Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear stress

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1Medical Pharmacology and Physiology, University of Missouri, Columbia, Missouri; 2Nutrition and Exercise Physiology, University of Missouri, Columbia, Missouri; 3Kinesiology, University of Texas-Arlington, Arlington, Texas; 4Dalton Cardiovascular Research Center, University of Missouri, Columbia, Missouri; and 5Child Health, University of Missouri, Columbia, Missouri

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Restaino RM, Walsh LK, Morishima T, Vranish JR, Martinez-Lemus LA, Fadel PJ, Padilla J. Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear stress. Am J Physiol Heart Circ Physiol 310: H648–H653, 2016. First published January 8, 2016; doi:10.1152/ajpheart.00943.2015.—We and others have recently reported that prolonged sitting impairs endothelial function in the leg vasculature; however, the mechanism(s) remain unknown. Herein, we tested the hypothesis that a sustained reduction in flow-induced shear stress is the underlying mechanism by which sitting induces leg endothelial dysfunction. Specifically, we examined whether preventing the reduction in shear stress during sitting would abolish the detrimental effects of sitting on popliteal artery endothelial function. In 10 young healthy men, bilateral measurements of popliteal artery flow-mediated dilation were performed before and after a 3-h sitting period during which one foot was submerged in 42°C water (i.e., heated) to increase blood flow and thus shear stress, whereas the contralateral leg remained dry and served as internal control (i.e., nonheated). During sitting, popliteal artery mean shear rate was reduced in the nonheated leg (pre-sit, 42.9 ± 4.5 s⁻¹; and 3-h sit, 23.6 ± 3.3 s⁻¹; P < 0.05) but not in the heated leg (pre-sit, 38.9 ± 3.4 s⁻¹; and 3-h sit, 63.9 ± 16.9 s⁻¹; P > 0.05). Popliteal artery flow-mediated dilation was impaired after 3 h of sitting in the nonheated leg (pre-sit, 7.1 ± 1.4% vs. post-sit, 2.8 ± 0.9%; P < 0.05) but not in the heated leg (pre-sit: 7.3 ± 1.5% vs. post-sit, 10.9 ± 1.8%; P > 0.05). Collectively, these data suggest that preventing the reduction of flow-induced shear stress during prolonged sitting with local heating abolishes the impairment in popliteal artery endothelial function. Thus these findings are consistent with the hypothesis that sitting-induced leg endothelial dysfunction is mediated by a reduction in shear stress.

NEW & NOTEWORTHY

Data from the present study reveal that preventing the reduction of shear stress during prolonged sitting with local heating abolishes the impairment in popliteal artery endothelial function. Therefore, this study provides evidence that a reduction in shear stress mediates sitting-induced leg endothelial dysfunction.

THE AUGMENTED PROPENSITY TO ATHEROSCLEROSIS IN THE VASCULATURE OF THE LOWER EXTREMITIES...
vided written informed consent. Subjects were recreationally active, nonsmokers, with no history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease as determined from a detailed medical health history questionnaire. No subjects were using prescribed or over-the-counter medications.

Experimental procedures. A schematic of the study design is presented in Fig. 1 illustrating the sequence of events and various positions in which measurements were made. Subjects were 2-h postprandial upon arrival to the laboratory. All study visits were performed in a temperature-controlled room kept at 21–22°C. Upon arrival to the laboratory, subjects were placed in a supine position and instrumented with an automated sphygmomanometer (SphygmoCor XCEL, AtCor Medical, Itasca, IL) for periodic measurements of arterial blood pressure (BP) after resting quietly for 10 min. Popliteal artery diameter and blood velocity were measured using duplex-Doppler ultrasound (Logiq P5; GE Medical Systems, Milwaukee, WI). An 11-MHz linear array transducer was placed over the popliteal artery just distal to the popliteal fossa. Simultaneous diameter and velocity signals were obtained in duplex mode at a pulsed frequency of 5 MHz and corrected with an insolation angle of 60°. Sample volume was adjusted to encompass the entire lumen of the vessel without extending beyond the walls, and the cursor was set at midvessel. Popliteal artery FMD was assessed in the entire lumen of the vessel without extending beyond the walls, and the order of assessments was randomized between nonheated and heated leg within each subject.

