J Appl Physiol 141: 578–590, 2026.
First published July 13, 2026; doi:10.1152/japplphysiol.00065.2026

american physiological society | JOURNAL OF APPLIED PHYSIOLOGY

RESEARCH ARTICLE

Women's Health Research and Novel Perspectives on Sex as an Investigative Variable

Limb- and sex-specific determinants of vascular function assessed by flow-mediated dilation and passive limb movement

Elisa Fioraso,[1,2] Anna Pedrinolla,[2,3] Jillian M. Lang,[4] Elena S. Shostak,[4] Stephen J. Ives,[4] and Massimo Venturelli[1,5]

[1]*Department of Neuroscience, Biomedicine and Movement Science, University of Verona, Verona, Italy;* [2]*Center of Research for Mountain, Sport and Health—CeRiSM, University of Verona and University of Trento, Rovereto, Italy;* [3]*Department of Psychology and Cognitive Sciences—DIPSCO, University of Trento, Trento, Italy;* [4]*Health and Human Physiological Sciences, Skidmore College, Saratoga Springs, New York, United States; and* [5]*Center on Aging, University of Utah, Salt Lake City, Utah, United States*

Abstract

Assessing regional vascular function is crucial for understanding systemic cardiovascular health. This study primarily investigated whether flow-mediated dilation (FMD) and passive limb movement (PLM) yield congruent, interchangeable patterns across the upper limbs (ULs) and lower limbs (LLs). Secondarily, we evaluated sex-specific influences on these regional vascular relationships. Twenty healthy individuals (10 males and 10 females) underwent brachial and popliteal FMD, alongside axillary and femoral PLM, in a counterbalanced single session. Baseline diameters, FMD%, allometrically scaled FMD, and blood flow (BF) PLM parameters [peakBF, ΔpeakBF, area under the curve (AUC)] were analyzed. Regarding our primary aim, FMD% was significantly higher in UL than LL (7.7 ± 2.9% vs. 4.9 ± 1.8%; $P = 0.002$) and was not correlated between limbs ($r = 0.11$, $P = 0.650$). However, when analyzed using an allometric mixed model with baseline diameter as a covariate, this limb difference was no longer significant ($P = 0.334$). PLM ΔpeakBF was lower in UL than LL (ΔUL − LL = −68.3%; $P < 0.001$) but showed significant interlimb correlations ($r = 0.50$, $P = 0.024$). No limb × sex interaction was found for FMD% ($P = 0.889$) or allometric FMD% ($P = 0.815$). However, a significant limb × sex interaction emerged for PLM AUC ($P < 0.001$), driven by a higher absolute AUC in the LL for males ($P < 0.001$) and a higher volume-normalized AUC in the UL for females ($P = 0.003$). In conclusion, brachial and popliteal FMD are not interchangeable, providing complementary, region-specific information heavily influenced by vessel size, and FMD% is not affected by sex. PLM_{UL} and PLM_{LL} provide partially convergent but not equivalent information, warranting caution when comparing limbs, particularly when sex is a variable of interest.

NEW & NOTEWORTHY This study directly compares flow-mediated dilation and passive limb movement across upper and lower limbs, providing novel insight into limb-specific vascular function. Flow-mediated dilation and passive limb movement are not interchangeable between limbs, impacting future experimental and clinical evaluation. Vascular function differs between sexes for passive limb movement, highlighting the need to consider sex-specific effects in study design.

endothelial function; limb specificity; sex differences; vascular function

INTRODUCTION

Cardiovascular disease (CVD) is the main cause of morbidity and mortality worldwide, and multiple risk factors, such as hypertension, diabetes, obesity, dyslipidemia, tobacco smoking, and sedentary lifestyle, are known for their negative effects on cardiovascular health (1). The vascular system is a complex network of structures that respond acutely and chronically to hemodynamic stimuli, influencing vascular function and cardiovascular disease risk (2). The endothelium, which is the internal part of the vascular wall, is recognized to have a fundamental role in the regulation of vascular function (3). Endothelial function is strongly influenced by lifestyle factors, including physical activity, diet, and smoking habits (4, 5). The endothelium produces antiatherosclerotic substances, like nitric oxide (NO), which have a protective role in the cardiovascular system (3). Therefore, assessment of endothelial function may facilitate the early identification of preclinical CVD in individuals without overt disease, where promoting lifestyle changes may prevent future cardiovascular events.

Based on this premise, two noninvasive tests assess vascular function by quantifying vasodilatory capacity and hyperemic

Correspondence: A. Pedrinolla (anna.pedrinolla@unitn.it).
Submitted 21 January 2026 / Revised 23 June 2026 / Accepted 8 July 2026

responses in the peripheral circulation to predict CVD risk before clinical presentation (6). The first test, flow-mediated dilation (FMD), is an endothelium-dependent test that is widely used to assess vascular function (7–9). Typically performed on the brachial artery, it is considered the gold standard for vascular function assessment in both clinical and preclinical or research settings (10). The popliteal FMD, though less commonly used than brachial FMD, is performed to assess vascular function in the lower limb. Another noninvasive test of vascular function is passive limb movement (PLM), which assesses microvascular function, representing a different aspect compared with FMD, and is commonly performed in the lower limb (11–13). PLM-induced hyperemia is largely (~80%) nitric oxide (NO) dependent (12, 14). In contrast, FMD primarily reflects macrovascular (conduit artery) endothelial function and appears to be influenced by factors beyond NO bioavailability alone, notably the magnitude and temporal profile of the shear stimulus produced by the reactive hyperemia that follows cuff release (15). Both tests are widely used in research settings to investigate vascular function (9, 12, 14); however, their interchangeability between upper limb and lower limb remains unclear. In addition, in some individuals, the application of the tests may be unfeasible due to health-related, anatomical (e.g., limb amputation, brachial artery bifurcation, or surgically induced arteriovenous anastomoses such as dialysis fistulae), or individual limitations. In such cases, the availability of a valid alternative—namely, assessing the upper limb instead of the lower limb, or vice versa—becomes essential.

Another important factor to consider in vascular assessments is the possible effect of sex. Studies have shown that the risk of CVD is lower in females compared to males, at least in the premenopausal years (16). Specifically, estrogens in females provide a protective effect on the vascular system, lowering CVD risk in premenopausal females (17). In line with this, the literature suggests that males and females exhibit different results in FMD, with females generally showing better vascular function despite the potential influence of fluctuating hormone levels during the menstrual cycle (18, 19). This female advantage is primarily attributed to the protective effect of estrogens on the vascular endothelium (20). However, sex-related differences in FMD also appear to be region-specific: although consistently reported in the brachial artery, evidence in the lower limb is more limited and partly conflicting, with some studies suggesting that an age-related divergence between upper- and lower-limb endothelial function emerges in males but not in females (21). Evidence regarding sex differences in passive limb movement (PLM) is more limited. In the lower limb, comparable PLM responses have been reported between premenopausal females and males independently of menstrual cycle phase, whereas emerging evidence suggests that sex differences may be more pronounced in the upper-limb microvasculature (22). Notably, the association between upper- and lower-limb vascular function, previously described in young males, does not appear to hold in young females (23), suggesting that sex-related differences may emerge specifically when both regions are concurrently evaluated.

A further aspect that warrants consideration is that vascular function is not uniform across the arterial tree, and FMD responses may differ between upper- and lower-limb conduit arteries. Although brachial artery FMD is widely considered the gold-standard noninvasive marker of systemic endothelial function and a strong predictor of cardiovascular events (7), it does not necessarily reflect endothelial function in the lower limb. In healthy young adults, the magnitude of FMD in upper-limb conduit arteries is not predictive of FMD in lower-limb arteries, suggesting that the two regions may be regulated by partially independent mechanisms, possibly related to differences in local hemodynamics and arterial structure (24). Consistently, vascular adaptations and responses are region-specific across the arterial tree, with different conduit arteries showing distinct patterns of function and remodeling (25). Despite this evidence, direct upper- versus lower-limb comparisons within the same individuals—particularly combining macrovascular (FMD) and microvascular (PLM) assessments—remain scarce. Thus, understanding the interplay of region specificity and sex in these assessments of vascular function is critical to clarify whether they provide complementary or redundant information on endothelial health.

To enhance understanding of the limb specificity of FMD and PLM as assessments of vascular function, the primary aim of this study was to compare FMD and PLM responses between the upper and lower limbs. A secondary aim was to examine the influence of sex on FMD and PLM responses. As an additional, exploratory analysis, we also compared the two tests (FMD and PLM) within the same limb, since these macro- and microvascular assessments are typically compared across different limbs rather than within the same region. We hypothesized that, despite known region-specific differences in vascular regulation, outcomes obtained from the same test would show a degree of concordance between limbs within individuals. In addition, based on the protective vascular effect of estrogens, we hypothesized that females would exhibit superior vascular function compared with males, with potentially region-specific differences between upper and lower limbs.

METHODS

Participants and General Procedures

To be included in this study protocol, participants must have been healthy and between 18 and 35 yr. All participants were screened and determined to be free of acute and chronic disease. The healthy status and sex of the participants were assessed using a self-reported medical history. The exclusion criteria were cardiovascular, pulmonary, metabolic or neural disease, current or recent smokers, use of hormonal methods for birth control, or pregnancy. All female participants were premenopausal and were tested within 7 days after the start of their menstrual cycle (11, 26). Participants were asked to avoid caffeine and physical activity during the day preceding the experimental visit and refrain from eating at least 4 h before the visit. All participants gave written informed consent after a complete explanation of the study's experimental procedure. The local institutional review board reviewed and approved this protocol (IRB No. 00038036). All the procedures

conformed to the most recent revisions to the Declaration of Helsinki standards.

Experimental Design

When the participants arrived at the laboratory, height was assessed with the stadiometer (Seca, Mt. Pleasant, SC) and weight with the scale (RD-545, Tanita, Arlington Heights, IL). Participants were then asked to rest quietly for 10 min. The order of the vascular function tests (FMD and PLM) and limb (upper and lower extremities) was counterbalanced to avoid possible biases based on test order (Fig. 1). There were 15 min of rest between each test. All participants, both male and female, were tested within the same time window (9:00 AM to 1:00 PM) to avoid potential effects of the time of day on vascular function (27).

Mean Arterial Pressure and Body Mass Index

As part of the self-reported medical history, participants provided systolic and diastolic blood pressure, body weight, and height, from which mean arterial pressure (MAP) and body mass index (BMI) were calculated. These values were then compared with laboratory measurements to confirm the self-reported medical history and verify that participants were in good health.

