Impact of acute exposure to increased hydrostatic pressure and reduced shear rate on conduit artery endothelial function: a limb-specific response

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Padilla J, Sheldon RD, Sitar DM, Newcomer SC. Impact of acute exposure to increased hydrostatic pressure and reduced shear rate on conduit artery endothelial function: a limb-specific response. Am J Physiol Heart Circ Physiol 297: H1103–H1108, 2009.—Unlike quadrupeds, humans exhibit a larger hydrostatic pressure in the lower limbs compared with the upper limbs during a major part of the day. It is plausible that repeated episodes of elevated pressure in the legs may negatively impact the endothelium, hence contributing to the greater predisposition of atherosclerosis in the legs. We tested the hypothesis that an acute exposure to increased hydrostatic pressure would induce conduit artery endothelial dysfunction. In protocol 1, to mimic the hemodynamic environment of the leg, we subjected the brachial artery to a hydrostatic pressure gradient (~15 mmHg) by vertically hanging the arm for 3 h. Brachial artery flow-mediated dilation (FMD) was assessed in both arms before and following the intervention. In protocol 2, we directly evaluated popliteal artery FMD before and after a 3-h upright sitting (pressure gradient ~48 mmHg) and control (supine position) intervention. Our arm-hanging model effectively resembled the hemodynamic milieu (high pressure and low shear rate) present in the lower limbs during the seated position. Endothelium-dependent vasodilation at the brachial artery was attenuated following arm hanging (P < 0.05); however, contrary to our hypothesis, upright sitting did not have an impact on popliteal artery endothelial function (P > 0.05). These data suggest an intriguing vascular-specific response to increased hydrostatic pressure and reduced shear rate. Further efforts are needed to determine if this apparent protection of the leg vasculature against an acute hydrostatic challenge is attributable to posture-induced chronic adaptations.

blood pressure; shear rate; endothelium; high-resolution ultrasound

THE AUGMENTED PROPENSITY to atherosclerosis in the vasculature of the lower extremities, relative to the upper extremities, has been described extensively (15, 22, 37, 40). However, the mechanisms underlying the heterogeneous distribution of atherosclerosis between limb vasculatures remain to be delineated. The focal and independent nature of atherosclerosis may be due to factors that create unique local environments. For example, a discrepancy in blood pressure between upper and lower extremities has been postulated as a mechanism contributing to the vascular limb differences (11, 23, 26, 31, 44). Blood pressure in the upper and lower extremities is similar in the supine posture; however, because of the earth’s gravitational force, blood pressure is greater in the lower limbs compared with the upper limbs during seated or standing positions (20). Because the average American spends two-thirds of the day in an upright posture (13, 18), the legs are exposed to greater pressures than the arms for the majority of the day. The rationale for proposing blood pressure as an underlying mechanism for vascular limb discrepancies is supported by epidemiological data indicating a strong connection between systemic hypertension and atherosclerotic disease (32, 38). Indeed, chronic increases in blood pressure following coartation in animals elicit an impairment of the endothelium (2, 3, 16, 19, 21), a state that is central in the pathogenesis of atherosclerosis (36). Reciprocally, the improvement in femoral artery endothelial function following bed rest (5) could be explained, at least in part, by the removal of the hydrostatic pressure in the lower extremities. It is possible that repeated episodes of elevated hydrostatic pressure at the lower limbs may negatively impact the endothelium, hence contributing to the greater predisposition to atherosclerosis in this vasculature.

The present study consisted of two protocols designed to examine the impact of acute exposure to increased hydrostatic pressure on conduit artery endothelium-dependent vasodilation. In protocol 1, we transiently subjected the brachial artery to a hydrostatic pressure gradient by vertically hanging the arm. This model was designed to mimic, although to a lesser magnitude, the hemodynamic forces present in the lower extremities during upright posture in a vessel (brachial artery) that is relatively unexposed to elevated hydrostatic pressure. In protocol 2, we directly evaluated popliteal artery endothelial function before and following an episode of upright posture, thus determining the effect of hydrostatic pressure in a vessel that is recurrently exposed to higher pressures. We hypothesized that both the brief period of arm hanging and upright posture would result in impairment of the brachial and popliteal artery endothelial function, respectively.