Data analysis. Mean blood flow was calculated from continuous diameter and mean blood velocity recordings at each of the experimental time points using the following equation: 3.14·(diameter/2)^2·mean blood velocity·60. Hyperemic blood flow area under the curve (AUC) was calculated for the entire period in which blood flow was above baseline values using the sum of trapezoids method (20). Popliteal artery FMD percent change was calculated using the following equation: 90·FMD = (peak diameter − baseline diameter)/baseline diameter·100. Shear rate was defined as 8·mean blood velocity/diameter (22). Hyperemic shear rate AUC up to peak diameter was calculated as stimulus for FMD, as previously described (2, 33).

Statistical analysis. The two-way (time × leg), repeated-measures ANOVA test was performed using SPSS software (version 23). Significance was accepted at P ≤ 0.05. Data are expressed as means ± SE. Based on data from our previous sitting study (28), we performed a power calculation and determined that eight subjects would be needed to detect a statistically significant (P < 0.05) effect of sitting on leg endothelial function with a power of 0.8.

RESULTS

Over the course of the sitting period, popliteal artery blood flow (Table 1) and shear rate (Fig. 2A) were progressively reduced under the nonheated control condition (P < 0.05). In contrast, blood flow and shear rate in the heated leg were not reduced during sitting and were higher than in the nonheated leg (Fig. 2A, P < 0.05). These results indicate the effectiveness of limb heating in preventing the decline in blood flow and shear rate during the sitting period. As illustrated in Fig. 2B, several subjects actually exhibited an increase in blood flow and shear rate during sitting in the heated leg with the magnitude of this increase being variable among subjects. Nonetheless, the impairment in popliteal artery FMD after 3 h of sitting in the nonheated leg (7.1 ± 1.4 to 2.8 ± 0.9%; Cohen’s d = 1.16; P < 0.05) was prevented in the heated leg (7.3 ± 1.5 to 10.9 ± 1.8%; Cohen’s d = 0.68; P > 0.05; Fig. 3). Hyperemic shear rate AUC and blood flow AUC were similar between legs before sitting and reduced in both legs after sitting (Table 1). FMD corrected for hyperemic shear rate AUC using ANCOVA did not affect the interpretation of the main findings (Table 1). No changes were observed in popliteal artery diameter over time, and no differences between legs were detected across time points (Table 1). BP was unaffected by the period of sitting (pre-sit: systolic BP = 123.6 ± 2.8 mmHg, diastolic BP = 76.4 ± 3.6 mmHg, and mean arterial pressure = 92.1 ± 3.1 mmHg; and post-sit: systolic BP =
Table 1. Popliteal artery hemodynamics in nonheated and heated legs before, during, and after sitting for 3 h

