Reliability of low-flow vasoreactivity in the brachial artery of adolescents

ABSTRACT

Purpose: Macrovascular endothelial function is commonly assessed using flow-mediated dilation (FMD) and is nitric oxide (NO) dependent. However, the vasoreactivity to low-flow during the FMD protocol may complement FMD interpretation. This study aimed to investigate in adolescents: 1) the day-to-day reliability of low-flow-mediated constriction (L-FMC) and composite vessel reactivity (CVR); and 2) the relationship between L-FMC and FMD.

Methods: A retrospective analysis of data on 27 adolescents (14.3 ± 0.6 y, 12 males) was performed. Participants had two repeat measures, on separate days, of macrovascular function using high-resolution ultrasound for assessment of L-FMC, FMD and CVR.

Results: On average, the L-FMC response was vasoconstriction on both days (-0.59 ± 2.22 % and -0.16 ± 1.50 %, respectively). In contrast, an inconsistent response to low flow (vasoconstriction, dilation or no change) was observed on an individual level. Cohen's Kappa revealed poor agreement for classifying the L-FMC measurement between visits (k=0.04, P>0.05). Assessment of the actual vessel diameter was robust with a coefficient of variation of 1.7 % (baseline and peak) and 2.7 % (low-flow). The between-day correlation coefficient between measures was r=0.18, r=0.96 and r=0.52 for L-FMC, FMD and CVR, respectively. No significant correlation between FMD and L-FMC was observed for either visit (r=-0.06 and r=-0.07, respectively; P>0.05).

Conclusion: In adolescents, the low-flow vasoreactivity is inconsistent between days. Whereas the actual vessel diameter is reproducible, the measurement of L-FMC and CVR has

poor between-day reliability compared to FMD. Finally, L-FMC and FMD are not significantly correlated.

Keywords: vascular function, FMD, L-FMC, low-flow-mediated constriction, repeatability

INTRODUCTION

Cardiovascular disease (CVD) is the major cause of non-communicable deaths worldwide [1]. Although the clinical implications of CVD are not evident until later adulthood, its origins can be found in childhood [2]. Endothelial dysfunction is the initial stage in the pathophysiology of atherosclerosis [3] and can be assessed non-invasively by flow-mediated dilation (FMD), typically performed at the brachial artery [4]. The FMD technique is both accurate and reproducible [5] and guidelines of best practice are available [6,7]. Briefly, the measurement of baseline artery diameter is followed by a 5 min period of cuff-induced local ischaemia. During this ischaemic time span, blood flow through the vessel is low followed by a period of high flow when the cuff is released. The peak arterial diameter post-occlusion is compared to baseline diameter and the change typically expressed as a percentage. The FMD response, which is nitric oxide (NO) dependent [8], has been subject to many investigations in children and adolescents, such as establishing endothelial function in children at risk of CVD [4] or the benefits of exercise [9,10].

The vasoreactivity to the low-flow condition during the cuff-occlusion phase of the FMD protocol has recently been subject to some investigation. When examined at the radial artery, studies consistently reported vasoconstriction during cuff-occlusion [11,12] which led to the term 'low-flow-mediated constriction' (L-FMC). It has been suggested that low-flow vasoreactivity is complementary to the traditional FMD measure as it enhances prognostic value [12,13]. Furthermore, measuring L-FMC may provide additional mechanistic insight of endothelial function as it is NO independent [12]. Finally, the combination of FMD and L-FMC

to create a vasoactive range (composite vessel reactivity, CVR) may aid to establish a more comprehensive image of vascular health [12].

