, suggesting that patients with greater disease severity have lesser constriction [4]. This difference between brachial and radial arteries in relation to the "direction" of association with the disease state has been previously reported [1, 15, 16, 18]. Interestingly, the results of "stimulus-response" characterization show that as much as 63% of the variation in artery diameter during occlusion could be explained by the changes in the flow profile and a possible reason for the conflicting results of the previous studies could be due to differences in the flow profile during occlusion. Increase in OSI was observed during occlusion suggesting that the "low-flow" state during occlusion is, in effect, a state of "altered" flow, with an increase in the retrograde and oscillatory shear. The association between change in retrograde and oscillatory shear and FVR indicate that this increase is probably mediated by the increase in FVR during occlusion. Resting retrograde flow has been previously reported to correlate with age-induced changes in forearm vascular conductance [19]. Exposure to retrograde flow has been shown to increase ET-1 expression in an endothelial cell culture model [12]. In humans, infusion of ET_A receptor antagonist attenu-

ated the constriction response during occlusion, suggesting that the vasoconstriction could be mediated by an increase in ET-1 [20]. The results of the current study indicate that the increase in RSR during occlusion may “actively” stimulate the endothelium to release vasoconstrictors, like ET-1 leading to constriction. ET-1 is stored in the endothelial cells and can be secreted rapidly in response to a stimulus [21], which makes it a plausible mediator of occlusion-induced vasoconstriction. Furthermore, ET-1 release has been linked to both duration and level of shear stimulus in cell culture models [22].

There is also a possibility of a “dose-response” like relationship between the flow profile, which may trigger an increase or decrease in the ET-1 release depending on the duration and amplitude of anterograde and retrograde shear stimulus, with the change in the artery diameter that may explain the differences in the results obtained for radial and brachial arteries. Based on these results, the term “Low flow mediated constriction” appears to be a misnomer and “Retrograde flow mediated constriction” may be more closely indicative of the phenomenon observed during occlusion.

The acute effect of drugs affecting vascular function was avoided in the current study by asking the patients to withhold these for 24 h before the assessment. In a meta-analysis, better FMD has been reported in patients taking angiotensin-converting enzyme inhibitors, calcium channel blockers, angiotensin receptor blockers, diuretics, and beta-blockers [23]. Additionally, in a study on patients with hypercholesterolemia, lipid-lowering therapy was found to reduce brachial artery constriction after 3 months [24]. However, in the current study, no difference was observed in the percentage of subjects taking angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, beta-blockers, diuretics, or nitrates in those who showed constriction versus those who did not show constriction during occlusion (online suppl. Table 3).

A significant positive association was observed between LFMC and FMD, and similar results have been reported previously [16, 25–27]. However, in the current study, no association was observed between FMD and severity of myocardial PDs (data not shown). In a previous study, FMD was found to be lower in patients with greater number of PDs, but its association with the severity of ischemia was not reported [28]. Interestingly, while FMD was comparable in the patient and healthy groups in the present study, it was significantly lower in the constrictors than in those who did not show constriction during occlusion. It is possible that presence of constriction during occlusion in the

brachial artery is indicative of underlying endothelial dysfunction or a predisposition for endothelial dysfunction even in apparently healthy subjects without cardiovascular risk factors. Indeed, there was a trend toward lower FMD in healthy constrictors versus healthy nonconstrictors (delta FMD $p = 0.051$, % FMD $p = 0.049$, online suppl. Fig. 1). Greater release of ET-1 during occlusion in the population showing constriction could inhibit the post-release NO-mediated vasodilation leading to lower FMD. Though the current sample size is small for this sub-group comparison, these results are of potential clinical significance. These findings would need to be further evaluated in prospective studies on a larger sample size. The association between LFMC and fasting blood sugar levels and systolic blood pressure in the current study, further suggests its association with underlying disease processes. These results suggest that in addition to being an indicator of disease severity in patients, LFMC may also be suggestive of subclinical disease in healthy individuals, with the presence of constriction indicating underlying vascular dysfunction.

A limitation of the current study is that basal ET-1 levels were not measured. ET-1 can be higher in atherosclerosis and spillover of ET-1 from endothelial cells and vascular smooth muscle cells have been suggested to be involved in vascular pathology. Estimation of basal levels may provide further insight into the factors associated with constriction during occlusion.

Conclusion

The major conclusion from the current study is that constriction during occlusion is associated with the severity of myocardial PD and mediated by an altered flow profile of which a major contribution is by the increase in RSR and additional effects by the decrease in ASR. Additionally, presence of constriction during occlusion may be indicative of subclinical disease and help in identifying individuals at risk of CVDs, which needs to be further investigated.

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Statement of Ethics

The study was approved by the All India Institute of Medical Sciences, New Delhi, Institute Ethics Committee for Postgraduate Research for Clinical Science, for research on human subjects (Ref

No. IECPG/288/27.04.2016, RT-37/29.06.2016), and the investigations conformed to the Declaration of Helsinki. A written, informed consent was obtained from all patients and subjects before starting the study.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Author Contributions

S.B. contributed to conception and design of the study, acquisition, analysis and interpretation of data, drafting the article, and final approval of the submitted manuscript. D.S.C. contributed to

conception and design of the study, interpretation of data, revising the article, and final approval of the submitted manuscript. A.K.J. contributed to conception and design of the study, revising the article, and final approval of the submitted manuscript. R.N. contributed to conception and design of the study, revising the article, and final approval of the submitted manuscript. C.P. contributed to conception and design of the study, acquisition, analysis and interpretation of data, revising the article, and final approval of the submitted manuscript. K.K.P. contributed to conception and design of the study, revising the article, and final approval of the submitted manuscript.

Data Availability Statement

Data generated and analyzed for the study are available from the corresponding author upon request.

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