MATERIALS AND METHODS

Subjects

Eleven healthy adults (10 men, 1 woman) participated in protocol 1, and eight adults (all men) participated in protocol 2. All subjects were free of recognized cardiovascular, pulmonary, and metabolic diseases, nonhypertensive (resting blood pressure >140/80 mmHg), nonobese (body mass index <30 kg/m2), nonsmokers, physically active, and had no family history of heart disease. Subjects were not taking medications, including contraceptives. All procedures were approved by the Purdue University Committee for the Protection of Human Subjects. Written informed consent was obtained from each subject before participation in the study.
Study Procedures

Before initiation of study procedures, subjects completed a medical history/health habits questionnaire. In addition, all subjects were familiarized with the equipment and experimental procedures. In protocol 1, brachial artery flow-mediated dilation (FMD), a noninvasive index of endothelial function, was assessed in both arms before and following a 3-h intervention. During this intervention, the subject was positioned prone with one arm vertically hanging while the opposite arm was maintained at heart level to serve as the control. The arm to be hung was randomized across subjects. In protocol 2, popliteal artery FMD was measured in the left leg before and after a 3-h upright sitting (lower legs) intervention. Interventions were conducted on separate days (<1 wk apart), and the order of interventions was randomized.

During both protocols, individuals were instructed to report to the laboratory fasted for 4 h, abstain from caffeine and vitamin supplements for 4 h, and abstain from strenuous physical activity for 12 h. Subjects were positioned comfortably supine in a dark, climate-controlled quiet room (22–24°C). Each subject underwent an acclimation phase of ~60 min to obtain a hemodynamic steady state. An automatic cuff (E-20 rapid cuff inflator; D.E. Hokanson, Bellevue, WA) was placed around the forearm (protocol 1) or lower leg (protocol 2) as previously described (30). The ultrasound image of the brachial and popliteal arteries were obtained longitudinally by a two-dimensional (2D) high-resolution ultrasound system (Terason T3000; Teratech, Burlington, MA), using a 5 to 12-MHz multifrequency linear-array transducer. The brachial artery was imaged 2–5 cm above the antecubital fossa, whereas the popliteal artery was imaged immediately proximal to the bifurcation (usually at or slightly above the popliteal fossa). For easier positioning of the popliteal artery in protocol 2, the subject was repositioned prone during the assessment. Once a satisfactory image was obtained, the studied limb was secured, and the transducer was stabilized using a clamp. The location of the transducer was marked on the skin to ensure consistent placement during subsequent measurements. Doppler velocity was also measured via ultrasound. Doppler flow signals were corrected at an insonation angle of 60°, and measurements were performed with the sample volume placed in midartery. Ultrasound parameters were not changed during the study. Simultaneous Doppler measurements for blood velocity and 2D ultrasound imaging for diameter were continuously taken at baseline (1 min) and immediately following 5 min of forearm cuff occlusion (220 mmHg) for 2 min. Ultrasound images were recorded at 5 frames/s using Camtasia (TechSmith, Okemos, MI) and converted into an AVI file. R-wave gated frames were not captured exclusively because we demonstrated that continuous assessment of diameter at 5 frames/s yields the same FMD as measured with 2 frames/s (30). The brachial and popliteal artery diameters has recently been incorporated by our group (29) and other well-established laboratories (4).

The hydrostatic column was determined by measuring the vertical distance (cm) between the heart level and the site of FMD assessment. The heart level was considered as the intersection between the midaxillary line and the fourth intercostals. To convert to units of millimeters mercury, the distance between the two points was multiplied by a factor 0.766, which includes a correction for the specific gravity of blood (9, 12, 41). Measurements of brachial and popliteal artery blood pressure, arterial diameter, and blood velocity were taken periodically (every hour) throughout the study period. Blood pressure readings were auscultated by a technician who was blinded to the condition for each image. The occipital blood pressure was recorded using an automatic cuff (E-20 rapid cuff inflator; D.E. Hokanson, Okemos, MI) and converted into an AVI file. R-wave gated frames were not captured exclusively because we demonstrated that continuous assessment of diameter at 5 frames/s yields the same FMD results. This approach of employing continuous assessment of diameters has recently been incorporated by our group (29) and other well-established laboratories (4).

Statistical Analysis

Descriptive statistics were used to summarize the demographic information. A one-way repeated-measure ANOVA was used to evaluate the time course of blood pressure, arterial diameter, and shear rate. When a significant time effect was found, Dunnet’s post hoc procedure was used to identify what time points deviated from preintervention. A 2 × 2 (arm × time; protocol 1) and a 2 × 2 (arm × time; protocol 2) ANOVA were performed first. Figure 1 illustrates the experimental design for both protocols.