Gori, et al. [14] described good repeatability of the L-FMC measurement on the radial artery (intraclass correlation coefficient (ICC) of 0.8) in 25 healthy young adults. In contrast to the radial artery, there is no homogeneous vasoreactivity to the low-flow condition in the brachial artery, with reports of vasodilation, vasoconstriction and no alteration [13,15-17]. Bell, et al. [18] reported good between-day reliability of the L-FMC measurement in the brachial artery of adults (ICC of 0.87). Additionally, Aizawa, et al. [15] reported a significant association between L-FMC and FMD in adults, suggesting that low-flow vasoreactivity contributes to the magnitude of the FMD response. In children, only Thijssen, et al. [19] have investigated L-FMC and found a significant[WC1], yet small (~ 0.04 mm), increase of the brachial artery diameter when compared to baseline. However, the low-flow vasodilation was only reported as a group mean and inter-individual differences were not presented. Furthermore, whereas the FMD measurement is reliable in adolescents [20], no previous study has assessed whether L-FMC of the brachial artery is reliable between days or examined its relationship with FMD in adolescents.

The aims of the study were to address the following in an adolescent population: 1) to describe the vasoreactivity to low flow at the brachial artery and to document the day-to-day reliability of L-FMC; and 2) to characterise the magnitude of the relationship between L-FMC and FMD.

METHODS

Participants

The data of the current investigation were obtained retrospectively from previous work (reference[wc2]) and reanalysed statistically. An analysis of the low-flow data was not presented in previous publications. The original sample comprised 40 participants but 13 participants were excluded from analysis due to poor image quality or movement during the low flow period. Therefore, relevant data for the current investigation were available on twenty-seven 12- to 15-year-old adolescents (twelve boys). The original investigations were approved by the institutional ethics committee and both participants and their parents provided written informed assent and consent, respectively, before commencement of the studies. Exclusion criteria involved the use of any medication or substance known to influence vascular function.

Description and reliability of low-flow vasoreactivity

On the first visit to the laboratory, body mass and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, before participants were familiarized to all measurements. For the assessment of their fitness, participants performed a combined ramp and supramaximal exercise protocol [21] in order to determine maximal oxygen uptake ($\dot{V}O_{2max}$). Pulmonary $\dot{V}O_2$ was monitored throughout the test (Cortex Metalyzer III B, Leipzig, Germany). All exercise was performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Definitions of low fitness and overweight/obesity were made based on age- and sex-appropriate $\dot{V}O_{2max}$ [22] and body mass index (BMI) [23] cut points, respectively. Participants' pubertal status was determined by a self-assessment of secondary sexual characteristics using adapted drawings of the five stages of pubic hair development [24].

On two occasions separated by approximately one week, participants were transported to the laboratory at 08:00 h following a ~ 12 h overnight fast and then consumed 30 g of commercially available corn flakes with 130 mL of skimmed milk. The macronutrient contribution of this breakfast is unlikely to have influenced endothelial function [25]. At 08:45 h, participants rested in a darkened, temperature-controlled room (24° C) for 10 min before the assessment of vascular function.

Vascular assessment

High-resolution Doppler and B-mode images of the brachial artery were simultaneously acquired (Sequoia 512; Acuson; Siemens Corp, Aspen, CO, USA) with a 13 MHz linear array transducer in duplex mode, in accordance with recent guidelines [7] and our earlier work (reference). Following a 10 min acclimatization period to the temperature-controlled room (24° C) in the supine position, baseline arterial diameter was measured for 1.5 min. Low-flow brachial artery diameter was measured during the last 30 s of a 5 min ischaemic stimulus [11,15] induced by rapid forearm pneumatic cuff inflation (Hokanson, Bellevue, WA, USA) to 220 mmHg. Endothelium-dependent vasodilation of the brachial artery was measured for 3 min after the 5 min occlusion period. Baseline, low-flow and post-occlusion brachial artery diameters were assessed during end diastole using validated ECG-gating software (Medical Imaging Applications LLC, Coralville, IA, USA) [7,26]. All analyses were performed by the same investigator. The estimated shear rate for the low-flow period was calculated by averaging shear during the last 30 s of cuff occlusion. The area under the curve for estimated shear rate for FMD was calculated from the time of cuff deflation until peak dilation (SR_{AUC}) [7].