Mean arterial pressure (MAP) was continuously recorded throughout the testing session using a Finapres NOVA (Finapres Medical Systems, Amsterdam, The Netherlands). The data were exported in .txt format and subsequently analyzed offline.

BMI was calculated using the standard formula (28):

$$\mathrm{BMI} = \frac{\mathrm{weight\,(kg)}}{\mathrm{height(m)}^2}$$

Flow-Mediated Dilation

The FMD on the brachial artery in the arm ($\mathrm{FMD_{UL}}$) was performed using a duplex ultrasound system (LogiQ-7, GE Medical Systems, Milwaukee, WI). Testing was performed in a quiet room, on the right arm, with the participant in a supine position, following the published guidelines (26). A linear probe with a 4–12 MHz frequency range was used to imagine the artery and record blood velocities. A blood pressure cuff was placed distal to the probe on the forearm, near the elbow. Once the participant had been instrumented and was in a resting state for at least 15 min, 30 s of baseline were recorded. Then, 5 min of occlusion was performed at a suprasystolic cuff pressure (220 mmHg). The 2 min after the cuff release was recorded, following the guidelines (26, 29). The diameter and blood velocity were measured at baseline and continuously for 2 min after the cuff release. The same protocol was done for the FMD on the popliteal artery in the leg ($\mathrm{FMD_{LL}}$). The cuff was placed distal to the probe, the same one used for the FMD on the brachial artery and was put behind the knee on the popliteal artery.

Brachial artery images and blood flow velocity were recorded for 2 min after cuff release. During baseline and cuff release, images were sent in real time to off-line software and later analyzed using automated edge detection analysis. To minimize investigator bias and eliminate interobserver variability, all subsequent analyses were performed by a single, experienced investigator using an objective approach (Brachial Analyzer, Medical Imaging Applications, Coralville, IA). Diameters were then averaged every 4 s for the first 20 s after the cuff release and every 10 s for the remaining 2 min. The diameters were measured as the distance (mm) between the deep and superficial intimamedial borders of the vessel's walls. The dilation is expressed as a percent change of the peak diameter in relation to the baseline. It was calculated with the following formula (26):

$$\mathrm{FMD(\%)} = (\mathrm{peak\,diameter} - \mathrm{baseline\,diameter}) / \mathrm{baseline\,diameter} \times 100$$

To reduce the influence of vessel caliber on the vasodilatory response, an allometric scaling approach was applied to the peak diameter data, based on the model (30)

$$D_{\mathrm{peak}} = \mathrm{a} \times D_{\mathrm{base}}^{\mathrm{b}}$$

where D_{peak} is the peak arterial diameter, D_{base} is the baseline diameter, a is a constant, and b is the allometric scaling exponent describing the relationship between baseline and peak diameter (30). Details of the statistical implementation are provided in *Statistical Analysis*.

The time to peak (TTP) was measured as the time the vessel needed to reach peak vasodilation and is expressed in seconds.

The shear rate identified as the integral of the shear rate over time and it is usually identified as the area under

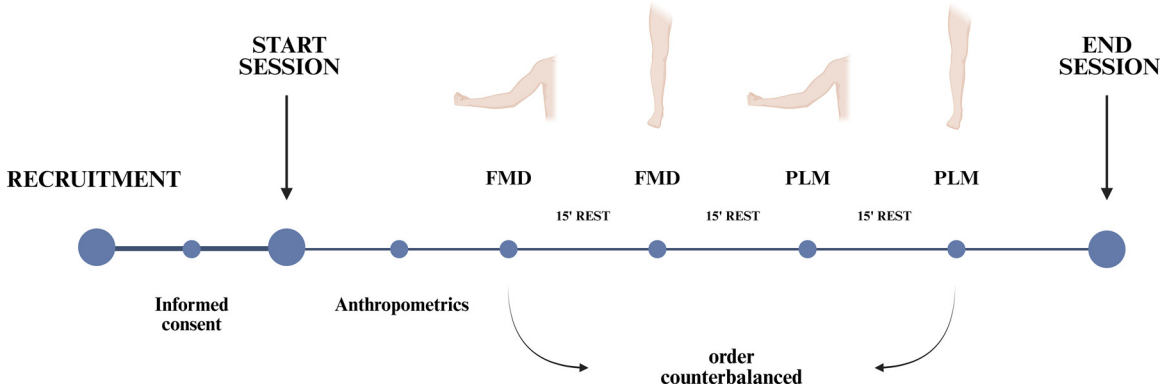

Figure 1. Experimental design.

the curve (AUC). It was calculated using the trapezoidal rule (26):

$$\sum \left[yi(x(i+1) - x(i)) + (1/2)(y(i+1) - y(i))(x(i+1) - x(i)) \right]$$

where x is time, y is shear, x_i is the initial point, and y_i is the initial blood velocity.

Passive Limb Movement

The PLM was performed on the lower limb following the published guidelines (11). The PLM was done on the femoral artery (PLM_{LL}) with the participant in the semiupright position, with support for the legs. We used the same ultrasound system that was used for the previously described FMD. We performed the PLM using a range of motion from an extended position (180°) to a 90° flexed position. After 15 min of rest, with the participant in a resting condition, 30 s of baseline were recorded, measuring the diameter of the vessel and the intensity-weighted mean blood velocity. After this we started with the passive movement at a frequency of 1 Hz while recording and measuring the mean blood velocity until the end of the minute.

The passive limb movement was also performed on the upper limb (PLM_{UL}) with the same protocol used for the lower limb. The participant was lying in a supine position with the arm abducted and placed on a lateral table. The range of motion utilized for the PLM_{UL} was from fully extended to 90° flexed position. The probe was placed over the axillary artery (AA) to reduce the probe movement caused by the passive movement of the muscle mass. The axillary artery was specifically selected for the upper-limb passive limb movement (PLM) protocol to establish a methodologically sound, structurally matched comparison with the lower-limb protocol (femoral artery). Unlike the distal brachial artery, the axillary artery offers a larger baseline vessel caliber that more closely approximates the dimensions of the lower-limb conduit vessels, thereby minimizing geometry-dependent confounding factors. Furthermore, measuring blood flow at the axillary level mirrors the lower-limb setup by positioning the ultrasound sample volume immediately proximal to the primary axis of rotation (the shoulder joint) (31, 32). This ensures that the primary hyperemic response feeding the active muscle beds of the entire moving limb is accurately captured, in accordance with established regional hemodynamic assessment models (12, 33).

The mean blood velocity (Vmean) was analyzed on a Doppler ultrasound system (GE LogiQ-7 ultrasound system, GE Medical Systems, Milwaukee, WI) for 30 s at rest and second by second for the next 60 s of passive movement. Resting arterial diameter and resting mean blood velocity were determined to calculate the blood flow at baseline. The arterial diameter was measured as distance (mm) between the intimalumen interfaces of the vessel's walls; 3 measures were taken during baseline and every 2 s during the 60 s of PLM. The blood flow was calculated with the following formula (11):

$$\text{Blood flow(mL/min)} = \text{Vmean} \times \pi \times (\text{vessel diameter}/2)^2 \times 60.$$

The peak blood flow was the maximum value of BF reached during the 60 s of PLM. The relative change in blood flow (Δ peak) was calculated by subtracting basal blood flow from the peak blood flow. The area under the curve (AUC) was calculated for 60 s using the trapezoidal rule, according to the following equation (26):

$$\sum \left[yi(x(i+1) - x(i)) + (1/2)(y(i+1) - y(i))(x(i+1) - x(i)) \right]$$

Anthropometrics

Anthropometric measurements were taken on subjects to estimate the limb volume of the leg and arm. Three measures were taken: the limb length (joint to joint), three circumferences for each segment (proximal, middle, and distal), and skinfold thickness at the middle distance (34). Limb volumes (LVs) were estimated with the following formulas previously used in published works (35):

$$\text{Limb Volume} = (L/12_{\Pi}) \times \left(C1^2 + C2^2 + C3^2\right) - \left[(S - 0.4)/2\right] \times L \times C1 + C2 + C3)]/3]$$

where L refers to the length; $C1$, $C2$, and $C3$ refer to the proximal, middle, and distal circumferences, respectively, and were measured at the most proximal part of the limb, at half of its length, and at the most distal part. S represents the skinfold thickness of the limb. Measurements were taken for the thigh, lower leg, upper arm, and forearm.

Statistical Analysis

An a priori power analysis was performed using G*Power (Düsseldorf, Germany). For the primary objective of assessing inter-limb differences, a conservative within-subject framework assuming a large effect size (Cohen's $d = 0.8$), alpha = 0.05, and a power of 0.80 indicated a required sample size of 12 participants. For the secondary objective investigating sex-specific interactions, assuming an effect size $f =$ 0.4 for the interaction factor, a minimum of 16 participants was required to achieve a power of 0.80. Although these standard frameworks served as conservative estimates for sample size determination, the final analysis was executed using linear mixed models, which further enhance statistical power and precision by accounting for within-subject correlations and interindividual variance.

The statistical analyses were completed using IBM SPSS Statistics (version 32.0, IBM Corp., Armonk, NY). The Shapiro–Wilk test was used to evaluate whether the data were normally distributed. Data points differing by two standard deviations or more from the mean were identified as outliers and adjusted using the Winsorized Mean method; no data points were removed from the analyses. To assess the effects of sex, limb, and their interaction on the vascular and blood flow outcomes, a linear mixed model was used, with sex as a between-subjects factor, limb (UL vs. LL) as a within-subjects factor, and their interaction; sex and limb were included as fixed factors, and subject was entered as a random effect. Significant interactions were further examined using Bonferroni-corrected post hoc comparisons. In the absence of a significant limb × sex interaction, sex comparisons were additionally performed as exploratory analyses. To account for the influence of baseline diameter on the flow-mediated response, an allometric analysis was performed using a linear mixed model, following the approach proposed by Atkinson and Batterham (30). The difference

Table 1. *Participant characteristics*

Parameter	Male (n = 10)	Female (n = 10)	P Value
Age, yr	24.40 ± 3.10	22.80 ± 2.10	0.195
Height, m	1.80 ± 0.04	1.65 ± 0.07	**<0.001**
Weight, kg	75.56 ± 6.40	62.10 ± 6.09	**<0.001**
BMI, kg/m^2	23.39 ± 1.91	22.75 ± 1.67	0.434
Heart rate, beats/min	77.20 ± 8.40	68.50 ± 7.93	**<0.001**
Mean arterial pressure, mmHg	87.24 ± 6.41	82.06 ± 6.23	**0.015**

Bold values indicate statistically significant differences ($P < 0.05$).

between log-transformed peak diameter and log-transformed baseline diameter was entered as the dependent variable, log-transformed baseline diameter as a covariate, sex and limb as fixed factors, and subject as a random effect. The estimated marginal means generated by the model were back-transformed into a percentage-equivalent value by calculating the antilogarithm, subtracting 1, and multiplying by 100. To examine correlations between the tests, Pearson's correlation was used for normally distributed variables, whereas Spearman's rank correlation was used for variables that violated the assumption of normality (baseline diameter, peak diameter, shear rate, AUC, peak BF normalized for limb volume, and Δpeak BF normalized for limb volume). All data are expressed as means ± standard deviation unless noted otherwise. Alpha was set at 0.05.