<table>
<thead>
<tr>
<th></th>
<th>Basal Diameter, cm</th>
<th>Basal Blood Flow, ml/min</th>
<th>Hyperemic Blood Flow AUC, arbitrary units</th>
<th>Hyperemic Shear Rate AUC, arbitrary units</th>
<th>ANCOVA-Corrected FMD, %</th>
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<tbody>
<tr>
<td></td>
<td>Nonheated Heated</td>
<td>Nonheated Heated</td>
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<td>Nonheated Heated</td>
</tr>
<tr>
<td>Supine Pre-sit</td>
<td>0.59 ± 0.03 0.59 ± 0.03</td>
<td>58.9 ± 11.2 47.1 ± 5.0</td>
<td>45,990 ± 8,986 42,552 ± 6,725</td>
<td>34,259 ± 6,155 30,640 ± 4,018</td>
<td>6.9 ± 1.5 7.2 ± 1.5</td>
</tr>
<tr>
<td>Sitting 0 h</td>
<td>0.58 ± 0.02 0.57 ± 0.02</td>
<td>36.2 ± 5.5 79.4 ± 13.7</td>
<td>23.8 ± 3.7 72.8 ± 15.5</td>
<td>25.1 ± 3.3 64.7 ± 12.4</td>
<td>20.715 ± 5.17 ± 8,253</td>
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<td></td>
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<td></td>
<td>Time, P = 0.474</td>
<td>Leg, P = 0.648</td>
<td>ANOVA Leg, P = 0.526</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Interaction, P &lt; 0.001</td>
<td>Interaction, P &lt; 0.001</td>
<td>Interaction, P = 0.001</td>
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<tr>
<td>1 h</td>
<td>0.59 ± 0.02 0.57 ± 0.02</td>
<td>32.3 ± 6.7 79.5 ± 16.3</td>
<td>28,749 ± 8,253</td>
<td>Leg, P = 0.003</td>
<td>Interaction, P = 0.003</td>
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<td>Leg, P = 0.664</td>
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<td>2 h</td>
<td>0.57 ± 0.02 0.57 ± 0.02</td>
<td>23.8 ± 3.7 72.8 ± 15.5</td>
<td>18,281 ± 3,846</td>
<td>Leg, P = 0.048</td>
<td>Interaction, P = 0.944</td>
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<td>Leg, P = 0.006</td>
<td>Interaction, P = 0.399</td>
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<tr>
<td>3 h</td>
<td>0.57 ± 0.02 0.56 ± 0.02</td>
<td>25.1 ± 3.3 64.7 ± 12.4</td>
<td>16,807 ± 4,531</td>
<td>Leg, P = 0.048</td>
<td>Interaction, P = 0.798</td>
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<tr>
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<td>Time, P &lt; 0.001</td>
<td>Leg, P = 0.006</td>
<td>Interaction, P = 0.011</td>
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<tr>
<td>Supine Post-sit</td>
<td>0.58 ± 0.02 0.59 ± 0.03</td>
<td>30.8 ± 4.7 56.2 ± 11.8</td>
<td>29 ± 1.5 11.0 ± 1.5</td>
<td>Leg, P = 0.048</td>
<td>Interaction, P = 0.003</td>
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<td></td>
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<td>Time, P = 0.001</td>
<td>Leg, P = 0.006</td>
<td>Interaction, P = 0.003</td>
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<td>Leg, P = 0.648</td>
<td>Interaction, P &lt; 0.001</td>
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Values are means ± SE. ANCOVA-corrected flow-mediated dilation (FMD) data are adjusted for hyperemic shear rate area under the curve (AUC). *P < 0.05, vs. pre-sit; †P < 0.05, between legs.
vascular dysfunction with sitting requires attention. Given our earlier finding that sitting was accompanied with a marked reduction in popliteal artery blood flow and shear rate (28), our next logical step was to test the hypothesis that the sustained reduction of shear stress during sitting was the mediator of leg endothelial dysfunction. This was accomplished by increasing leg vascular conductance with local heating of one foot during sitting in an experimental setting where the contralateral leg served as an internal control. Consistent with our hypothesis, we provide evidence that leg endothelial dysfunction following sitting can be abrogated by preventing the decrease in shear during sitting.

The notion that maintenance of shear stress is critical for sustaining optimal vascular health is supported by a plethora of both in vitro and in vivo studies. For example, studies using the proatherogenic apolipoprotein-E null mice demonstrate that chronic experimental reduction of carotid artery shear stress impairs endothelial function and promotes atherosclerotic lesions (3, 18). Similarly, studies in humans also demonstrate that short-term (i.e., 30 min) experimental induction of low (and oscillatory) conduit artery shear stress by inflation of a distal cuff blunts FMD in both upper and lower extremities and causes endothelial inflammation (11, 12, 31, 34, 37). Notably, recent data indicate that these effects of low shear stress on brachial artery endothelial function persist when the reduction in shear stress via forearm compression is maintained for 2 wk (35), thus further emphasizing the importance of shear stress for maintaining optimal endothelial health.