Mean arterial diameter over 1.5 min before cuff-occlusion (baseline) and its associated 95 % confidence intervals (CI) were determined in order to classify L-FMC. L-FMC was calculated as mean diameter over the last 30 s of the low-flow period and defined as vasoconstriction (diameter < lower CI of mean baseline diameter), no response (diameter within CI of mean baseline diameter), and vasodilation (diameter > upper CI of mean baseline diameter). FMD, L-FMC, and CVR were calculated using the following equations:

FMD (%) = (Peak post-occlusion diameter - Mean baseline diameter) / (Mean baseline diameter) x 100%

L-FMC (%) = (Mean diameter during last 30 s of occlusion - Mean baseline diameter) / (Mean baseline diameter) x 100%

CVR (%) = (Peak post-occlusion diameter - mean diameter during last 30 s of occlusion) / (Mean baseline diameter) x 100%

Control for confounding variables

With parental supervision, participants were asked to replicate their evening meal prior to each laboratory visit. Furthermore, they also completed a food diary during the 48 h period immediately preceding each visit, which were subsequently assessed for total energy and macronutrient intake (CompEat Pro; Nutrition Systems, Banbury, UK). Participants were also instructed to avoid strenuous exercise and wear a triaxial accelerometer on the wrist of their non-dominant hand (GENEActiv; Activinsights Ltd, Cambridge, UK) during the 48 h prior to each visit. Time spent performing moderate-to-vigorous physical activity was determined using validated cut points for paediatric groups [27].

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159 **Statistical analyses**

160 All data are presented as mean and standard deviation (SD) unless otherwise stated. Given
161 the recent suggestion of adjusting FMD allometrically for baseline diameter [28], Pearson's
162 correlation coefficient (*r*) was applied to examine the relationship between both FMD and L-
163 FMC and baseline diameter. However, as there were no significant correlations between
164 FMD and baseline diameter (r = -0.06 and r = 0.01, both $P > 0.7$) or L-FMC and baseline
165 diameter (r = 0.01 and r = 0.1, ~~all~~ both $P > 0.6$), allometric scaling was not undertaken.
166 Descriptive statistics and Cohen's Kappa were employed to analyse the day-to-day reliability
167 of the vasoreactivity (i.e. classified as vasoconstriction, vasodilation and no response) to the
168 low-flow condition. The magnitude of agreement was classified according to Fleiss [29] with k
169 > 0.75 as excellent, k between 0.40 and 0.75 as fair to good and $k < 0.40$ as poor agreement.
170 The reliability of the vascular measurements was examined using the typical error (TE), the
171 TE expressed as a coefficient of variation (CV) and the ICC [30]. Pearson's correlation
172 coefficient (*r*) was employed for the analysis of the relationship between L-FMC and FMD.
173 Statistical significance was accepted when $P < 0.05$. IBM SPSS Statistics software (Version
174 22; IBM Corporation, Armonk, NY) was used for all statistical analyses.

175

176 **RESULTS**

177 Characteristics for participants (n = 27) are presented in Table 1. Maturation status for boys
178 and girls was as follows: Tanner stage 2, n = 1 and 0, stage 3, n = 6 and 1, stage 4, n = 3 and
179 11, stage 5, n = 2 and 3, respectively. No significant mean differences in total energy intake,

individual macronutrient contribution, or time spent performing moderate-to-vigorous physical activity were apparent during the 48 h preceding each visit (all $P > 0.05$, data not reported).