RESULTS

Participant Characteristics

Twenty healthy Caucasian individuals were recruited for the study, including men ($n = 10$) and women ($n = 10$). The mean age was 24 ± 3 yr for males and 23 ± 2 yr for females. Mean height and body mass were 1.80 ± 0.05 m and 75.6 ± 6.8 kg for males, and 1.65 ± 0.06 m and 62.0 ± 6.1 kg for females, respectively (Table 1). All participants were healthy.

Limb Differences in FMD: FMD_{UL} versus FMD_{LL}

A linear mixed model was performed on baseline diameters and peak diameters data (Table 2). Regarding baseline diameters, an effect was found for sex ($P < 0.001$; $F = 18.4$) and for limb ($P < 0.001$; $F = 41.0$), but no interaction limb × sex ($P = 0.925$; $F = .01$). The peak diameter showed an effect for sex ($P < 0.001$; $F = 19.2$) and for limb ($P < 0.001$; $F = 41.5$), but no interaction sex × limb was found ($P = 0.981$; $F = .001$). The linear mixed model performed on TTP showed an effect for sex ($P = 0.037$; $F = 5.05$), but no effect for limb ($P = 0.669$; $F = .19$) or interaction limb × sex ($P = 0.179$; $F = 1.96$). The linear mixed model performed on FMD/shear rate showed an effect for limb ($P = 0.002$; $F = 13.35$), but no effect for sex ($P = 0.266$; $F = 1.32$) or the interaction limb × sex ($P = 0.515$; $F = 0.44$).

The linear mixed model, performed on FMD% results, shows a significant effect for limb ($P = 0.002$; $F = 11.7$; Fig. 2), but not for sex ($P = 0.766$; $F = 0.09$; Fig. 2) or interaction limb v sex ($P = 0.889$; $F = .1$). No correlation between FMD_{UL}% and FMD_{LL}% was found ($P = 0.650$). When peak diameter was analyzed using the allometric mixed model, with baseline diameter included as a covariate, no significant effect of sex ($P = 0.060$; $F = 3.9$), limb ($P = 0.334$; $F = .97$), or limb × sex interaction ($P = 0.815$; $F = .06$) was found. The linear mixed model analysis performed on shear rate showed no effects for sex ($P = 0.073$; $F = 3.6$), an effect was found for limb ($P < 0.001$; $F = 22.8$), but no interaction was found for limb × sex ($P = 0.175$; $F = 2.0$).

Table 2. *FMD and PLM outcomes in the upper and lower limb*

Parameter	Limb	Male	Female	P Value Between
Basal diameter, cm	UL	0.44 ± 0.04	0.34 ± 0.06	**<0.001**
	LL	0.58 ± 0.06	0.47 ± 0.11	**0.019**
Peak diameter, cm	UL	0.47 ± 0.04	0.36 ± 0.06	**<0.001**
	LL	0.61 ± 0.05	0.49 ± 0.11	**0.012**
FMD, %	UL	7.59 ± 2.51	7.46 ± 3.14	0.921
	LL	5.56 ± 1.84	4.81 ± 2.04	0.398
FMD adjusted	UL	7.56 (6.11–9.03)	5.91 (4.12–7.73)	0.940
	LL	6.88 (5.11–8.66)	4.93 (3.50–6.38)	0.514
TTP, s	UL	48.75 ± 9.91	39.00 ± 14.49	0.538
	LL	52.50 ± 10.35	37.00 ± 18.29	0.171
Shear rate AUC	UL	28551.38 ± 9763.52	45221.50 ± 28122.11	0.107
	LL	11657.62 ± 5714.79	14432.40 ± 11228.02	0.508
FMD/shear rate	UL	0.00037 ± 0.00040	0.00018 ± 0.00007	0.238
	LL	0.00057 ± 0.00030	0.00049 ± 0.00032	0.607
Peak blood flow, mL/min	UL	234.40 ± 83.87	189.90 ± 68.45	0.211
	LL	760.20 ± 118.70	610.20 ± 192.27	0.053
Δ Peak blood flow, mL/min	UL	105.60 ± 42.52	70.00 ± 55.36	0.125
	LL	528.80 ± 121.60	417.60 ± 93.84	**0.035**
AUC blood flow	UL	50.90 ± 48.96	100.20 ± 39.90	0.347
	LL	249.50 ± 162.85	80.90 ± 249.71	**<0.001**
Peak blood flow/LV, mL/min/L	UL	125.32 ± 52.23	156.93 ± 70.99	0.273
	LL	115.40 ± 39.04	140.56 ± 58.10	0.273
Δ Peak blood flow/LV, mL/min/L	UL	67.78 ± 41.60	68.36 ± 51.99	0.978
	LL	80.82 ± 33.52	96.74 ± 37.92	0.333
AUC blood flow/LV	UL	33.38 ± 37.51	66.11 ± 50.36	**0.003**
	LL	40.71 ± 22.13	6.34 ± 38.89	**0.038**

Bold values indicate statistically significant differences ($P < 0.05$). AUC, area under the curve; FMD, flow-mediated dilation; LV, limb volume; TTP, time-to-peak.

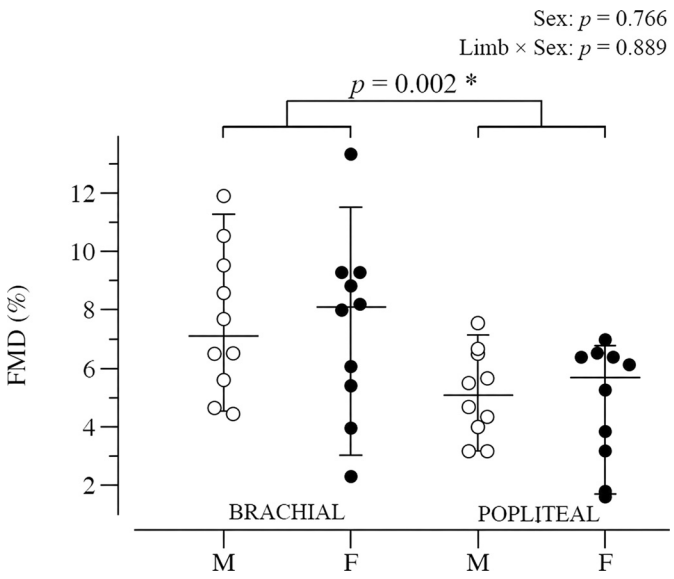

Figure 2. Comparison of flow-mediated dilation (FMD, %) between the brachial (upper limb) and popliteal (lower limb) arteries in male (M, ○) and female (F, ●) participants. Individual data points are presented alongside the median and interquartile range. *P* values from the linear mixed model are reported for the main effects of limb and sex, and for the limb × sex interaction. *$P < 0.05$.

Limb Specificity in Passive Movement-Induced Hyperemia: PLM_{LL} versus PLM_{UL}

The BF responses over time for both the UL and LL are presented in Fig. 3, *A* and *B*, whereas BF responses normalized for limb volumes are presented in Fig. 3, *C* and *D*. The peak BF was analyzed with a linear mixed model (Table 2). An effect was found for limb ($P < 0.0001$; $F = 145.8$) and for sex ($P = 0.02$; $F = 6.4$), but no interaction limb × sex was found ($P = 0.173$). When normalized for limb volume, no significant effects were observed for sex ($P = 0.167$; $F = 2.1$), limb ($P = 0.412$; $F = 0.7$), or interaction limb × sex ($P = 0.839$; $F = 0.04$). The Δpeak BF showed an effect for limb ($P < 0.001$; $F = 208.7$) and for sex ($P = 0.001$; $F = 7.6$), but no interaction limb × sex ($P = 0.165$). When normalized for limb volume, an effect was found for limb ($P = 0.022$; $F = 6.2$), but no effect for sex ($P = 0.518$; $F = 0.4$) or the interaction limb × sex ($P = 0.540$; $F = 0.4$). The AUC showed an interaction for limb and sex ($P < 0.001$; $F = 19.7$), an effect was found for limb ($P = 0.033$; $F = 5.4$) and for sex ($P = 0.024$; $F = 6.1$). Post hoc analysis (Bonferroni) showed a difference between UL and LL in males ($P < 0.001$; $F = 18.8$), but no difference in females ($P = 0.181$; $F = 1.9$); when comparing sexes within each limb, a difference was found in the LL ($P < 0.001$; $F = 22.5$), but not in the UL ($P = 0.347$; $F = .9$). The AUC normalized for LV showed an effect for limb ($P = 0.005$; $F = 8.8$), no effect for sex ($P = 0.4$; $F = 0.6$) and a significant interaction limb·× sex ($P < 0.001$; $F = 14.4$). Post hoc analysis (Bonferroni) showed a difference between UL and LL in females ($P < 0.001$; $F = 22.9$), but no difference in males ($P = 0.564$; $F = .3$); when comparing sexes within each limb, a difference was found in both the UL ($P = 0.003$; $F = 10.4$) and the LL ($P = 0.038$; $F = 4.6$).

A significant correlation was found between limbs for the peak BF ($r = 0.45$; $P = 0.046$; Fig. 4*A*) and Δpeak BF ($r = 0.50$; $P = 0.024$; Fig. 4*B*). No correlation between upper and lower limb AUC ($r = 0.081$; $P = 0.734$; Fig. 4*C*). The results were normalized for LV and showed no correlations between limbs for peak BF ($r = 0.28$; $P = 0.226$), Δpeak BF ($r = 0.02$; $P = 0.934$), or AUC ($r = -0.25$; $P = 0.293$).