Data Analysis

Brachial and popliteal artery diameter, blood velocity, and shear rate. Off-line analyses of diameters and velocities were performed using edge-detection Brachial Analyzer software (Medical Imaging Applications, Coralville, IA). In brief, the software allows the user to select the region of interest (ROI) on the portion of the image where the vessel walls are most clear. The arterial wall borders are then detected by an optimal graph search-based segmentation that uses a combination of pixel density and image gradient as an objective function. Each sequence of images was reviewed by the technician and interactively edited when needed to ensure that diameter measurements were always calculated from the intima-lumen interface at the far and near vessel wall. Similarly, for determination of blood velocity, the ROI was selected around the Doppler waveform, and the trace of the velocity-time integral was used to calculate mean velocity for each cardiac cycle. In addition, maximum (peak anterograde) and minimum (peak retrograde) velocities were measured for each cardiac cycle with the use of a calibrated caliper. All measurements were performed by a technician who was blinded to the condition for each image sequence. The postocclusion peak dilation was determined as the highest 3-s average and was represented as a percent change from baseline diameter (FMD; %). Shear rate (s⁻¹) was calculated using the following formula: 4 × BV/D, where BV is blood velocity (cm/s), and D is arterial diameter (cm) (25). For calculations of mean, maximal, and minimum shear rate, mean, maximal and minimum blood velocities were used, respectively.

To quantify the hyperemic stimulus used for FMD normalization, shear rate area under the curve (AUC; s⁻¹·s) above baseline was individually calculated for the duration of time-to-peak dilation (29, 34). Briefly, the AUC was calculated by summing the areas of successive postocclusion trapezoids (each with a base of 3 s) until peak dilation. Normalization of FMD to shear rate was calculated as the ratio between FMD and shear rate AUC (29, 34).

Fig. 1. Experimental design for protocol 1 and protocol 2. BP, blood pressure; FMD, flow-mediated dilation (in protocol 1, FMD was performed on both arms consecutively).
HYDROSTATIC PRESSURE AND ENDOTHELIAL FUNCTION

RESULTS

The characteristics of subjects in protocol 1 (age = 23.6 ± 1.6 yr; body mass index = 23.4 ± 0.6 kg/m) and protocol 2 (age = 24.0 ± 1.7 yr; body mass index = 23.7 ± 0.6 kg/m) were similar. All subjects were physically active according to the United States Surgeon General’s guidelines; that is, they perform at least 30 min of moderate physical activity on most days of the week. The calculated hydrostatic column created at the brachial artery with arm hanging was 14.9 ± 0.8 mmHg, whereas the pressure column imposed at the popliteal artery during upright sitting was 48.6 ± 1.7 mmHg. Discrepancies between calculated and measured (Figs. 2A and 3A) hydrostatic columns could be attributed to slight differences in the auscultation site or measurement error. Figure 2A shows the time course of measured brachial artery blood pressure, arterial diameter, and shear rate throughout the 3-h arm hanging period. As indicated, a significant increase in blood pressure and a reduction in mean and maximum shear rate was observed during upright sitting. However, contrary to arm hanging, upright sitting did not impair popliteal artery FMD [F(1,14) = 1.95, P = 0.184]. As noted, the slight trend toward a difference was fully removed after normalizing FMD to shear rate [F(1,14) < 0.001; P = 0.99] (Fig. 3B). Tables 1 and 2 display means for the remaining dependent variables, including baseline arterial diameter, shear rate AUC, time-to-peak dilation, mean arterial pressure, and heart rate. No significant differences were observed for any of these variables.

DISCUSSION

The heterogeneous distribution of atherosclerosis between limb vasculatures is well documented (15, 22, 37, 40); however, the mechanisms underlying this divergence remain controversial. It is conceivable that repeated episodes of elevated hydrostatic pressure in the lower limbs may negatively impact the endothelium, hence contributing to the greater predisposition of atherosclerosis. To further explore this concept, we conducted novel experiments aimed at examining the impact of acute exposure to increased hydrostatic pressure on conduit artery endothelium-dependent vasodilation. First, we transiently submitted the brachial artery to a hydrostatic pressure gradient by vertically hanging the arm. This model was designed to mimic, although to a lesser degree, the hemodynamic forces present in lower extremities during upright posture. Second, we directly evaluated popliteal artery endothelial function fol-
following a brief period of upright sitting. Consistent with our hypothesis, brachial artery endothelium-dependent vasodilation was impaired following a hydrostatic gradient of ~15 mmHg imposed by arm hanging. However, contrary to our premise, popliteal artery endothelial function was unaltered after a short-term episode of upright sitting during which a pressure gradient of ~48 mmHg was created. Together, these results suggest an intriguing limb-specific response to increased hydrostatic pressure. The apparent protection of the popliteal artery against a hydrostatic challenge could be attributed to the fact that the leg is chronically exposed to higher pressures and, therefore, may have developed adaptations to deal with these particular hydrostatic influences. Likewise, the increased sensitivity in the brachial artery may be due to its naturally lesser exposure to hydrostatic pressure.