The reproducibility of macrovascular outcomes is illustrated in Table 2. The average response on visit 1 was vasoconstriction (-0.59 ± 2.22 % of baseline diameter), which was observed in 15 participants (56.6 %). Vasodilation was apparent in eight participants (29.6 %), and four (14.8 %) did not show any response to the low-flow condition. On the second visit, participants demonstrated on average vasoconstriction (-0.16 ± 1.50 % of baseline diameter). In contrast to visit 1, 12 participants (44.4 %) showed vasoconstriction on the second visit, while vasodilation was exhibited by 10 participants (37.0 %). No alteration in vasoreactivity during cuff inflation was apparent in 5 participants (18.5 %). Eleven participants (40.7 %) presented the same low-flow vasoreactivity response on both visits. Cohen's Kappa revealed a poor agreement for the classification of L-FMC between measurements ($k = 0.04$, $P = 0.79$). Average shear rate during the low-flow period was 191.3 ± 71.1 $cm \cdot s^{-1}$ (visit 1) and 201.4 ± 96.9 $cm \cdot s^{-1}$ (visit 2), respectively. SR_{AUC} was 739.9 ± 277.4 (visit 1) and 674.7 ± 209.3 (visit 2), respectively. There was no significant correlation between L-FMC and FMD on visit 1 ($r = -0.06$, $P = 0.75$) or on visit 2 ($r = -0.07$, $P = 0.72$) (Figure 1).

DISCUSSION

The current investigation is the first study to show that the average L-FMC response in the brachial artery in an adolescent population is vasoconstriction. However, the response is variable between participants and its subsequent classification into 'vasoconstriction', 'no response' or 'vasodilation' is not reliable between days. Compared to FMD and CVR, the

measurement of L-FMC also has a poorer reliability in adolescents. Nevertheless, this study supports the view that the measurement of L-FMC may add complementary information to the FMD measurement due to the lack of a significant correlation between them.

Previous studies have shown that the vascular response to low flow is artery specific [17] and the reactivity in the brachial artery is non-uniform in adults [13,15,16]. The only study concerned with low-flow vasoreactivity in a paediatric population reported vasodilation (~ 0.04 mm) of the brachial artery in children (9 – 10 y) [19], however, individual responses were not reported. In the current study, vasoconstriction was apparent on both visits (-0.59 ± 2.22 % and -0.16 ± 1.50 %, respectively) but an inconsistent reactivity to low flow was observed. Across visits 1 and 2, vasoconstriction during low flow was apparent in the majority of participants (56.6 % and 44.4 %, respectively), followed by vasodilation (29.6 % and 37.0 %, respectively) and no response (14.8 % and 18.5 %, respectively).

Gori, et al. [14] measured L-FMC in the radial artery on two separate occasions with ≥ 24 h between assessments and reported good reproducibility of the measurement with an ICC of 0.80. With regards to the brachial artery, Spiro, et al. [13] investigated the within-day (2 h apart) reproducibility of L-FMC and FMD and found no significant differences in healthy young volunteers, concluding that L-FMC can be measured reliably. Furthermore, Bell, et al. [18] reported an ICC of 0.87 for L-FMC between days in their laboratory, however, within a very small sample size (n = 5). These results concur with our findings in the brachial artery of adolescents showing no significant mean differences (i.e. absence of an order effect) in L-FMC, FMD and CVR between-days. A possible explanation for this finding could ~~be seen in~~ the different methodological approaches regarding the assessment of reliability. The analysis by Spiro, et al. [13] is limited to a mean difference only and did not take into account ~~the~~ within-subject variation. The small sample size in the study by Bell, et al. [18] may also act to inflate the ICC, especially for a heterogeneous sample. From a physiological perspective, a

possible explanation could be the age-related difference in arterial wall features, in particular the previously reported increase in arterial stiffness with advancing age [19,31,32]. Furthermore, the general decrease in endothelial function with age [19,33] might also be considered to explain the difference between adults and adolescents.