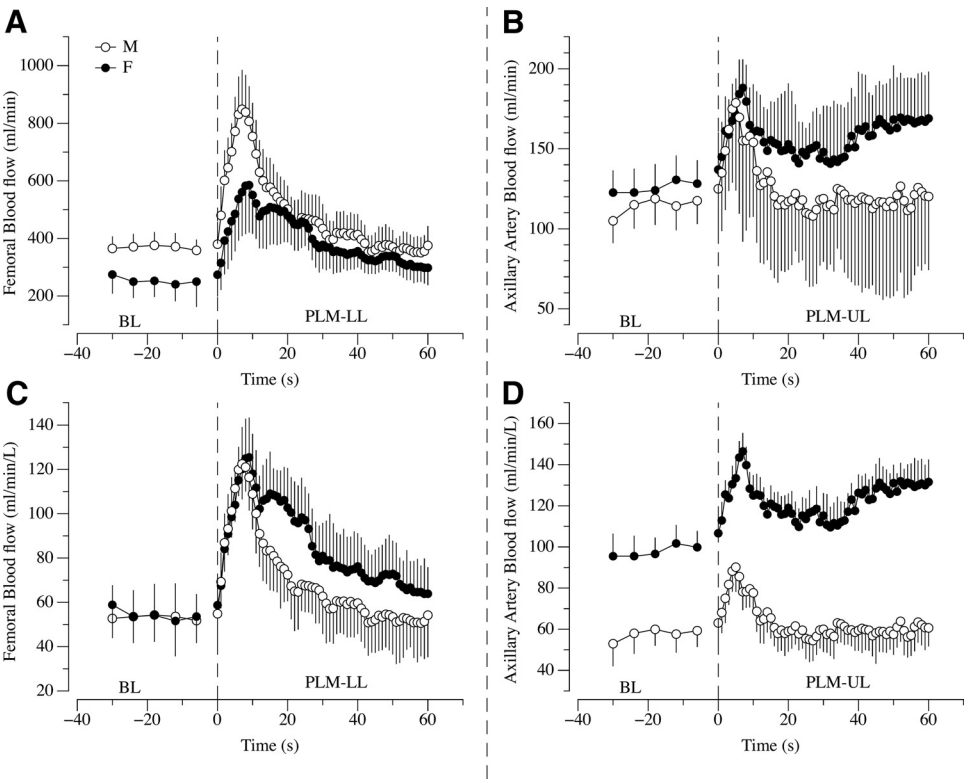

Figure 3. Dynamic second-by-second blood flow responses to passive limb movement (PLM) in male (M, ○) and female (F, ●) participants. Panels show absolute femoral blood flow during lower limb movement (PLM_{LL}) (*A*) and absolute axillary artery blood flow during upper limb movement (PLM_{UL}) (*B*). *C* and *D* represent, respectively, *A* and *B* data normalized for limb volume. BL indicates the 30-s baseline recording before movement onset; the dashed vertical line indicates the onset of passive movement (*time 0*). Each panel shows the group-mean response at each time point. Because individual peak responses occur at different time points, the maximum of these averaged traces is lower than the mean peak blood flow reported in Table 2, where each participant's individual peak—taken at that participant's own peak time point—is averaged across the group. Note that the responses represent the averaged time course of all participants; therefore, the maximal peak values are underestimated compared with the individual peak responses.

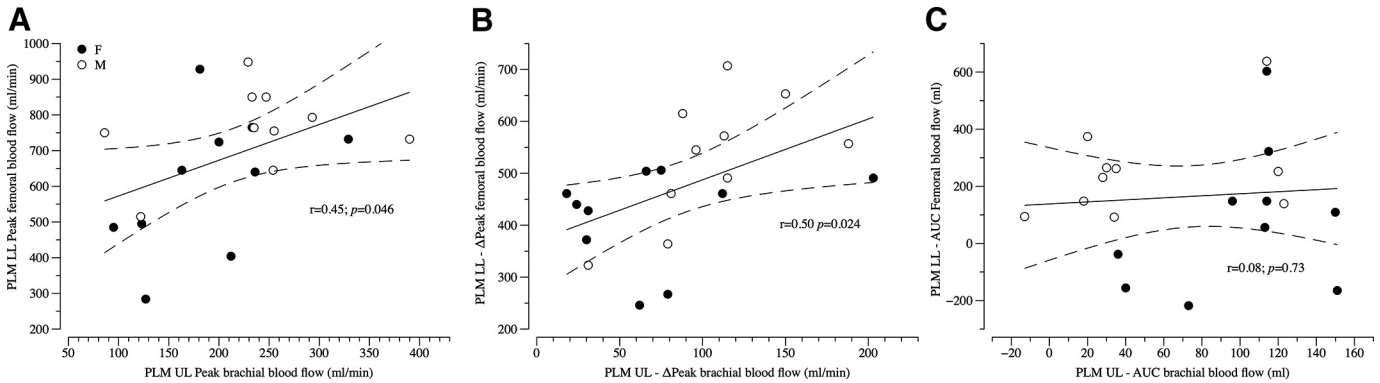

Figure 4. Correlation analyses between upper limb (PLM_{UL}) and lower limb (PLM_{LL}) hyperemic responsiveness. Panels illustrate the relationships for peak blood flow (*A*), Δpeak blood flow (*B*), and total hyperemic area under the curve (AUC) (*C*) across male (M, ○) and female (F, ●) subjects. The solid line represents the regression line, and the dashed lines indicate the 95% confidence intervals. Correlation coefficients (*r*) and relative *P* values are reported for each parameter. PLM, passive limb movement.

Within-Limb Associations: Correlation Structure of Vascular and Blood Flow Variables

To investigate possible correlations between variables within the same limb, correlation matrices were generated separately for each sex and limb (Fig. 5, *A–D*). As anticipated, absolute flow variables (peak BF, Δpeak BF, and AUC) were strongly correlated with their respective volume-normalized indices (peak BF/LV, Δpeak BF/LV, AUC/LV) in most groups, and baseline and peak diameter were almost perfectly correlated in all four conditions (r = 0.96–1.00, all $P < 0.001$); the complete set of coefficients is shown in Fig. 5.

In the upper limb of males (Fig. 5*A*), Δpeak BF was strongly and inversely correlated with shear rate ($r = -0.81$, $P = 0.004$), and shear rate was also inversely related to Δpeak BF/LV ($r = -0.70$, $P = 0.025$). In the lower limb of males (Fig. 5*B*), limb volume was inversely correlated with both PBF/vol ($r = -0.89$, $P = 0.001$) and Δpeak BF/LV ($r = -0.92$, $P < 0.001$), as well as with Δpeak BF (r = −0.70, $P = 0.023$), whereas PBF was inversely related to FMD ($r = -0.65$, $P = 0.041$).

In the upper limb of females (Fig. 5*C*), AUC was inversely correlated with both baseline and peak diameter ($r = -0.71$, $P = 0.022$ and $r = -0.78$, $P = 0.008$), and both diameters were inversely correlated with AUC/vol ($r = -0.91$ and −0.94, both $P < 0.001$); FMD was positively correlated with shear rate ($r = 0.79$, $P = 0.006$). In the lower limb of females (Fig. 5*D*), baseline and peak diameter were inversely correlated with shear rate ($r = -0.75$, $P = 0.012$ and $r = -0.74$, $P = 0.015$), and limb volume was inversely related to peak BF/LV ($r = -0.69$, $P = 0.028$) and Δpeak BF/LV ($r = -0.78$, $P = 0.008$).

Upper limb: FMD_{UL} versus PLM_{UL}.

No correlation was found between FMD_{UL}% and PLMUL-induced peak BF ($r = -0.123$; $P = 0.607$). Also, PLM_{UL}-induced Δpeak and AUC were not correlated with FMD_{UL}% (Δpeak: $r = 0.203$, $P = 0.391$; AUC: $r = -0.183$, $P = 0.440$).

Lower limb: FMD_{LL} versus PLM_{LL}.

Contrary to the upper limb, in the lower limb, the FMD_{LL} % results correlated with PLM_{LL}-induced peak BF ($r = -0.493$; $P = 0.027$), but no correlations were found for Δ peak ($r = -0.413$; $P = 0.070$) or AUC ($r = -0.093$; $P = 0.697$).

DISCUSSION

This study investigated two noninvasive tests of vascular function—FMD and PLM—with the primary aim of determining whether each test yields comparable results when applied to the upper and lower limbs in young, healthy males and females. The results did not completely support our initial hypotheses. FMD was not interchangeable between limbs: FMD% differed significantly between the upper and lower limbs, and no correlation was found between upper- and lower-limb FMD%. PLM showed only partial interlimb agreement: peak blood flow and Δpeak blood flow were significantly correlated between the upper and lower limbs, whereas AUC was not, and none of the volume-normalized indices showed an interlimb correlation. The limb-dependent differences in FMD likely reflect inherent structural differences between the brachial and popliteal arteries, which underlie their distinct responses to a similar shear stimulus. Notably, we observed pronounced sex-specific differences in the vascular response to PLM: for AUC, males exhibited higher blood flow in the lower limb, whereas females showed higher blood flow in the upper limb, reflecting two distinct physiological responses that may stem from a combination of limb volume differences and additional, limb-specific mechanisms not directly assessed in the present study. Contrary to our second hypothesis, FMD% did not differ between sexes in either limb once vessel size and shear stimulus were accounted for, although sex influenced the PLM response in a limb-dependent manner. Collectively, these findings indicate that interchangeability between limbs is not supported for FMD and is only partially supported for PLM, being limited to peak and Δpeak blood flow.

