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Limb Position Influences Peripheral Arterial Stiffness Reduction with Reactive Hyperemia

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Abstract

The mechanism behind the acute reduction in peripheral arterial stiffness with reactive hyperemia is presumed to be flow-mediated; however, this has not been clearly demonstrated. We hypothesized that a larger reactive hyperemia magnitude would result in a greater reduction in peripheral arterial stiffness. Fourteen healthy young adults (5 females, 25 ± 5 yrs, mean ± SD) underwent reactive hyperemia with a rapid-release cuff on the upper arm inflated to 220 mmHg for 5 min: once with the arm positioned ~ 50° above heart level and once with the arm positioned ~ 50° below heart level. Brachial-radial pulse wave velocity (PWV) was measured with tonometers over brachial and radial arteries before cuff inflation and at 5, 15, and 30 min after release. Brachial blood flow was monitored with doppler ultrasound. At 5 min after reactive hyperemia, both the absolute (2.31 ± 1.22 vs 1.19 ± 1.19 m/sec, $p = 0.007$) and relative (24% vs 15%; $p = 0.043$) changes in brachial to radial PWV were greater with the arm below the heart compared to above the heart. The peak brachial blood flow was higher when the arm was below the heart compared to above the heart (477 ± 145 vs 369 ± 137 ml/min; $p < 0.001$). Reactive hyperemia acutely reduced peripheral arterial stiffness above and below heart level, with a greater decrease observed when the arm was positioned below the heart. These results demonstrate the contribution of conduit artery blood flow to the reduction of peripheral arterial stiffness after reactive hyperemia.

Keywords Pulse wave velocity, Arterial stiffness, Reactive hyperemia, Blood flow, Blood pressure

1 Introduction

Arterial stiffness is a consequence of a variety of factors, including changes in the composition of the arterial wall matrix [1, 2] and properties of the vascular smooth muscle [3–5]. Chronic changes in arterial wall stiffening usually occur via changes in structural characteristics of the arterial wall while acute changes in arterial wall stiffening usually occur via changes in vascular smooth muscle contractility. The gold standard method for evaluating arterial stiffness in vivo is through the measurement of pulse wave velocity (PWV), which indicates the speed at which arterial pressure waves propagate through the vasculature [6, 7].

The change in PWV velocity observed in response to a reactive hyperemia stimulus is referred to as flow-mediated slowing (FMS). FMS has emerged as an alternative method for evaluating endothelial function which has traditionally relied on measuring the vasodilation of a conduit artery (flow-mediated dilation) following an increase in shear stress induced by a reactive hyperemia stimulus [8, 9]. Proponents of the FMS approach argue that a decrease in PWV during reactive hyperemia is flow-dependent and serves as an indicator of endothelial function. However, the relationship between the increase in blood flow and the decrease in PWV deserves further exploration.

The magnitude of blood-flow increase with reactive hyperemia can be altered by manipulating limb position

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below or above heart level. Both Jasperse et al. [10] and Bartlett et al. [11] observed a greater magnitude of reactive hyperemia when the arm was positioned below heart level compared to when it was positioned above heart level. We took advantage of this approach to explore the mechanism of action for the reduction in peripheral arterial stiffness following reactive hyperemia. The purpose of this study was to examine the changes in PWV following reactive hyperemia-induced increases in conduit artery blood flow. We did not intend to evaluate FMS as a measure of endothelial function, rather to study the relationship between increases in blood flow and changes in PWV. We hypothesized that a larger magnitude of reactive hyperemia would result in a greater reduction in peripheral arterial stiffness.

2 Methods

2.1 Participants

Fourteen healthy young adults volunteered for this study. Participants were excluded for smoking, presence of cardiovascular, metabolic, and pulmonary diseases. Other exclusion criteria include hypertension or hypotension, diabetes, obesity (body mass index [BMI] > 35 kg/m^2), anti-inflammatory medication, and pregnancy. Females were studied between days 1–7 after menses begin to control for hormone status. We asked participants to refrain from exercise and caffeine for at least 24 h before their test. Participants were also asked to fast 4 h prior to their test. This study consisted of one visit in which all participants completed the experimental procedures. Height and weight were assessed using a standard stadiometer and an electronic scale, respectively.

