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IMPAIRED POPLITEAL ARTERY FLOW-MEDIATED DILATION CAUSED BY REDUCED DAILY PHYSICAL ACTIVITY IS PREVENTED BY INCREASED SHEAR STRESS

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Running Head: Physical inactivity and vascular dysfunction

Author contributions

A.L.T., J.P. and L.C.V. conception and design of research; A.L.T. and L.C.V. performed experiments; A.L.T. and L.C.V. analyzed data; A.L.T., J.P. and L.C.V. interpreted results of experiments; A.L.T. prepared figures; A.L.T., J.P. and L.C.V. drafted manuscript; J.P. and L.C.V. edited and revised manuscript; A.L.T., J.P. and L.C.V. approved final version of manuscript.

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ABSTRACT

We recently showed that five days of reduced daily physical activity impairs popliteal artery, but not brachial artery, flow-mediated dilation (FMD). However, the mechanisms by which physical inactivity causes leg vascular dysfunction are unclear. We reason that a reduction in leg blood flow-induced shear stress is a primary underlying mechanism by which reduced daily physical activity impairs popliteal artery FMD. Thus, the purpose of this study was to determine whether increased leg blood flow and shear stress during inactivity prevents the reduction in popliteal artery FMD. Bilateral popliteal artery FMD measures were performed at baseline and after five days of a transition from high (>10,000 steps/day) to low levels (<5,000 steps/day) of physical activity in 13 healthy and physically active men (20±2 years). During the inactive period, one foot was submerged in ~42°C water (i.e., heated leg), three times a day for 30-min each period, to increase blood flow and thus shear stress, whereas the contralateral leg remained dry and served as internal control (i.e., nonheated leg). During heating, popliteal artery mean shear rate was increased in the heated leg (Δ119.3 ± 26.4%, P<0.01), but slightly decreased in the nonheated leg (Δ-21.8 ± 7.5%, P=0.03). Popliteal artery FMD was impaired after five days of reduced daily physical activity in the control nonheated leg (P<0.01), but was unchanged in the heated leg (P=0.34). These results support the hypothesis that reduced leg blood flow-induced shear stress during physical inactivity is a key underlying mechanism mediating leg vascular dysfunction.

Key words: Physical inactivity; exercise; sedentary; flow-mediated dilation; shear stress.

New & Noteworthy

We found that the impairment in popliteal artery flow-mediated dilation caused by physical inactivity can be prevented by increased shear stress. These findings indicate that reduced leg blood flow-induced shear stress during physical inactivity may be a key underlying mechanism mediating the detrimental leg vascular effects of physical inactivity. Heating the feet area may be used as a non-pharmacological therapy to combat inactivity-induced leg vascular dysfunction, especially in people who are unable or unwilling to be active.

INTRODUCTION

It is estimated that a sedentary lifestyle contributes to 6-10% of all deaths from chronic diseases (5), which is equivalent to the number of deaths attributed to smoking (10). The impact of inactivity is especially profound in the cardiovascular system. Indeed, epidemiological data demonstrate that individuals who are most inactive have the highest risk for cardiovascular disease morbidity and mortality (4, 19). Despite the well-established relationship between inactivity and cardiovascular disease risk, there is a relative paucity of experimental data on the vascular effects of reduced daily physical activity. Furthermore, the mechanisms by which inactivity increases vascular burden remain largely unknown.

In an effort to evaluate the vascular effects of physical inactivity, we recently performed a study designed to emulate the degree of inactivity encountered in real-life (6). Specifically, regularly active individuals meeting current guidelines of more than 10,000 steps/day were transitioned into a period of reduced activity (<5,000 steps/day) for 5 days. We found that reduced activity impaired popliteal artery flow-mediated dilation (FMD). Interestingly, brachial artery FMD was not affected by 5 days of reduced physical activity (6). The finding of decreased popliteal but not brachial artery FMD may be related to the fact that popliteal arteries, upon reduction of locomotion, were subjected to a greater decrease in blood flow and thus shear stress, relative to the brachial arteries. Because shear stress is an important stimulus for maintaining endothelial health (7, 11, 15, 20, 25, 35), it is plausible that with reduced activity, “knock down” of shear stress in the legs is an underlying factor mediating the impairment in popliteal artery function. In this sense, the

development of non-pharmacological therapies to combat inactivity-induced detrimental leg vascular effects is needed. In addition, the assessment of leg vascular function is important in light of studies demonstrating that the leg vasculature is highly susceptible to atherosclerosis, relative to other disease-resistant vasculatures such as the brachial artery (1, 13, 14, 32, 33).