Limb Specificity in Vascular Function Assessed with FMD

FMD is a noninvasive ultrasound-based assessment of conduit-artery endothelial function (29). Brachial-artery FMD, in particular, is considered the gold-standard marker of systemic endothelial function and has been established as

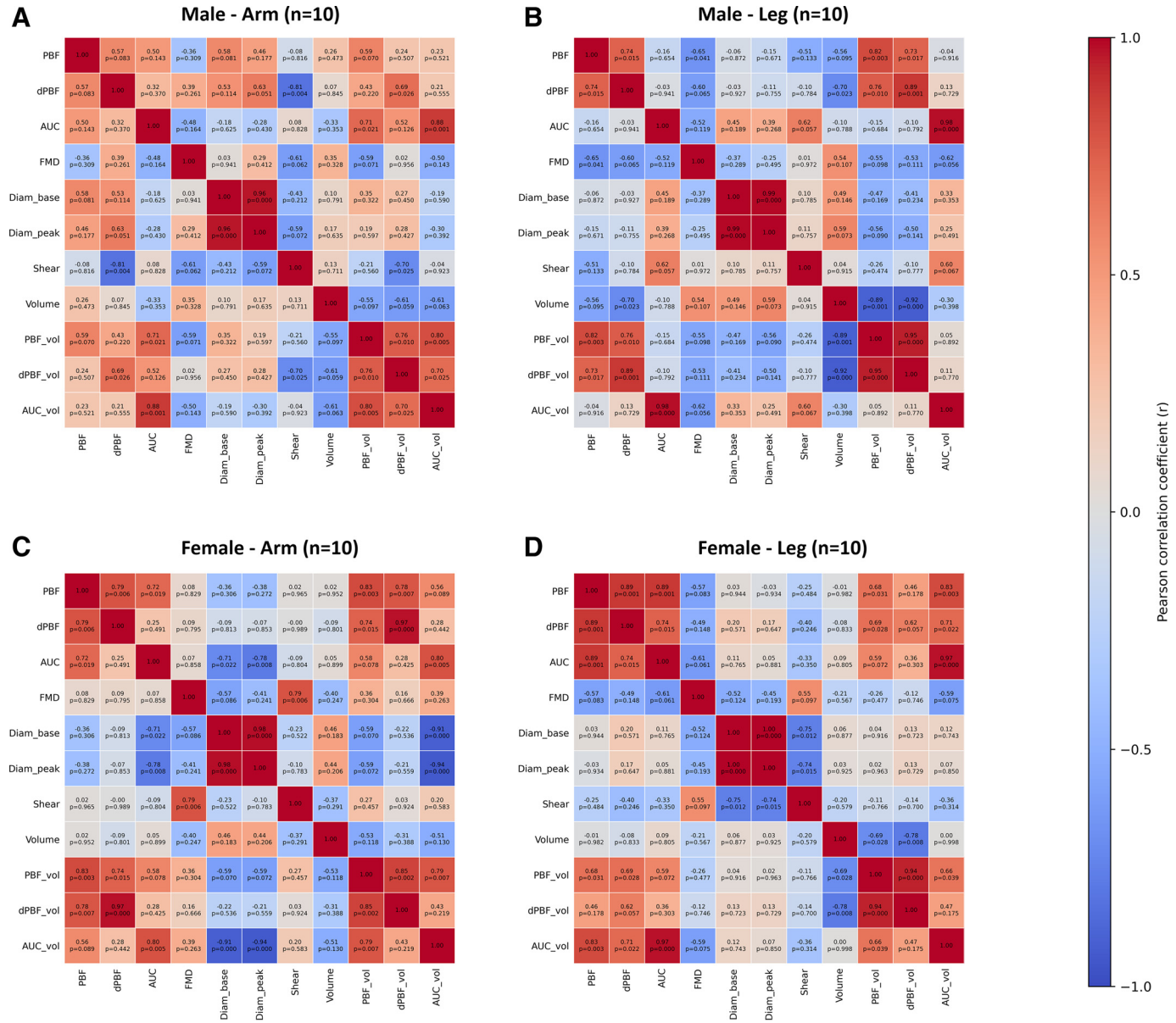

Figure 5. Correlation matrices showing the relationships among anthropometric, structural, and vascular functional variables, stratified by sex and limb: males—arm (*A*), males—leg (*B*), females—arm (*C*), females—leg (*D*). Variables shown on both axes are PBF, peak blood flow during passive limb movement; ΔPBF, change in peak blood flow from baseline; AUC, area under the blood-flow curve over the 60 s of passive movement; PBF/vol, ΔPBF/vol, and AUC/vol, the corresponding indices normalized for limb volume (mL/min/L); FMD, flow-mediated dilation (%); shear, shear rate area under the curve during reactive hyperemia; diam base, baseline arterial diameter (cm); diam peak, peak arterial diameter (cm); limb volume, anthropometrically estimated limb volume (*L*). The color scale bar indicates the strength and direction of the Pearson correlation coefficient (*r*), ranging from blue (strong negative correlation, −1.0) to red (strong positive correlation, +1.0).

an independent predictor of future cardiovascular events in multiple large prospective cohorts and meta-analyses (7, 36–38), and brachial FMD also tracks coronary-artery vasodilatory function (9). The prognostic value of FMD measured in lower-limb conduit arteries, such as the popliteal or femoral artery, for systemic cardiovascular events has not been established to the same extent. However, lower-limb FMD has been proposed as a region-specific biomarker of local vascular health and of the risk of peripheral artery disease (39, 40), an interpretation that is biologically plausible given that the leg vasculature is markedly more susceptible to atherosclerosis than the brachial artery (41). Brachial and popliteal FMD may therefore carry complementary, region-specific information on vascular health rather than equivalent information on systemic cardiovascular risk, which directly motivated the comparison performed in the present study.

The current study assessed FMD% in the brachial and popliteal arteries and found a significant limb effect ($P = 0.002$), with FMD_{UL}% higher than FMD_{LL}%. This finding is consistent with previous reports of limb-specific endothelial function (8, 42). The FMD_{UL}% values recorded here are within the range reported for young, healthy populations (19), supporting the validity of our assessment; with respect

to absolute FMD, our results are also aligned with those reported by Thijssen et al. (24).

The observed difference in vasodilatory response between the upper and lower limbs may stem from two related, but distinct, factors. First, baseline artery diameter was significantly larger in the lower than in the upper limb ($P < 0.001$) and, as described by Poiseuille's law (43), a larger baseline diameter generates a lower shear stimulus for a given flow. Consistent with this, the shear rate measured during reactive hyperemia was significantly higher in the upper limb ($P < 0.001$), which paralleled the higher FMD_{UL}%, whereas the FMD/shear rate was significantly greater in the lower limb ($P = 0.002$). This pattern is in close agreement with the framework described by Nishiyama et al. (8, 42) and with the lower-limb vascular data reported by Wang et al. (44): the higher relative dilation of the upper-limb conduit is largely attributable to the greater shear stimulus to which it is exposed, and once the dilatory response is expressed relative to its stimulus, the lower-limb conduit is not less responsive than the upper-limb conduit. Second, beyond its effect on the shear stimulus, baseline diameter also has a direct mathematical influence on FMD%, since FMD is computed as a relative change from baseline and is known to be inversely related to baseline vessel size (30). The limb difference in FMD% therefore reflects both a shear-stimulus difference and a baseline-diameter effect on the FMD% index itself. Supporting this interpretation, when peak diameter was analyzed with an allometric mixed model that included baseline diameter as a covariate, the limb effect was no longer significant ($P = 0.334$), indicating that the apparent limb difference in FMD% is largely explained by underlying differences in vessel size and shear, rather than by an intrinsically reduced dilator capacity of the lower-limb conduit.

Future assessments using endothelium-independent vasodilation, such as nitroglycerin administration, could help determine whether structural constraints contribute to these limb-specific differences. Finally, no correlation was observed between FMD_{UL}% and FMD_{LL}% ($P = 0.650$), indicating that conduit-artery function in the upper limb is not predictive of conduit-artery function in the lower limb. This finding aligns with the region-specific interpretation outlined earlier: brachial and popliteal FMD probe vascular beds with different structural and functional properties and different patterns of disease susceptibility, and they should be regarded as complementary rather than interchangeable assessments. Researchers should therefore not assume that one substitutes for the other when designing studies.

Limb Specificity in Vascular Function Assessed with Passive Limb Movement

The hyperemic response to PLM is predominantly nitric oxide-dependent (12, 14) and primarily reflects resistance-vessel (microvascular) function (11, 13), making it a noninvasive index of microvascular health that is complementary to, rather than equivalent to, the conduit-artery assessment provided by FMD.

In the present study, the lower limb exhibited significantly higher peak BF, Δpeak BF, and AUC than the upper limb (limb effect: peak BF $P < 0.001$; Δpeak BF $P < 0.001$), as expected given the larger muscle mass and vascular bed of the leg. Significant interlimb correlations were observed for peak BF ($r = 0.45$, $P = 0.046$) and Δpeak BF ($r = 0.50$, $P = 0.024$), whereas no correlation was found for AUC ($r = 0.08$, $P = 0.734$). When the responses were normalized for limb volume, none of the interlimb correlations remained significant (peak BF/LV: $r = 0.28$, $P = 0.226$; Δpeak BF/LV: $r = 0.02$, $P = 0.934$; AUC/LV: $r = -0.25$, $P = 0.293$). These findings indicate that the interlimb agreement of PLM is only partial: it is limited to the magnitude of the peak hyperemic response and is largely lost once the response is corrected for the size of the vascular bed. PLM_{UL} may therefore provide complementary, but not equivalent, information to PLM_{LL}, and the two assessments should not be assumed to be substitutable across limbs.

To our knowledge, only one previous study has directly compared the PLM response between upper and lower limbs (32). Burns et al. (32) reported a similar blood flow response to PLM in the two limbs, which is partly consistent with our interlimb correlations for peak BF and Δpeak BF; however, that study reported only PLM-induced changes in blood flow and did not provide peak BF, Δpeak BF, or AUC data, which precludes a direct quantitative comparison with the present results.

An additional aspect that warrants consideration is the sex-specific pattern of the PLM response. Although the absolute sex differences in peak BF and Δpeak BF observed in the lower limb were abolished once responses were normalized for limb volume, AUC displayed a significant limb × sex interaction ($P < 0.001$) that was clarified by the volume-normalized analysis, with females showing higher AUC/LV than males in the upper limb ($P = 0.003$) and the opposite pattern in the lower limb ($P = 0.038$). The physiological mechanisms potentially underlying these sex-specific patterns are discussed in detail in *The effect of sex on vascular function*.

Overall, PLM_{UL} and PLM_{LL} provide partially convergent but not equivalent information on vascular function. Caution is therefore warranted when comparing PLM responses between limbs, particularly when sex is a variable of interest. Future research is needed to clarify the mechanisms underlying movement-induced hyperemia in the upper limb, which remains substantially less characterized than its lower-limb counterpart.

Assessing vascular function in the upper limb and lower limb: FMD, PLM, or both?