2.2 Experimental Procedures

Participants lay supine in a dimly lit and temperature-controlled room for 10 min before instrumentation. Arterial beat-by-beat blood pressure was continuously monitored throughout the procedure using a finger cuff photoplethysmography sensor (Finometer, Pro, Finapres Medical System, Amsterdam, Netherlands) placed on the middle finger of the left hand. A rapid-release cuff (ED, Hokanson, Bellevue, Washington, USA) was positioned on the right upper arm which was supported by a stationary device (JawStand XP, Rockwell, Charlotte, North Carolina, USA), tilted to ~ 50° above and below heart level. Local pressure in the forearm at the two positions was estimated by adjusting for differences in the hydrostatic column between the heart and mid-forearm. Brachial arterial blood flow and brachial to radial PWV were measured as described below.

2.3 Pulse Wave Velocity

Two pulse tonometers (Millar Instruments, Houston, Texas, USA) were utilized to obtain arterial pressure waveforms between the brachial artery and radial artery of the right arm. The arterial pressure waveform signals were recorded in real time and stored at 1000 Hz using Powerlab (AD instruments, Colorado Springs, Colorado, USA). The calculation of PWV was made from the measured distance between the brachial and radial arterial sites and pulse transit time between arterial pressure waves. The distance between the placement of the pulse tonometer on the brachial and radial artery was measured in a straight line using a tape measure. Pulse transit time was determined by measuring the time delay between the foot of the brachial and radial arterial pulse waves. An average time delay was obtained from the second derivative of the pressure waves for at least seven consecutive brachial and radial pulse waves. Automated calculations were performed using a macro programmed in Powerlab. In our laboratory, the coefficient of variation for resting brachial-radial PWV is 1.7%.

2.4 Brachial Artery Blood Flow

Blood flow in the brachial artery of the experimental arm was assessed using high-resolution Doppler ultrasound (Prosound Alpha 7, Hitachi-Aloka, Japan) equipped with a linear probe operating between the 5–13 MHz range and 60° insonation angle. Mean blood velocity was analyzed with blood velocity analysis software (Cardiovascular Suite, Quipu, Pisa, Italy). Brachial artery blood flow (expressed in mL·min^{-1}) was calculated using the following formula: ([conduit artery diameter (in cm)/2]$^2 \times \pi \times$ mean blood velocity (in cm·s^{-1}) $\times 60$). One-second averages were used for the calculation of blood-flow measurements. The peak blood-flow response was defined as the highest one-second blood-flow value following the release of the cuff occlusion.

2.5 Reactive Hyperemia

Participants underwent two reactive hyperemia conditions on the same day: one with the arm positioned ~ 50° above heart level and one with the arm positioned ~ 50° below heart level, the order of which was counterbalanced. To perform reactive hyperemia, the rapid-release cuff on the right upper arm was inflated to 220 mmHg to occlude arterial inflow and sustained for 5 min. Brachial arterial blood flow was continuously monitored from 1 min before cuff inflation (e.g., baseline) to 3 min after release. PWV measurements were made before cuff inflation (e.g., baseline) and 5, 15, and 30 min after release of the cuff pressure.

2.6 Statistical Analysis

Statistical analyses were performed using IBM SPSS version 28.0. (IBM Corp., Armonk, New York, USA). Statistical significance was a-priori defined at $p \leq 0.05$. A two way (position × time) repeated measures ANOVA was used to evaluate differences in PWV between reactive hyperemia above and below heart level. When a significant effect was found, post-hoc analysis using Bonferroni adjustment was applied. Based on our previously published data which showed a nadir in PWV 5 min post-reactive hyperemia [12], we determined *a-priori* to specifically examine the magnitude of change in PWV from baseline to 5 min post. This comparison was accomplished with a paired sample T-test examining the absolute and relative changes in PWV. Paired sample T-tests were also performed to evaluate the absolute and relative changes in blood flow from baseline to peak values above and below heart level. Data are presented as mean ± standard deviation.