The measurement of the actual vessel diameter was robust with a CV of 1.7 % (baseline and peak diameter) and 2.7 % (low-flow diameter), respectively. However, when expressed as a change compared to baseline, L-FMC has inferior reliability compared to FMD and CVR. Due to the vasoconstrictive response on average to low-flow, a loglinear transformation for the calculation of a CV for L-FMC was not possible. However, the absolute TE for L-FMC was almost five times higher than that for FMD (1.74 % vs 0.36 %). Furthermore, the CV of 28.8 % for the CVR suggests larger variation in L-FMC considering that CVR is the sum of the absolute values of L-FMC and FMD, and the CV for FMD was only 5 %. However, the CV of CVR is consistent with previous FMD guidelines which stated that a CV of 20-30 % for FMD is a satisfactory level of repeatability [6]. Finally, there was a very strong correlation between the two FMD measurements (ICC = 0.95) in the current study whereas the correlation for L-FMC between days was weak (ICC = 0.17), resulting in a moderate correlation for CVR (ICC = 0.52). The inferior reproducibility of L-FMC may be due to the small magnitude of change, either positive or negative, in artery diameter during low flow and consequently presents considerable variation in L-FMC. In conclusion, despite excellent repeatability of the measurement of the low-flow diameter, the L-FMC measurement itself has poor reproducibility between-days in adolescents when compared to FMD and CVR. A practical consequence is that larger sample sizes will be needed in order to identify changes in the mean between conditions due to the greater noise caused by the large variation when contrasted to FMD and CVR.

Despite previous reports of non-uniform reactivity to low flow in the brachial artery [15,16,34] no study has explored whether the classification into 'vasoconstriction', 'vasodilation' and 'no response' is reliable. Harrison, et al. [16] reported a wide variation for L-FMC in healthy adults and adults with risk factors for coronary artery disease from -5.6 to 5.0 %. They concluded that the individual response to low flow 'cannot be assumed to remain unchanged' [16] but did not discuss this further. We showed that almost 60 % of the adolescent participants presented different responses to low flow and agreement between-days was poor [29]. As a consequence, our data show poor reliability of the categorisation of the low-flow response on a day-to-day basis. This inconsistent classification likely contributed to the poorer reliability of L-FMC compared to FMD.

While FMD measures the ability of the endothelium to recruit or stimulate vasomotor function following an increase in shear stress, only the L-FMC can measure the vascular response at rest, i.e. reduced shear stress [12]. The two different measurements have been proposed to complement each other to provide an extensive overview of vasomotor function [12]. We did not find any significant correlation between L-FMC and FMD either on the first or the second visit, which is in agreement with the results of Gori, et al. [14] using the radial artery and in patients with coronary atherosclerosis using the brachial artery [13]. These findings are likely to reflect observations that the measurement of L-FMC alongside FMD enhances prognostic value [12,13] and provides insight into NO-independent mechanisms of endothelial function. In contrast, others who measured L-FMC in the brachial artery reported a significant but weak to moderate correlations between L-FMC and FMD in healthy older adults (r = 0.41), those with increased CVD risk (r = 0.19) [15] or adults varying in age and coronary artery risk factors (r = 0.41) [16]. However, the sample population in the aforementioned studies differed significantly from the participants in the present study in terms of age and health status. Another study has found a~~n~~ significant inverse correlation

between L-FMC and FMD in which FMD increased with larger L-FMC in healthy adults [13]. However, the sample size in that study was ~~relatively~~ small (n = 10) and it appears that the direction of this correlation was caused by two of the participants[WC3].

Conclusion

On average, adolescents demonstrate vasoconstriction at the brachial artery during low flow. However, on an individual level adolescents present vasoconstriction, vasodilation or no change and these individual responses are not consistent between days. While the measurement of the vessel diameter in the low-flow condition has high reproducibility, the between-days assessment of L-FMC has poor reproducibility compared to FMD and CVR. No significant correlation was observed between L-FMC and FMD showing~~suggesting~~ the former provides complementary information about vascular endothelial function. However, the poorer reliability of L-FMC compared to FMD and CVR indicates that larger samples sizes will be needed to detect a given effect[WC4], at least in adolescents.

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397 **Table 1.** Participant characteristics.