It has been previously shown that local heating at 42°C is an effective stimulus for dilating the skin circulation, increasing limb blood flow and thus shear stress without producing major systemic cardiovascular effects (24, 26-28). Herein, we reasoned that if reduced shear stress is indeed a primary mechanism by which reduced physical activity impairs popliteal artery FMD, an increase in leg blood flow and shear stress during inactivity would produce a vascular protective effect. Specifically, we examined whether preventing the sustained reduction in shear stress by intermittent foot heating during five days of reduced daily physical activity would abolish the detrimental effects of inactivity on popliteal artery FMD.

METHODS

Participants

Thirteen apparently healthy (determined by a detailed heath history questionnaire), recreationally active men (age: 20 ± 2 y, height: 177.4 ± 7.5 cm, weight: 76.6 ± 10.5 kg, mean ± SD) were enrolled in this study. Physical activity was self-reported and recreationally active was defined as completing habitual physical activity for at least 6 consecutive months with a minimum frequency of 3 days per week in ≥30-min sessions and taking greater than 10,000 steps per day. Subjects had to be asymptomatic, non-smokers, normotensive and non-diabetic. All subjects confirmed to have no heart disease and were using no prescribed or over-the-counter medications.

All study procedures were approved by the University of Brasilia research committee (CAAE: 49631815.6.0000.0030) in accordance with the Declaration of Helsinki. All subjects participated in the present study voluntarily, receiving no financial incentive. Participants were informed that they could withdraw at any time and each subject read and signed a specific informed consent form.

Experimental protocol

Bilateral popliteal artery FMD was measured before and after five days of reduced daily physical activity. Before baseline measurements, participants wore an accelerometer (Garmin, vívofit, Kansas, USA) during five days and were asked to follow their normal physical activity patterns. Subjects were then instructed to reduce daily physical activity during five consecutive days by

transitioning from high (>10,000 steps/day) to low daily physical activity (<5,000 steps/day) (6). Subjects were also instructed to avoid any planned exercise sessions. Steps per day were measured using the accelerometer. To achieve the target goal of reduced steps, under certain circumstances, some subjects went home and/or commuted by taxi arranged by study personnel.

To test our initial hypothesis that shear stress is a primary underlying mechanism by which reduced daily physical activity impairs popliteal artery FMD, during the five days of inactivity, one foot was submerged in 40–42°C water (i.e., heated leg), whereas the contralateral foot remained in open air and served as internal control (i.e., nonheated leg). Leg assignment was randomized. The heating protocol was performed three times a day for 30-min each period and comprised of one foot being submerged up to the ankle in a commercially available foot spa (Britânia, Aqua Foot, Brazil) with temperature of the water maintained at ~42°C by using an automated thermometer (i.e., heated leg). A total of 15 sessions of heating were performed by each participant. All participants were trained in utilizing the heating device for weekend sessions. Compliance over the weekend was ensured by a phone call from one of the researchers. A total of 195 sessions of heating were performed by the participants, in which 62% (n=121) were performed at the laboratory and 38% (n=74) were conducted at home. The last heating session was performed 12-16-h before the post leg FMD.

In addition, bilateral popliteal artery Doppler ultrasound, beat-to-beat heart rate (HR – three lead electrocardiography) and oscillometric arterial blood pressure (BP) were measured before and during one session of heating using automated equipment (Dixtal, DX2022, Brazil). The popliteal artery and

hemodynamics data were obtained during the final 10-min period of protocol. Figure 1 provides the schematic illustration of experimental setup (panel A) and original Doppler ultrasound recordings from one subject showing the remarkable increase in popliteal artery blood flow in the heated leg compared to the nonheated leg during one session of heating (panel B).

[insert Figure 1 here]

The subjects were asked to refrain from consuming caffeine/alcohol and from engaging in physical exercise for 6 and 24 h, respectively, prior to the tests. Subjects were 2-h postprandial upon arrival to the laboratory. To avoid potential diurnal variations, subjects were always tested at the same time of day and in the same quiet, temperature-controlled room (~24°C). Upon arrival to the laboratory, height and weight were obtained via standard methods.

Popliteal artery FMD

Bilateral popliteal artery FMD was measured at baseline and after five days of reduced daily physical activity. Initially, subjects rested in a supine position during 15-min period to ensure the attainment and stabilizations of the cardiovascular variables. Popliteal artery diameter and blood velocity were measured using high-resolution duplex-Doppler ultrasound (Logiq P5; GE Medical Systems, Milwaukee, WI) following present guidelines (34). A 9-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 insonation 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 both legs in the supine position as previously described (6, 18, 29, 30). Briefly, a rapid inflating cuff was placed on the lower leg. Two minutes of baseline hemodynamics were recorded and then the cuff was inflated to a pressure of 220 mmHg for 5 min. Continuous diameter and blood velocity measures were recorded for 3 min following rapid cuff deflation. Recordings of all vascular variables were analyzed offline using specialized edge-detection software (Cardiovascular Suite, Quipu, Pisa, Italy). FMD percentage change was normalized to shear rate incremental area under the curve up to peak diameter (22, 23).