Sample size for this study was estimated based on the variability in the study by Naka et al. 2006. The results of the power analysis indicated that 10 participants would allow detection of a meaningful difference of 1 m/sec in PWV with > 80% power and an alpha at 0.05.

3 Results

A total of 14 participants were included in this study (5 females, 9 males) and descriptive characteristics are presented in Table 1.

Figure 1 depicts the brachial to radial PWV measurements following reactive hyperemia with the experimental arm positioned above and below heart level. There was a significant position × time interaction ($p = 0.028$). Brachial to radial PWV was higher at baseline and all subsequent time points when the arm was positioned below the heart than when it was positioned above the heart. The absolute change in brachial to radial PWV was greater with the arm below the heart compared to above the heart ($p = 0.007$, Fig. 2A). Given the baseline differences between positions, the percentage changes in brachial to radial PWV were also compared. At 5-min after reactive hyperemia, the percent change in PWV was greater when the arm was positioned below the heart compared to when positioned above the heart ($p = 0.043$, Fig. 2B). Mean arterial pressure (MAP) did not change ($p = 0.251$) between positions or across time points (Table 2). The estimated local pressure with the arm

Table 1 Descriptive characteristics of participants ($n = 14$)

Age, years	25 ± 5
Male/Female, n	9/5
Height, cm	169.9 ± 8
Weight, kg	71.2 ± 15
BMI, kg/m^2	24.9 ± 4

Data presented as mean ± SD

BMI body mass index

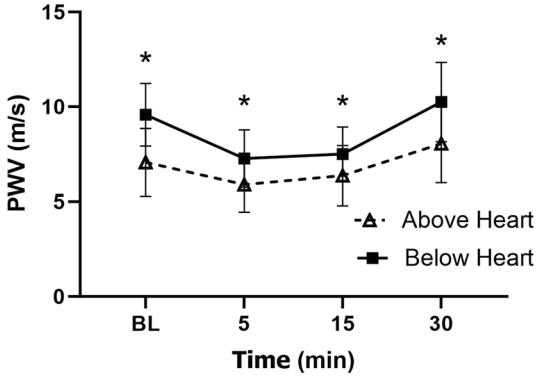

Fig. 1 Brachial to radial pulse wave velocity (PWV) measurements in the experimental arm following reactive hyperemia with the arm positioned above and below heart level. BL = baseline, *Significant differences between above and below heart level ($p < 0.001$), $n = 14$

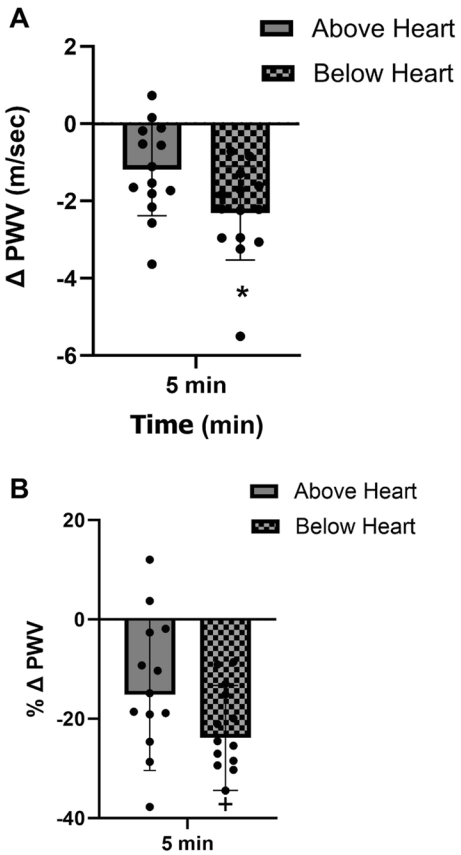

Fig. 2 Change in brachial to radial PWV from baseline to 5-min post. Panel A shows absolute changes and panel B shows relative changes. *$p = 0.007$, +$p = 0.043$ $n = 14$