	Participants (n = 27)
Age (years)	14.3 ± 0.6
Body mass (kg)	56.0 ± 11.0
Height (m)	1.64 ± 0.09
BMI ($kg \cdot m^{-2}$)	20.7 ± 2.4
Overweight (n (%))	2 (7%)
$\dot{V}O_{2\ max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	41.2 ± 6.7
Low fit (n (%))	10 (37%)

398 BMI, body mass index; $\dot{V}O_{2\ max}$, maximal oxygen uptake. Data are presented as mean ± SD.

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Table 2. Reproducibility of macrovascular measurements.

	Visit 1 Mean ± SD	Visit 2 Mean ± SD	Change in mean	*P* value	Typical error	Typical error as CV (%)	ICC
Baseline diameter (mm)	3.17 ± 0.35	3.18 ± 0.36	0.01	0.61	0.06	1.7	0.98*
Low-flow diameter (mm)	3.15 ± 0.35	3.17 ± 0.37	0.02	0.34	0.09	2.7	0.94*
Peak diameter (mm)	3.44 ± 0.38	3.44 ± 0.40	0.01	0.73	0.06	1.7	0.98*
FMD (%)	8.42 ± 1.51	8.34 ± 1.68	-0.09	0.39	0.36	5.0	0.95*
L-FMC (%)	-0.59 ± 2.22	-0.16 ± 1.50	0.43	0.37	1.74	#	0.17
CVR (%)	9.02 ± 2.75	8.51 ± 2.34	-0.51	0.31	1.80	28.8	0.52*

CV, coefficient of variation; ICC, intraclass correlation coefficient; FMD, flow-mediated dilation; L-FMC, low-flow-mediated constriction; CVR, composite vessel reactivity; # Negative values did not allow a loglinear transformation for the calculation of the typical error as CV (%); * significant correlation, $P < 0.01$

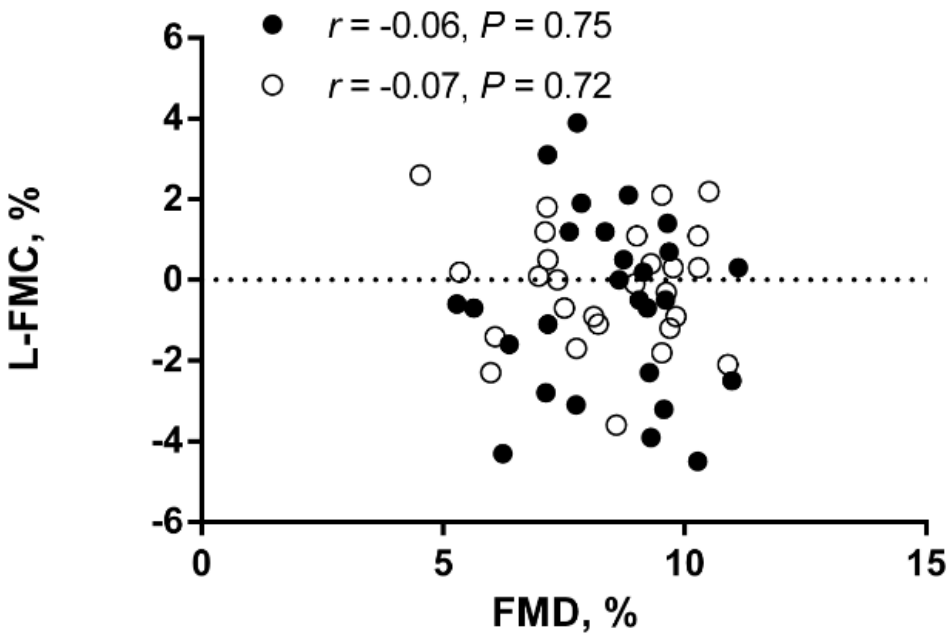

Figure 1. Correlation between flow-mediated dilation (FMD) and low-flow-mediated constriction (L-FMC) on visit 1 (●) and visit 2 (○). The lines of best fit are emitted for clarity.