The potential agreement between FMD and PLM has been investigated in recent years (13, 23, 33), but this area of research remains limited. Importantly, the two tests are not redundant: FMD assesses conduit-artery (macrovascular) endothelial function and is influenced by the shear stimulus generated by the reactive hyperemia that follows cuff release, whereas PLM assesses resistance-vessel (microvascular) function and is predominantly nitric oxide-dependent. Within each limb, the two tests also interrogate different vessels (e.g., the brachial or popliteal conduit artery for FMD vs. the downstream resistance vasculature for PLM), adding a vessel-level dimension to the limb-level comparison. Because macro- and microvascular measures of vascular health are frequently uncorrelated, FMD and PLM should be regarded as complementary, rather than substitutable,

assessments. Prior studies (13, 33) have compared FMD and PLM across different limbs (e.g., brachial FMD vs. lower-limb PLM); in the present study, we conducted an exploratory secondary analysis in which the two tests were compared within the same limb, to characterize—rather than to test the substitutability of—the relationship between conduit-artery and resistance-vessel function in the same vascular region.

In the upper limb, no significant correlation was observed between FMD_{UL}% and any PLM_{UL} outcome (peak BF: $r = -0.123$, $P = 0.607$; Δpeak BF: $r = 0.203$, $P = 0.391$; AUC: $r = -0.183$, $P = 0.440$). This indicates that, in the upper limb, conduit-artery vasodilator function and resistance-vessel hyperemic response capture largely independent aspects of vascular function.

In the lower limb, a significant negative correlation was found between FMD_{LL}% and PLM_{LL} peak BF ($r = -0.49$, $P = 0.027$), whereas no significant correlation was observed for Δpeak BF ($r = -0.413$, $P = 0.070$) or AUC ($r = -0.093$, $P = 0.697$). The negative direction of the FMD_{LL}%− PLM_{LL} peak BF correlation warrants careful interpretation. It indicates that individuals classified as having a "better" conduit-artery response by FMD tended to show a "lower" peak microvascular response by PLM, which is the opposite of the pattern that would be expected if the two tests provided equivalent information on vascular health. Rather than reflecting "intertest variability in response magnitude," this finding is most parsimoniously explained by the fact that FMD and PLM probe different vessels and different functional compartments of the vascular tree, and are therefore expected to be driven by distinct underlying mechanisms.

These results are not directly comparable with the positive across-limb correlation between FMD and PLM reported by Rossman et al. (33), since our analysis was conducted within the same limb rather than across limbs, and the two designs may capture different aspects of the macro–micro relationship. In particular, an across-limb design averages out region-specific differences in vessel structure and shear environment, whereas a within-limb design preserves them, which may explain why a positive association observed across limbs is not reproduced within a single limb.

Taken together with the absence of any association in the upper limb, the inverse association observed in the lower limb indicates that the relationship between FMD and PLM within the same limb is limb-dependent and does not support the use of the two tests as interchangeable assessments of vascular function. Brachial/popliteal FMD and PLM should be regarded as complementary, region-specific markers of macro- and microvascular health, respectively. This is consistent with the broader observation in the literature that macro- and microvascular measures of vascular function frequently do not correlate, and underscores the importance of selecting the assessment most appropriate to the specific aspect of vascular function under investigation.

The effect of sex on vascular function.

Contrary to our initial hypothesis, we observed no sex differences in FMD% in either limb (UL $P = 0.921$; LL $P = 0.398$), despite the well-documented female advantage in brachial endothelial function attributed to the protective effect of estrogens (20). Several factors of our experimental design and physiology may explain this divergence. Baseline diameter was significantly smaller in females than in males in both limbs (UL: 0.34 ± 0.06 vs. 0.44 ± 0.04 cm, $P < 0.001$; LL: 0.47 ± 0.11 vs. 0.58 ± 0.06 cm, $P = 0.019$), and peak diameter showed the same pattern. Because FMD% is expressed relative to baseline diameter and is mathematically inversely related to it (30), the smaller vessels of females would be expected to yield a higher relative dilation; the fact that FMD % was nonetheless comparable between sexes suggests that the magnitude of the dilatory stimulus, rather than vessel size alone, governed the response. Consistent with this, the shear rate AUC during reactive hyperemia tended to be higher in females, particularly in the upper limb (45,221 ± 28,122 vs. 28,551 ± 9,764, $P = 0.107$), without reaching significance, and the FMD/shear rate ratio did not differ between sexes (UL $P = 0.238$; LL $P = 0.607$). When peak diameter was analyzed with an allometric mixed model including baseline diameter as a covariate, the sex effect remained nonsignificant ($P = 0.060$). An effect of sex was, however, observed for time-to-peak (TTP), with females reaching peak diameter faster than males in both limbs (sex effect, $P = 0.037$). This pattern is consistent with previous observations of a longer TTP in males compared with females reported in children and adolescents (45) and may reflect subtle sex-related differences in vasodilatory kinetics not captured by the magnitude of FMD%. Taken together, these data indicate that, once vessel size and shear stimulus are accounted for, conduit-artery vasodilator function was largely comparable between young males and females, although a sex-related difference in dilatory kinetics persists. The expected female advantage in the magnitude of FMD may have been masked by characteristics of our sample—young, healthy individuals with preserved endothelial function in both sexes. In addition, by design, all female participants were tested within the first 7 days of the menstrual cycle (i.e., in the early follicular phase), when circulating estrogen levels are at their lowest. This deliberate standardization minimized hormonally driven within-group variability but may also have attenuated the estrogen-mediated enhancement of FMD typically reported in females, providing a parsimonious explanation for the absence of a sex difference in FMD% in our cohort. The mixed findings in the literature (19, 46) further suggest that factors such as baseline vessel size, vascular bed dimensions, and habitual physical activity contribute to the heterogeneity of sex-related FMD results.

In contrast with the FMD findings, the hyperemic response to PLM showed clear sex-specific differences with a limb-dependent pattern. In the lower limb, males exhibited higher peak BF, Δpeak BF, and AUC than females (sex effect: peak BF $P = 0.02$; Δpeak BF $P = 0.001$; LL AUC between sexes $P < 0.001$). This pattern is most parsimoniously explained by the greater muscle mass and limb volume of males, an interpretation that is directly supported by our own data: once peak BF and Δpeak BF were normalized for limb volume, the sex differences disappeared ($P = 0.167$ and $P = 0.518$, respectively), confirming that the absolute lower-limb sex difference is largely volume-driven. In the upper limb, the pattern was qualitatively different: no sex difference was observed for peak BF or Δpeak BF, but AUC displayed a significant limb × sex interaction ($P < 0.001$), and after normalization for limb volume, AUC/LV was significantly higher in females than in males in the upper limb (66.11 ± 50.36 vs. 33.38 ± 37.51, $P = 0.003$), with the opposite pattern in the lower limb (40.71 ± 22.13 vs.

6.34 ± 38.89, $P = 0.038$). This crossover cannot be explained by muscle mass alone and suggests additional limb-specific mechanisms.

Several nonmutually exclusive mechanisms may contribute to the sex-specific patterns observed. First, sex-related differences in mechanoreflex sensitivity have been documented during passive leg movement: Ives et al. (47) reported that, despite an attenuated central, mechanoreflex-mediated hemodynamic response in females during PLM, females achieved a movement-induced hyperemia comparable with that of males, possibly through augmented peripheral vasodilatory mechanisms—a pattern broadly consistent with our volume-normalized findings. Whether the mechanoreflex behaves similarly in the upper limb is currently unclear; recent work by Lehnen et al. (48) characterizing mechanoreflex activation via passive forearm stretching provides a relevant framework for interpreting upper-limb responses, which remain far less explored than lower-limb responses. Second, sex-related differences in neuromuscular activation patterns (49) may contribute to the limb-specific divergence, particularly in the upper-limb response where muscle mass cannot account for the observed crossover. Third, sex hormones, particularly estrogens, modulate endothelial nitric oxide bioavailability and smooth muscle responsiveness (20, 48) and may influence both the conduit-artery and the microvascular components of the response. In the present study, female participants were tested in the early follicular phase to control for hormonal variability; this choice minimizes the contribution of high estrogen states to the observed responses, but it also limits the generalizability of our sex-related findings to other phases of the menstrual cycle, in which a more pronounced female advantage may emerge. We also acknowledge that autonomic activity was not directly measured in the present study, so the contribution of autonomic mechanisms to the observed sex differences remains a working hypothesis to be tested in studies specifically designed for this purpose.

The within-limb correlation matrices stratified by sex and limb (Fig. 5) provide additional, sex-specific insights into the determinants of the vascular responses. In the upper limb of females, FMD% was positively correlated with shear rate ($r = 0.79$, $P = 0.006$), suggesting that conduit-artery dilation was tightly coupled to the dilatory stimulus in this group, in line with the shear-mediated mechanism of FMD. In the upper limb of males, Δpeak BF was strongly and inversely correlated with shear rate ($r = -0.81$, $P = 0.004$), pointing to a different relationship between the magnitude of the microvascular response and the shear environment. In the lower limb of males, limb volume was strongly and inversely correlated with both peak BF/LV ($r = -0.89$, $P = 0.001$) and Δpeak BF/LV ($r = -0.92$, $P < 0.001$), confirming that the volume normalization captures a substantial portion of the interindividual variability in PLM responses, and that limb volume itself shapes the relationship between absolute and volume-normalized variables. These sex- and limb-specific correlation structures support the broader interpretation that the regulators of conduit artery and resistance vessel function differ between sexes and between limbs, and that pooling data across sex or limb may mask physiologically relevant relationships.

Overall, these findings indicate that sex influences the PLM response—largely through differences in limb volume in the lower limb, and through additional, volume-independent mechanisms in the upper limb, whereas the magnitude of FMD% was not influenced by sex once vessel size and shear stimulus were taken into account, although the kinetics of the response (TTP) differed between sexes. Researchers and clinicians should therefore consider sex explicitly when interpreting both PLM and FMD outcomes, particularly in the upper limb, where the underlying physiological mechanisms remain to be clarified.