Our findings are well supported by the only human study designed to evaluate the acute effects of elevated arterial pressure on endothelial function. In this study, Jurva et al. (14) utilized leg press exercise to transiently increase blood pressure in a group of adults engaging in regular weight lifting and in a group of nonweight lifters. The authors reported a 65% brachial artery FMD reduction following leg press exercise in the nonweight lifters; however, FMD was unchanged in the regular weight lifters. Similar to our results, this differential response suggests that repeated episodes of increased pressure (in their case induced by resistance training) may protect the vasculature against subsequent bouts.

The impact of acute increases in blood pressure has also been studied in vitro with contradicting results. Ungvari et al. (43) exposed rat femoral arterial branches to normal (80 mmHg) and high (160 mmHg) intraluminal pressures for 30 min and found high pressure to impair endothelial function due to an overproduction of vascular superoxide radicals. Conversely, Woodman et al. (45) subjected soleus muscle feed arteries from young and old rats to low (90 cmH\textsubscript{2}O) and high (130 cmH\textsubscript{2}O) intraluminal pressure for 4 h and found high pressure to improve endothelial function in the old rats but not in the young rats. These discrepancies in findings could perhaps be explained by differences in studied vasculatures and length of pressure exposures.

Table 1. Hemodynamic information for each flow-mediated dilation measurement in protocol 1

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<th>Hanging Arm</th>
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<th>Control Arm</th>
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<td></td>
<td>Preintervention</td>
<td>Postintervention</td>
<td>Preintervention</td>
<td>Postintervention</td>
</tr>
<tr>
<td>BA baseline diameter, mm</td>
<td>4.0±0.1</td>
<td>3.9±0.2</td>
<td>3.9±0.2</td>
<td>3.8±0.2</td>
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<tr>
<td>BA shear rate AUC, AU</td>
<td>4,026.8±365.2</td>
<td>3,583.3±372.7</td>
<td>3,734.4±375.8</td>
<td>4,312.0±330.6</td>
</tr>
<tr>
<td>BA time-to-peak dilation, s</td>
<td>61.4±5.5</td>
<td>55.7±7.4</td>
<td>58.1±7.4</td>
<td>54.8±5.4</td>
</tr>
<tr>
<td>BA mean arterial pressure, mmHg</td>
<td>83.6±2.7</td>
<td>86.9±1.8</td>
<td>85.0±2.5</td>
<td>87.5±2.0</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>46.9±1.4</td>
<td>48.0±1.4</td>
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Values are means ± SE; n = 11 subjects. BA, brachial artery; AUC, area under the curve; AU, arbitrary units.
We are unable to exclusively attribute the vascular responses observed in this study to the increase in arterial pressure, since there was also a decrease in shear rate in both the brachial and popliteal arteries during arm hanging and upright sitting, respectively (Figs. 2A and 3A). A large body of evidence from in vitro (cell culture and intact vessel) and in vivo studies using animal models indicates that low shear negatively impacts the endothelium (6–8, 10, 17, 35, 39). The clear observation that upright sitting reduces shear rate prompts us to believe that disturbed flow patterns occurring in the leg vasculature during upright postures may be another factor contributing to vascular phenotypic differences between limbs. Evidently, our arm hanging model effectively resembled the hemodynamic milieu present in the lower limbs during the seated position. Because making the arm “look” like the leg resulted in blunted endothelium-dependent vasodilation at the brachial artery, this study supports the view that, when a “healthy” vessel is abruptly exposed to a proatherogenic hemodynamic environment, the endothelium becomes compromised. In contrast, our data suggest that a vessel that has been persistently exposed to detrimental forces (i.e., popliteal artery) may have developed protective adaptations and, as a result, may be capable of withstanding additional acute challenges. Alternatively, it is also possible that an acute impairment cannot be detected in a chronically dysfunctional endothelium. Hypothetically, therefore, arms and legs of crawling infants or quadrupeds should exhibit similar vascular function at baseline and in response to a given hemodynamic perturbation. Future research is needed to test this interesting hypothesis.