Although not the purpose of this study, a discussion on potential mechanisms contributing to the reduction in leg blood flow during sitting is deserved. It is possible that increased hydrostatic pressure within the leg vasculature provokes blood to pool within the venous circulation. In this regard, in our previous study we found an increase in calf circumference during sitting (28), thus suggesting that blood pooling is indeed occurring in the lower limbs. This effect may be exacerbated by reduced skeletal muscle activity during sitting, which eliminates any contribution of the muscle pump in facilitating venous return (6). Other factors that may contribute to an increase in leg vascular resistance during an orthostatic stress, such as sitting, are venous distension-induced arterial constriction and increased hydrostatic pressure-induced myogenic constriction (14). In addition, given that muscle sympathetic nerve activity is greater in the upright position compared with supine (27), adrenergic vasoconstriction may also contribute to the increased leg vascular resistance. In light of the present findings that sitting-induced reduction in blood flow and shear mediate the impairment in endothelial function, further research is needed to determine the mechanisms by which sitting increases leg vascular resistance. A better understanding of these mechanisms can lead to the development of strategies that prevent increased leg vascular resistance and thus the reduction in blood flow and shear during sitting.

Another salient observation of the present study is that reactive hyperemia, indicative of microvascular dilator function, was reduced after sitting in both legs. The finding that local heating-induced limb blood flow did not prevent the decline in microvascular dilator function with sitting may suggest that the skeletal muscle resistance vasculature within the lower limb was not exposed to a robust increase in shear stress with local heating. It is likely that most of the increase in limb blood flow was directed to skin as the heated area was limited to the foot. Future research is needed to determine if increasing the limb surface area subjected to heat (i.e., encompassing the calf) during sitting produces an increase in muscle blood flow (9), thus also preventing the impairment in microvascular dilator function associated with sitting.

Discussion of several considerations for the overall interpretation of the current findings is warranted. First, this study included only healthy young men, thus the generalizability of the findings remain limited to this population. The lack of a nonsitting control condition could be considered a potential limitation of the study. However, other studies show that over a 3-h period when subjects interrupt their sitting time, there is no decline in leg vascular function (17, 36). Lastly, although we aimed to maintain shear with heating during the prolonged sitting period, we observed an increase in mean shear during this intervention, although not statistically significant. Careful review of the individual data indicate that two big responders primarily drive the increase in mean shear with heating (Fig. 2B). Nevertheless, overall, heating was fairly effective in preventing the decrease in shear with prolonged sitting in the majority of subjects studied. Thus, although it remains unclear as to whether maintaining or slightly enhancing shear would be better during sitting, the current findings clearly demonstrate that preventing the reduction in shear with prolonged sitting also prevents the impairments in leg endothelial function.

The clinical relevance of the present findings should be highlighted. It is possible that the decline in leg endothelial function associated with sitting contributes to the increased propensity of atherosclerosis in the lower extremities (1, 15, 16, 30, 32); however, more research is needed to determine the long-term vascular ramifications of too much sitting. Although the prognostic value of popliteal artery FMD remains unknown, the finding that sitting lowered FMD by ~4% (absolute units) should be considered in light of epidemiological data suggesting that a 1% decrease in brachial artery FMD is associated with a 13% increase of cardiovascular events in low-risk and high-risk populations (7, 10). Importantly, the finding that preventing the decrease in popliteal artery shear rate during sitting via heating of the
foot prevented the decline in leg endothelial function may stimulate creation of simple therapeutic strategies (e.g., local heating) used to offset or alleviate the detrimental vascular effects of prolonged sitting. These interventions could be particularly favorable in clinical populations that are susceptible to peripheral artery disease such as patients with type 2 diabetes or spinal cord injury, who are limited in their physical activity and spend a large portion of their day sitting.

In conclusion, the present study revealed that preventing the reduction of shear stress during prolonged sitting with local heating abolishes the impairment in popliteal artery endothelial function. Therefore, this study provides evidence that a reduction in shear stress mediates sitting-induced leg endothelial dysfunction.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