Limitations

This study has some limitations. The first one is the range of motion (ROM) of the PLM. As mentioned in a previous paper on PLM on different joints (32), the 90 degrees of ROM on the knee is not the same relative proportion for the elbow, though matched in absolute terms between limbs, this relatively lower ROM in the UL could have influenced the results. The second aspect of the ROM is that it can increase and decrease over time if the participant does a specific kind of training or activity, which, alongside individual differences between the participants, it is possible that 90 degrees of ROM relative to the individual maximal joint ROM may have also played a role in the hyperemic response to limb movement. The position of the participant for the PLM is an additional important context, as there are studies with PLM performed in the supine position and others that use an upright position. We used the upright position because it is the one usually adopted for the PLM and the one suggested in the guidelines (11), but it could be difficult to compare results obtained with different positions (upright vs. supine) because of the different central and peripheral hemodynamic responses to the different positions (31). Regarding physical activity (PA), there are many papers in the literature that document that a higher level of PA helps to increase vascular function in healthy young or older individuals (50, 51). This study did not control participants' PA, or the specificity of the PA (UL vs. LL), which could have affected the results. An additional limitation is that the participants' health status was verified only through self-reported medical history, together with MAP and BMI; no further clinical or laboratory measures were available to confirm the absence of underlying conditions. The last methodological limit is the relatively small sample size; 20 participants completed the study, 10 males and 10 females. With a larger sample size, some of the correlations that did not reach significance but trended in a direction could become significant. Though the effect size indicators (e.g., r values) are useful, further research with a larger sample is needed.

Finally, it must be acknowledged that the primary scope of this study was strictly methodological and comparative. Our main objective was to evaluate the degree of agreement and concordance between FMD and PLM across different limbs and sexes, rather than to dissect specific cellular pathways or provide new, direct insights into the underlying potential physiological explanations. Consequently, further mechanistically oriented studies are warranted to fully

elucidate the exact biological networks driving these regional and sex-specific vascular variations.

Conclusions

This study compared two widely used methods of vascular function assessment—flow-mediated dilation and passive limb movement—applied to the upper and lower limbs in young, healthy males and females. FMD was not interchangeable between limbs, with significantly different FMD hx0025; values and no correlation between FMD_{UL}% and FMD_{LL}%, supporting the view that brachial and popliteal FMD provide complementary, region-specific information. Sex did not influence FMD% once vessel size and shear stimulus were accounted for, although vasodilatory kinetics (time-to-peak) were faster in females than in males. The interlimb agreement of PLM was only partial, limited to peak BF and Δpeak BF and lost after volume normalization, so that PLM_{UL} and PLM_{LL} should be regarded as complementary rather than fully interchangeable indices. Males and females also exhibited distinct PLM response patterns, mainly driven by limb volume in the lower limb and by additional, volume-independent mechanisms in the upper limb. Future studies should clarify the physiological mechanisms underlying these sex-specific responses, particularly during passive limb movement.

DATA AVAILABILITY

Data will be made available upon reasonable request.

GRANTS

The American Heart Association (AHA) has provided support (to S.J.I.) (https://doi.org/10.58275/AHA.24AIREA1247045.pc.gr.189804).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.F. and M.V. conceived and designed research; E.F., A.P., J.M.L., and E.S.S. performed experiments; E.F. analyzed data; E.F., A.P., and S.J.I. interpreted results of experiments; E.F. and M.V. prepared figures; E.F. drafted manuscript; E.F., A.P., J.M.L., E.S.S., S.J.I., and M.V. edited and revised manuscript; E.F., A.P., J.M.L., E.S.S., S.J.I., and M.V. approved final version of manuscript.

REFERENCES

1. **Arnett DK**, **Blumenthal RS**, **Albert MA**, **Buroker AB**, **Goldberger ZD**, **Hahn EJ**, **Himmelfarb CD**, **Khera A**, **Lloyd-Jones D**, **McEvoy JW**, **Michos ED**, **Miedema MD**, **Muñoz D**, **Smith SC**, **Virani SS**, **Williams KA**, **Yeboah J**, **Ziaeian B.** 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation* 140: e596–e646, 2019. doi:10.1161/CIR.0000000000000678/SUPPL_FILE/WEB.
2. **Green DJ**, **Hopman MT**, **Padilla J**, **Laughlin MH**, **Thijssen DH.** Vascular adaptation to exercise in humans: role of hemodynamic stimuli. *Physiol Rev* 97: 495–528, 2017. doi:10.1152/PHYSREV.00014.2016.
3. **Versari D**, **Daghini E**, **Virdis A**, **Ghiadoni L**, **Taddei S.** Endothelial dysfunction as a target for prevention of cardiovascular disease. *Diabetes Care* 32, *Suppl* 2: S314–S321, 2009. doi:10.2337/DC09-S330.
4. **Papageorgiou N**, **Tousoulis D**, **Androulakis E**, **Giotakis A**, **Siasos G**, **Latsios G**, **Stefanadis C.** Lifestyle factors and endothelial function. *Curr Vasc Pharmacol* 10: 94–106, 2012. doi:10.2174/157016112798829788.
5. **Wang J**, **Widlansky ME.** Lifestyle choices and endothelial function: risk and relevance. *Curr Vasc Pharmacol* 7: 209–224, 2009. doi:10.2174/157016109787455581.
6. **Gokce N**, **Keaney JF Jr**, **Hunter LM**, **Watkins MT**, **Nedeljkovic ZS**, **Menzoian JO**, **Vita JA.** Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events inpatients with peripheral vascular disease. *J Am Coll Cardiol* 41: 1769–1775, 2003. doi:10.1016/S0735-1097(03)00333-4.
7. **Inaba Y**, **Chen JA**, **Bergmann SR.** Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *Int J Cardiovasc Imaging* 26: 631–640, 2010. doi:10.1007/S10554-010-9616-1/METRICS.
8. **Nishiyama SK**, **Wray DW**, **Berkstresser K**, **Ramaswamy M**, **Richardson RS.** Limb-specific differences in flow-mediated dilation: the role of shear rate. *J Appl Physiol (1985)* 103: 843–851, 2007. doi:10.1152/JAPPLPHYSIOL.00273.2007/ASSET/IMAGES/LARGE/ZDG0090774410005.JPEG.
9. **Broxterman RM**, **Witman MA**, **Trinity JD**, **Groot HJ**, **Rossman MJ**, **Park SY**, **Malenfant S**, **Gifford JR**, **Kwon OS**, **Park SH**, **Jarrett CL**, **Shields KL**, **Hydren JR**, **Bisconti AV**, **Owan T**, **Abraham A**, **Tandar A**, **Lui CY**, **Smith BR**, **Richardson RS.** Strong relationship between vascular function in the coronary and brachial arteries. *Hypertension* 74: 208–215, 2019. doi:10.1161/HYPERTENSIONAHA.119.12881.
10. **Ahn Y**, **Aung N**, **Ahn HS.** A comprehensive review of clinical studies applying flow-mediated dilation. *Diagnostics (Basel)* 14: 2499, 2024. doi:10.3390/DIAGNOSTICS14222499.
11. **Gifford JR**, **Richardson RS.** CORP: ultrasound assessment of vascular function with the passive leg movement technique. *J Appl Physiol (1985)* 123: 1708–1720, 2017. doi:10.1152/japplphysiol.00557.2017.-As.
12. **Trinity JD**, **Groot HJ**, **Layec G**, **Rossman MJ**, **Ives SJ**, **Runnels S**, **Gmelch B**, **Bledsoe A**, **Richardson RS.** Nitric oxide and passive limb movement: a new approach to assess vascular function. *J Physiol* 590: 1413–1425, 2012. doi:10.1113/JPHYSIOL.2011.224741.
13. **Groot HJ**, **Broxterman RM**, **Gifford JR**, **Garten RS**, **Rossman MJ**, **Jarrett CL**, **Kwon OS**, **Hydren JR**, **Richardson RS.** Reliability of the passive leg movement assessment of vascular function in men. *Exp Physiol* 107: 541–552, 2022. doi:10.1113/EP090312.
14. **Mortensen SP**, **Askew CD**, **Walker M**, **Nyberg M**, **Hellsten Y.** The hyperaemic response to passive leg movement is dependent on nitric oxide: a new tool to evaluate endothelial nitric oxide function. *J Physiol* 590: 4391–4400, 2012. doi:10.1113/JPHYSIOL.2012.235952.
15. **Tschakovsky ME**, **Pyke KE.** Counterpoint: flow-mediated dilation does not reflect nitric oxide-mediated endothelial function. *J Appl Physiol (1985)* 99: 1235–1238, 2005. doi:10.1152/japplphysiol.00607.2005.
16. **Rodgers JL**, **Jones J**, **Bolleddu SI**, **Vanthenapalli S**, **Rodgers LE**, **Shah K**, **Karia K**, **Panguluri SK.** Cardiovascular risks associated with gender and aging. *J Cardiovasc Dev Dis* 6: 19, 2019. doi:10.3390/JCDD6020019.
17. **Anand SS**, **Islam S**, **Rosengren A**, **Franzosi MG**, **Steyn K**, **Yusufali AH**, **Keltai M**, **Diaz R**, **Rangarajan S**, **Yusuf S**; **INTERHEART Investigators.** Risk factors for myocardial infarction in women and men: insights from the INTERHEART study. *Eur Heart J* 29: 932–940, 2008. doi:10.1093/EURHEARTJ/EHN018.
18. **Levenson J**, **Pessana F**, **Gariepy J**, **Armentano R**, **Simon A.** Gender differences in wall shear-mediated brachial artery vasoconstriction and vasodilation. *J Am Coll Cardiol* 38: 1668–1674, 2001. doi:10.1016/S0735-1097(01)01604-7.
19. **Holder SM**, **Bruno RM**, **Shkredova DA**, **Dawson EA**, **Jones H**, **Hopkins ND**, **Hopman MTE**, **Bailey TG**, **Coombes JS**, **Askew CD**, **Naylor L**, **Maiorana A**, **Ghiadoni L**, **Thompson A**, **Green DJ**, **Thijssen D.** Reference intervals for brachial artery flow-mediated dilation and the relation with cardiovascular risk factors. *Hypertension* 77: 1469–1480, 2021. doi:10.1161/HYPERTENSIONAHA.120.15754/SUPPL_FILE/HYP_HYPE-2020-15754_SUPP3.PDF.
20. **Stanhewicz AE**, **Wenner MM**, **Stachenfeld NS.** Sex differences in endothelial function important to vascular health and overall

cardiovascular disease risk across the lifespan. *Am J Physiol Heart Circ Physiol* 315: H1569–H1588, 2018. doi:10.1152/AJPHEART.00396.2018.