Statistical analysis

The Shapiro-Wilk normality test and Levene's test of homogeneity of variance were used to assess the normality of the distribution of data. All variables presented a normal distribution and equal variance. A 2 × 2 ANOVA for repeated measures was applied: time (pre × post) and condition (heated × nonheated leg). We then tested the sphericity of the data and used LSD post-hoc test to detect differences when necessary. As an additional FMD analysis, peak (D_{peak}) and baseline (D_{base}) popliteal artery diameters were logarithmically transformed, and differences between them were determined. Logged scale differences between diameters were entered into an ANCOVA model in which

time and condition constituted a fixed factor and the logarithmically transformed D_{base} constituted a covariate. Covariate-adjusted estimate of the means was then back-transformed and calculated as percentage changes according to previous reports (2, 3). A paired *t* test was used to compare hemodynamic data within group at rest (pre-heating) and during the heating protocol. SPSS (version 19; SPSS, USA) was used to perform all analyses. Data are reported as means ± SE and the significance level adopted was $P<0.05$.

RESULTS

The number of steps significantly decreased in the reduced daily physical activity phase compared with baseline (reduced daily physical activity phase: 3842 ± 123 steps/day vs. active phase: 13103 ± 455 steps/day, mean ± SE, $P<0.01$) (Fig. 2A, B).

Table 1 shows popliteal artery Doppler measurements and hemodynamics during one experimental session of heating. Baseline diameter was not impacted by heating in the heated or nonheated leg ($P=0.93$). Both blood flow and velocity increased in the heated leg (Δ110.7 ± 27.6%, $P<0.01$ and Δ104.7 ± 24.6%, $P<0.01$, respectively), and slightly decreased in the nonheated leg (Δ-25.7 ± 8.1%, $P<0.01$ and Δ-25.7 ± 6.7%, $P=0.01$, respectively) (Table 1). The heating protocol increased popliteal artery mean shear rate in the heated leg (Δ119.3 ± 26.4%, $P<0.01$), and slightly decreased in the nonheated leg (Δ-21.8 ± 7.5%, $P=0.03$) (Table 1 and Fig. 3). HR, systolic, diastolic and mean BP were not affected by heating ($P>0.05$ vs. pre-heating; Table 1).

[insert Figure 2 here]

[insert Table 1 here]

[insert Figure 3 here]

Consistent with our hypothesis, five days of reduced physical activity decreased popliteal artery FMD in the control nonheated leg (pre: 6.69 ± 0.45%

vs. post: 2.93 ± 0.36%, P<0.01) and this impairment was prevented by intermittent local heating in the heated leg (pre: 6.82 ± 0.30% vs. post: 6.47 ± 0.36%, P=0.34) (Figure 4). Likewise, popliteal artery FMD normalized to shear rate area under the curve was unchanged in heated leg (P=0.64), but significantly decreased after five days of inactivity in nonheated leg (P<0.01, Table 2). The hyperemic stimulus for FMD, shear rate area under the curve, was not significantly affected by inactivity or heating (Table 2). In addition, after five days of reduced daily physical activity, baseline popliteal artery diameter was not significantly different between legs (P=0.25, Table 2). However, baseline diameter numerically trended to decrease in both the heated (Δ-2.16 ± 0.87%) and nonheated leg (Δ-0.7 ± 0.91%) which could inflate the post-intervention FMD values. To address this, we performed an ANCOVA model in which the difference in diameter is the outcome and D_{base} is a covariate in a logarithmic-linked generalized linear model. The D_{base}–adjusted FMD% remained unchanged in heated leg (pre: 13.5 ± 0.6% vs. post: 12.7 ± 0.9%, P=0.34), but was decreased in non-heated leg (pre: 13.3 ± 0.8% vs. post: 6.1 ± 0.7%, P<0.01).

[insert Figure 4 here]

[insert Table 2 here]

DISCUSSION

The most salient finding of the present investigation is that the impairment in popliteal artery FMD caused by short-term physical inactivity can be prevented by increasing leg blood flow and shear stress. Indeed, we found that increasing leg blood flow-induced shear stress with intermittent foot heating throughout a 5-day period of reduced activity abolished the impairment in popliteal artery FMD. This finding supports the hypothesis that reduced leg vascular shear stress during inactivity may be an underlying physiological mechanism by which physical inactivity causes vascular dysfunction in the lower extremities.