Table 2 Mean arterial pressure (mmHg) across timepoints

	Baseline	5-min	15-min	30-min
Above Heart Level	91 ± 11	87 ± 9	91 ± 10	92 ± 10
Below Heart Level	88 ± 7	88 ± 8	85 ± 10	91 ± 7

Data presented as mean ± SD

below the heart was 100 mmHg and with the arm above the heart was 54 mmHg.

Figure 3 displays the ensemble averages of the blood-flow responses to the two reactive hyperemia conditions. Immediately following the release of the cuff, there was a rapid increase in blood flow in both positions with the pattern of the responses being similar. Peak blood flows were used to quantitatively assess the positional differences (see Fig. 4). Peak brachial artery blood flow was greater with the arm below the heart (477 ± 145 vs 369 ± 137 ml/min; $p < 0.001$).

4 Discussion

The objective of this study was to explore how increases in conduit artery blood-flow impact peripheral arterial stiffness. A unique aspect of this investigation was manipulating blood-flow responses following reactive hyperemia by altering the limb position, with the arm positioned above and below heart level. The primary findings of the study can be summarized as follows: First, there was an acute reduction in peripheral arterial stiffness following reactive hyperemia, both above and below heart level. Second, there was a greater reduction in peripheral arterial stiffness and a larger blood-flow response when the arm was positioned below the heart rather than above it. These data suggest that the mechanism for position-related differences in the reduction in peripheral arterial stiffness with reactive

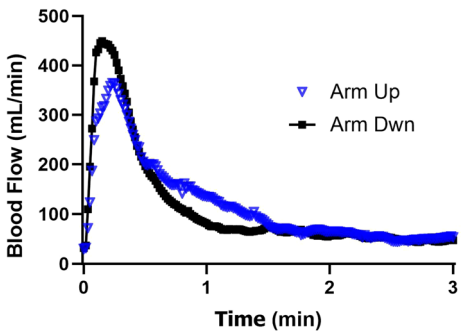

Fig. 3 Ensemble average of individual participants' brachial blood-flow responses to reactive hyperemia above and below heart level. Cuff release occurred at time 0. $n = 14$

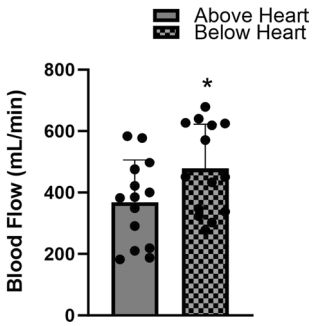

Fig. 4 Peak brachial blood-flow responses to reactive hyperemia when the experimental arm was above and below heart level. *Significant difference between above and below heart level. ($p < 0.001$). $n = 14$

hyperemia is associated with the magnitude of increase in blood flow.

In the current study, the application of reactive hyperemia, whether conducted above or below heart level, resulted in a reduction in peripheral arterial stiffness at 5-min. However, the reduction was ephemeral, returning to baseline levels by 30-min. It might be interesting to ask how quickly the change in PWV can occur after an acute change in flow. However, it is not technically feasible to simultaneously measure brachial artery blood flow with doppler ultrasound and measure brachial-radial PWV with tonometry. Although previous studies did not manipulate limb position, they reported similar outcomes regarding changes in peripheral arterial stiffness in response to reactive hyperemia. In the study conducted by Naka et al. [13], there was an acute decrease in peripheral PWV by 14% following cuff occlusion in healthy individuals, with PWV levels returning to baseline within 10 min. Similarly, Cauwenberghs et al. [14] observed a peak reduction in brachial-radial PWV of ~ 17% following a 5-min occlusion in younger individuals. Ellins et al. [15] found that reactive hyperemia led to an 18% reduction in brachial-radial PWV in healthy individuals. As previously reported by our laboratory [12] reactive hyperemia with the arm at heart level led to an acute reduction in peripheral arterial stiffness of ~ 17% following a 5-min cuff occlusion. Thus, this body of literature consistently indicates that reactive hyperemia results in an acute reduction in peripheral arterial stiffness.