21. **Nakamura A**, **Kajitani S**, **Sato K**, **Kanazawa M**, **Kondo M**, **Endo H**, **Nozaki E.** Decline of popliteal artery flow-mediated dilation with aging and possible involvement of asymmetric dimethylarginine in healthy men. *J Med Ultrason (2001)* 46: 503–511, 2019. doi:10.1007/S10396-019-00946-2.
22. **Weggen JB**, **Hogwood AC**, **Decker KP**, **Darling AM**, **Chiu A**, **Richardson J**, **Garten RS.** Vascular responses to passive and active movement in premenopausal females: comparisons across sex and menstrual cycle phase. *Med Sci Sports Exerc* 55: 900–910, 2023. doi:10.1249/MSS.0000000000003107.
23. **D'Agata MN**, **Hoopes EK**, **Witman MA.** Associations between noninvasive upper- and lower-limb vascular function assessments: extending the evidence to young women. *J Appl Physiol (1985)* 133: 886–892, 2022. doi:10.1152/JAPPLPHYSIOL.00177.2022.
24. **Thijssen DH**, **Rowley N**, **Padilla J**, **Simmons GH**, **Laughlin MH**, **Whyte G**, **Cable NT**, **Green DJ.** Relationship between upper and lower limb conduit artery vasodilator function in humans. *J Appl Physiol (1985)* 111: 244–250, 2011. doi:10.1152/japplphysiol.00290.2011.
25. **Padilla J**, **Simmons GH**, **Bender SB**, **Arce-Esquivel AA**, **Whyte JJ**, **Laughlin MH.** Vascular effects of exercise: endothelial adaptations beyond active muscle beds. *Physiology (Bethesda)* 26: 132–145, 2011. doi:10.1152/PHYSIOL.00052.2010.
26. **Harris RA**, **Nishiyama SK**, **Wray DW**, **Richardson RS.** Ultrasound assessment of flow-mediated dilation: a tutorial. *Hypertension* 55: 1075–1085, 2010. doi:10.1161/HYPERTENSIONAHA.110.150821.
27. **Etsuda H**, **Takase B**, **Uehata A**, **Kusano H**, **Hamabe A**, **Kuhara R**, **Akima T**, **Matsushima Y**, **Arakawa K**, **Satomura K**, **Kurita A**, **Ohsuzu F.** Morning attenuation of endothelium-dependent, flow-mediated dilation in healthy young men: possible connection to morning peak of cardiac events? *Clin Cardiol* 22: 417–421, 1999. doi:10.1002/clc.4960220610.
28. **Bray GA.** Beyond BMI. *Nutrients* 15: 2254, 2023. doi:10.3390/NU15102254.
29. **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* 39: 257–265, 2002 [Erratum in *J Am Coll Cardiol* 39: 1082, 2002]. doi:10.1016/S0735-1097(01)01746-6.
30. **Atkinson G**, **Batterham AM.** The percentage flow-mediated dilation index: a large-sample investigation of its appropriateness, potential for bias and causal nexus in vascular medicine. *Vasc Med* 18: 354–365, 2013. doi:10.1177/1358863X13508446;CTYPE:STRING:JOURNAL.
31. **Trinity JD**, **McDaniel J**, **Venturelli M**, **Fjeldstad AS**, **Ives SJ**, **Witman MAH**, **Barrett-O'Keefe Z**, **Amann M**, **Walter Wray D**, **Richardson RS.** Impact of body position on central and peripheral hemodynamic contributions to movement-induced hyperemia: implications for rehabilitative medicine. *Am J Physiol Heart Circ Physiol* 300: H1885–H1891, 2011. doi:10.1152/AJPHEART.00038.2011/ASSET/IMAGES/LARGE/ZH40051198450002.JPEG.
32. **Burns KJ**, **Pollock BS**, **McDaniel J.** The cardiovascular response to passive movement is joint dependent. *Physiol Rep* 4: e12721, 2016. doi:10.14814/phy2.12721.
33. **Rossman MJ**, **Groot HJ**, **Garten RS**, **Witman MA**, **Richardson RS.** Vascular function assessed by passive leg movement and flow-mediated dilation: initial evidence of construct validity. *Am J Physiol Heart Circ Physiol* 311: H1277–H1286, 2016. doi:10.1152/ajpheart.00421.2016.
34. **Casadei K**, **Kiel J.** Anthropometric Measurement [Online]. https://www.ncbi.nlm.nih.gov/books/NBK537315/ [6 Aug. 2024].
35. **Pedrinolla A**, **Colosio AL**, **Magliozzi R**, **Danese E**, **Kirmizi E**, **Rossi S**, **Pogliaghi S**, **Calabrese M**, **Gelati M**, **Muti E**, **Cè E**, **Longo S**, **Esposito F**, **Lippi G**, **Schena F**, **Venturelli M.** The vascular side of chronic bed rest: when a therapeutic approach becomes deleterious. *J Clin Med* 9: 918, 2020. doi:10.3390/JCM9040918.
36. **Ras RT**, **Streppel MT**, **Draijer R**, **Zock PL.** Flow-mediated dilation and cardiovascular risk prediction: a systematic review with meta-analysis. *Int J Cardiol* 168: 344–351, 2013. doi:10.1016/j.ijcard.2012.09.047.
37. **Matsuzawa Y**, **Kwon TG**, **Lennon RJ**, **Lerman LO**, **Lerman A.** Prognostic value of flow-mediated vasodilation in brachial artery and fingertip artery for cardiovascular events: a systematic review and meta-analysis. *J Am Heart Assoc* 4: e002270, 2015. doi:10.1161/JAHA.115.002270;REQUESTEDJOURNAL:JOURNAL:JAHA;WGROUP:STRING:PUBLICATION.
38. **Yeboah J**, **Crouse JR**, **Hsu FC**, **Burke GL**, **Herrington DM.** Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the cardiovascular health study. *Circulation* 115: 2390–2397, 2007. doi:10.1161/CIRCULATIONAHA.106.678276;WEBSITE:WEBSITE:AHA-SITE;WGROUP:STRING:PUBLICATION.
39. **Bapir M**, **Untracht GR**, **Hunt JEA**, **McVey JH**, **Harris J**, **Skene SS**, **Campagnolo P**, **Dikaios N**, **Rodriguez-Mateos A**, **Sampson DD**, **Sampson DM**, **Heiss C.** Age-dependent decline in common femoral artery flow-mediated dilation and wall shear stress in healthy subjects. *Life (Basel)* 12: 2023, 2022. doi:10.3390/LIFE12122023.
40. **Daniele A**, **Lucas SJE**, **Rendeiro C.** Variability of flow-mediated dilation across lower and upper limb conduit arteries. *Eur J Appl Physiol* 124: 3265–3278, 2024. doi:10.1007/S00421-024-05517-Z.
41. **Criqui MH**, **Aboyans V.** Epidemiology of peripheral artery disease. *Circ Res* 116: 1509–1526, 2015 [Erratum in *Circ Res* 117: e12, 2015]. doi:10.1161/CIRCRESAHA.116.303849.
42. **Nishiyama SK**, **Wray DW**, **Richardson RS.** Aging affects vascular structure and function in a limb-specific manner. *J Appl Physiol (1985)* 105: 1661–1670, 2008. doi:10.1152/JAPPLPHYSIOL.90612.2008/ASSET/IMAGES/LARGE/ZDG0110881950006.JPEG.
43. **Slaaf DW**, **Vrielink HH**, **Tangelder GJ**, **Reneman RS.** Effective diameter as a determinant of local vascular resistance in presence of vasomotion. *Am J Physiol Heart Circ Physiol* 255: H1240–H1243, 1988. doi:10.1152/AJPHEART.1988.255.5.H1240.
44. **Wang E**, **Rossman MJ**, **Groot HJ**, **Garten RS**, **Jarrett CL**, **Helgerud J**, **Hoff J**, **Richardson RS.** Leg vascular function with advancing age in men: the impact of physical activity and endurance exercise training. *J Appl Physiol (1985)* 139: 473–481, 2025. doi:10.1152/japplphysiol.00784.2024.
45. **Hopkins ND**, **Dengel DR**, **Stratton G**, **Kelly AS**, **Steinberger J**, **Zavala H**, **Marlatt K**, **Perry D**, **Naylor LH**, **Green DJ.** Age and sex relationship with flow-mediated dilation in healthy children and adolescents. *J Appl Physiol (1985)* 119: 926–933, 2015. doi:10.1152/JAPPLPHYSIOL.01113.2014.
46. **Johns JA**, **O'Brien MW**, **Bungay A**, **Kimmerly DS.** Sex and light physical activity impact popliteal, but not brachial artery flow-mediated dilation in physically active young adults. *Appl Physiol Nutr Metab* 45: 1387–1395, 2020. doi:10.1139/APNM-2020-0308.
47. **Ives SJ**, **McDaniel J**, **Witman MA**, **Richardson RS.** Passive limb movement: evidence of mechanoreflex sex specificity. *Am J Physiol Heart Circ Physiol* 304: H154–H161, 2013. doi:10.1152/AJPHEART.00532.2012.
48. **Lehnen GCS**, **Guerrero RVD**, **Cunha GMR**, **Araujo MS**, **Carmo JC**, **Drew RC**, **Silva BM**, **Trajano GS**, **Vianna LC.** Cardiovascular and autonomic responses in young adults during and after mechanoreflex activation via passive forearm stretching: are there sex differences? *J Appl Physiol (1985)* 139: 1282–1292, 2025. doi:10.1152/japplphysiol.00645.2025.
49. **Shultz SJ**, **Perrin DH.** Using surface electromyography to assess sex differences in neuromuscular response characteristics. *J Athl Train* 34: 165–176, 1999.
50. **Pahkala K**, **Heinonen OJ**, **Simell O**, **Viikari JS**, **Rönnemaa T**, **Niinikoski H**, **Raitakari OT.** Association of physical activity with vascular endothelial function and intima-media thickness. *Circulation* 124: 1956–1963, 2011. doi:10.1161/CIRCULATIONAHA.111.043851.
51. **Payvandi L**, **Dyer A**, **Mcpherson D**, **Ades P**, **Stein J**, **Liu K**, **Ferrucci L**, **Criqui MH**, **Guralnik JM**, **Lloyd-Jones D**, **Kibbe MR**, **Liang ST**, **Kane B**, **Pearce WH**, **Verta M**, **McCarthy WJ**, **Schneider JR**, **Shroff A**, **Mcdermott MM.** Physical activity during daily life and brachial artery flow-mediated dilation in peripheral arterial disease. *Vasc Med* 14: 193–201, 2009. doi:10.1177/1358863X08101018.