We speculate that the reduction in shear rate associated with arm hanging and upright sitting could be because of an alteration in microvascular regulation secondary to hydrostatic forces to maintain constant flow, or an increase in venous congestion. These alterations in the experimental limb may also have contributed to the modest decrease in postischemia reactive hyperemic stimulus (shear rate AUC) (Table 1). Reciprocally, Bahadir et al. (1) showed that, when forearm venous volume is reduced by transiently elevating the arm, the hyperemic response to 5 min of forearm occlusion is augmented. The trends of reduced shear stimulus found in our study could have been exclusively responsible for the attenuated FMD response following arm hanging; however, after normalizing FMD to shear rate, the vasodilation response remained blunted.

This study has a number of limitations that are important to note. First, while the arm hanging model appeared to successfully reproduce the hemodynamic forces present in the leg during upright posture, a 3-h period may not be sufficient to fully represent the pathophysiological responses. Second, because endothelium-independent dilation was not evaluated, we can only presume that the vascular changes observed are endothelium and not smooth muscle mediated. Further studies administering a nitric oxide donor and/or inhibiting endothelial nitric oxide synthase may provide valuable mechanistic information. Third, we did not measure shear rate in the control arm during the intervention period because of our inability to accurately scan the brachial artery when the subject was positioned prone. However, we currently have no reason to believe that the control arm had an alteration in shear rate as that occurred in the hanging arm. Fourth, because different subjects were used for protocols 1 and 2, direct comparisons between arm and leg should be done with caution. A purposeful effort was made to maintain the characteristics of the subjects similar between protocols. In addition, it is important to note that all of the individuals were physically active; therefore, these findings cannot be generalized to the sedentary population whose chronic adaptation to environmental influences may be different. It could be speculated that physically inactive subjects would not have developed the necessary adaptations in the resistance vessels to “handle” increases in load imposed by upright posture. Because the only female subject followed the same response trend as the other individuals, we do not consider the imbalance between men and women a limitation to the study.

A plethora of recent studies have carefully characterized vascular function differences between the arm and the leg (23, 24, 26–28, 30, 33, 42, 46). At present, it appears fundamental to advance from this descriptive research to a more mechanistic approach. Understanding the mechanisms underlying the evolution toward limb discrepancies in vascular diseases may have significant implications for new therapeutic strategies aimed at primary prevention. For the first time, our data demonstrates an intriguing limb-specific response to increased hydrostatic pressure and reduced shear rate. Using novel approaches, future research should continue focusing on delineating the biological mechanisms governing this captivating vascular limb-differences phenomenon.

ACKNOWLEDGMENTS

We thank all of the subjects for their time, effort, and willingness to participate in the study. We also thank Chris Kapp, Megan Dubenetzky, Ian Skarbek, and David Maurer for assistance in data analysis.

Table 2. Hemodynamic information for each flow-mediated dilation measurement in protocol 2

<table>
<thead>
<tr>
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<th>Upright Sitting</th>
<th>Postintervention</th>
<th>Control</th>
<th>Postintervention</th>
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<tbody>
<tr>
<td>PA baseline diameter, mm</td>
<td>6.0±0.3</td>
<td>5.7±0.2</td>
<td>5.9±0.3</td>
<td>5.7±0.2</td>
</tr>
<tr>
<td>PA shear rate AUC, AU</td>
<td>2.724.5±505.5</td>
<td>2.043.6±366.4</td>
<td>2.880.1±295.5</td>
<td>3.108.6±502.7</td>
</tr>
<tr>
<td>PA time-to-peak dilation, s</td>
<td>78.8±14.3</td>
<td>74.3±13.4</td>
<td>86.3±7.1</td>
<td>91.5±13.4</td>
</tr>
<tr>
<td>PA mean arterial pressure, mmHg</td>
<td>91.8±1.8</td>
<td>96.3±5.4</td>
<td>92.3±4.3</td>
<td>93.8±3.6</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>54.0±2.3</td>
<td>54.0±2.5</td>
<td>56.3±1.6</td>
<td>57.0±2.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects. PA, popliteal artery.
REFERENCES