The proposed mechanism underlying the acute reduction in peripheral arterial stiffness to reactive hyperemia is thought to be flow-mediated. Importantly, none of the aforementioned studies by Naka [13], Cauwenberghs [14], and Ellins [15] measured or manipulated blood flow. In our previous study [12] we assessed brachial artery blood flow and manipulated the

blood-flow response to reactive hyperemia via restricting arterial inflow. The results showed that brachial to radial PWV did not change significantly in response to reactive hyperemia when the blood-flow increase was blunted. In the current study to gain further understanding of how changes in blood-flow impact peripheral arterial stiffness, we measured brachial artery blood flow and altered the magnitude of increase in blood flow by manipulating limb position. The results show a larger reduction in peripheral PWV when the arm was positioned below the heart where the increase in blood flow was greater. This finding supports our previous conclusion that the reduction in peripheral arterial stiffness is influenced by the magnitude of blood-flow increase.

Manipulating limb position impacts the magnitude of blood-flow changes following reactive hyperemia. Previous research has revealed that reactive hyperemia elicits a higher blood-flow response when the arm is positioned below heart level [10, 11]. To the best of our knowledge, no prior study has investigated the influence of limb position on peripheral arterial stiffness following reactive hyperemia. The current study highlights the utility of using limb position to manipulate blood flow. When the arm was positioned below the heart, there was a larger increase in blood flow with reactive hyperemia and a greater reduction in peripheral arterial stiffness.

An interesting finding in our study is the observed PWV differences between limb positions. Notably, peripheral PWV was higher when the arm was positioned below the heart compared to when it was above the heart. Our findings align with a study by Zieff et al. [16], which investigated the pressure dependence of arterial stiffness measures and similarly observed that PWV varied based on limb position. Together our results demonstrate that local pressure in the vessels influences vessel stiffness. In the current study, when the arm was below the heart PWV was greater, indicating a stiffer vessel. The stiffer vessels had a greater reduction in PWV after reactive hyperemia. Our expectation is that, given the same stimulus in the absence of flow, stiffer vessels would exhibit a smaller reduction in stiffness than more compliant vessels. Although this reasoning is plausible, we acknowledge the lack of a flow-independent control makes it difficult to verify. Nevertheless, we attribute the greater reduction in peripheral PWV below the heart to the change in blood flow and not the baseline arterial stiffness.

This research broadens our understanding of how PWV responds to reactive hyperemia. A key strength of this study is the use of applanation tonometry for PWV measurement, a method acknowledged as the gold standard for PWV measurement [6, 7]. There are limitations to this study. The researchers were unblinded as to limb position when PWV analyses were completed. However, the use of an automated macro for calculations diminished the possibility of investigator bias. The participants were young healthy adults. Since age is a factor that can influence arterial stiffness, future studies should investigate PWV changes following reactive hyperemia in diverse age groups and clinical conditions.

5 Summary/Conclusion

In summary, the findings indicate: 1) an acute reduction in peripheral arterial stiffness following reactive hyperemia, both above and below heart level, and 2) a greater decrease in peripheral arterial stiffness coupled with a greater blood-flow response when the arm is positioned below the heart rather than above it. These results highlight the impact of conduit artery blood flow on peripheral arterial stiffness as measured by brachial-radial PWV.

Authors' Contributions

The experiments for the purpose of this study were performed at the Integrative Physiology Laboratory at the University of Illinois at Chicago. RJ conceived and designed research, performed experiments, analyzed data, interpreted results of experiments, prepared figures, drafted manuscript, edited and revised manuscript, approved final version of manuscript. NL conceived and designed research, performed experiments, interpreted results of experiments, edited, and revised manuscript and approved final version of manuscript. SS performed experiments, edited and revised manuscript, and approved final version of manuscript. BH performed experiments, edited and revised manuscript, and approved final version of manuscript. PC conceived and designed research, interpreted results of experiments, edited and revised manuscript, approved final version of manuscript.

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Availability of Data and Materials

Data supporting the results of the present study are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest

The authors declare no competing interest.

Ethics Approval and Consent to Participate

All study procedures were approved by the Institutional Review Board at the University of Illinois at Chicago (2022–0996) and in accordance with guidelines set forth by the Declaration of Helsinki except for registration as a database. Verbal and signed written consents were obtained from each participant before participating in this study.

Consent for Publication

Not applicable.

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References

1. Avolio A, Jones D, Tafazzoli-Shadpour M. Quantification of alterations in structure and function of elastin in the arterial media. Hypertension. 1998;32(1):170–5.
2. Katsuda S, Kaji T. Atherosclerosis and extracellular matrix. J Atheroscler Thromb. 2003;10(5):267–74.
3. Folkow B. Structural factors: the vascular wall. Consequences of treatment. Hypertension. 1983. https://doi.org/10.1161/01.HYP.5.5_Pt_2.III58.
4. Heagerty AM, Aalkjaer C, Bund SJ, Korsgaard N, Mulvany MJ. Small artery structure in hypertension. Dual processes of remodeling and growth. Hypertension. 1993;21(4):391–7.
5. Rizzoni D, Porteri E, Castellano M, Bettoni G, Muiesan ML, Muiesan P, et al. Vascular hypertrophy and remodeling in secondary hypertension. Hypertension. 1996;28(5):785–90.
6. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J. 2006;27(21):2588–605.
7. Pereira T, Correia C, Cardoso J. Novel methods for pulse wave velocity measurement. J Med Biol Eng. 2015;35(5):555–65.
8. Thijssen DHJ, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. Am J Physiol-Heart Circ Physiol. 2011;300(1):H2-12.
9. Bots ML, Westerink J, Rabelink TJ, de Koning EJ. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. Eur Heart J. 2005;26(4):363–8.
10. Jasperse JL, Shoemaker JK, Gray EJ, Clifford PS. Positional differences in reactive hyperemia provide insight into initial phase of exercise hyperemia. J Appl Physiol. 2015;119(5):569–75.
11. Bartlett MF, Oneglia A, Jaffery M, Manitowabi-Huebner S, Hueber DM, Nelson MD. Kinetic differences between macro- and microvascular measures of reactive hyperemia. J Appl Physiol. 2020;129(5):1183–92.
12. Jackson RE, Lima NS, Sherman SR, Clifford PS. Reduction in peripheral arterial stiffness after reactive hyperemia is dependent on increases in blood flow. Physiol Rep. 2023;11(24): e15894.
13. Naka KK, Tweddel AC, Doshi SN, Goodfellow J, Henderson AH. Flow-mediated changes in pulse wave velocity: a new clinical measure of endothelial function. Eur Heart J. 2006;27(3):302–9.
14. Cauwenberghs N, Heyrman Y, Thijs L, Yang WY, Wei FF, Zhang ZY, et al. Flow-mediated slowing of brachial-radial pulse wave velocity: methodological aspects and clinical determinants. Artery Res. 2018;21:29–37.
15. Ellins EA, New KJ, Datta DB, Watkins S, Haralambos K, Rees A, et al. Validation of a new method for non-invasive assessment of vasomotor function. Eur J Prev Cardiol. 2016;23(6):577–83.
16. Zieff GH, Heffernan K, Stone K, Fryer S, Credeur D, Hanson ED, et al. The pressure-dependency of local measures of arterial stiffness. J Hypertens. 2019;37(5):956.