Consistent with our previous work (6), we found that when regularly active individuals engaging in more than 10,000 steps per day reduce their number of steps to ~4,000 steps per day (i.e., the American average) for 5 days, popliteal artery FMD becomes impaired. The finding that impaired FMD manifested in the popliteal artery but not in the brachial artery (6) suggests that the mechanisms altering vascular function with changes in activity levels are largely regulated by local factors. A well-known local factor responsible for the modulation of vascular function is shear stress. With reduced locomotion, the vasculature of the lower extremities becomes exposed to a marked reduction in blood flow and thus shear stress, relative to the vasculature of the arms which largely retain similar levels of activity. Thus, it is likely that inactivity-induced reduction in popliteal artery FMD is attributable to the loss of shear stress. The concept that vascular adaptations can be mediated by shear stress-dependent mechanisms is indeed supported by cell and organ culture, as well as by *in vivo*

animal and human studies experimentally altering shear stress (7, 9, 11, 15, 20, 25, 35).

In support of the hypothesis that reduced shear stress with inactivity is a prime underlying mechanism of impaired popliteal artery FMD, herein we found that subjecting one leg to increased blood flow with foot heating throughout the inactivity period prevented the decline in popliteal artery FMD in that leg. That is, the present data indicate that leg vascular dysfunction caused by inactivity can be eliminated by "replenishing" the shear stress stimulus lost during inactivity. However, based on the current data, we cannot exclude the possibility that inactivity impairs popliteal artery FMD through other mechanisms that are independent of shear stress and that increased shear stress with heating restores popliteal artery FMD back to normal.

As shown recently, acute (30, 31, 36) and chronic (8, 21) limb-specific heating improves vascular dilator function in both upper (8, 21, 36) and lower (30, 31) extremities of healthy humans through a shear stress-dependent mechanism. However, heating *per se*, independent of changes in physical activity or muscular contractions (8, 21), may also produce beneficial vascular effects. In this regard, previous studies indicate that elevated body core temperature increases expression of heat shock proteins, which are known to play a role in the modulation of vascular health (12, 16). Importantly, in the present study heating was applied to only the foot, minimizing the direct effects of heat on the upstream popliteal artery.

Some methodological aspects of the present study should be considered. First, we studied only healthy, young and physically active men, thus the generalizability of the findings remain limited to this population.

298 Second, part of our results should be interpreted with caution given the potential
299 for a type II error. For example, a non-significant (P=0.25) reduction in baseline
300 popliteal artery diameter was observed between legs after the inactivity period.
301 However, the additional allometric scaling analysis proposed by Atkinson and
302 Batterham (2), in which logarithmically transformed resting popliteal artery
303 diameter represent a covariate, reinforced our main findings that leg vascular
304 function was impaired after five days of reduced daily physical activity in the
305 non-heated leg, but remained unchanged in the heated leg. Third, we cannot
306 guarantee that 2 hours postprandial is sufficient to eliminate the confounding
307 effects of hyperglycemia and hyperinsulinemia on the FMD response. However,
308 we believe that this is more indicative of normal daily life than the fasted state.
309 Importantly, our approach is in line with others previous recent studies about
310 physical inactivity and FMD (6, 17, 18, 29, 30, 37). Fourth, although the
311 prognostic value of popliteal artery FMD remains unknown, the finding that
312 reduced daily physical activity lowered FMD by 3.8% (absolute units) in the
313 nonheated leg should be considered in light of studies demonstrating that the
314 leg vasculature is highly susceptible to atherosclerosis, relative to other
315 disease-resistant vasculatures such as the brachial artery (1, 13, 14, 32, 33).
316 Given this, the use of foot heating as a non-pharmacological therapy to combat
317 inactivity-induced popliteal artery dysfunction may be particularly relevant in
318 patients with increased risk factors for peripheral artery disease who are unable
319 or unwilling to be active. Future studies, however, should be conducted in these
320 populations to confirm this hypothesis.

321 In conclusion, we found that 5 days of reduced physical activity impairs
322 popliteal artery FMD and that this impairment can be abrogated by increasing

leg vascular shear stress with recurrent foot heating. These findings support the hypothesis that reduced leg blood flow-induced shear stress during physical inactivity may be a key underlying mechanism mediating detrimental leg vascular effects associated with inactivity. Thus, our study presents initial evidence that foot heating may be used as a non-pharmacological therapy to combat inactivity-induced leg vascular dysfunction.

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Disclosures

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

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457

458 **Figure legends**

459

460 **Figure 1.** Schematic diagram of experimental protocol (panel A) and original
461 Doppler ultrasound recordings from one subject illustrating the remarkable
462 increase in popliteal artery blood velocity in the heated leg compared to the
463 nonheated leg during one session of local heating (panel B).

464

465 **Figure 2.** Number of daily steps during the five days of baseline (pre) and five
466 days of reduced physical activity (post; Panel A). Panel B shows the five-day
467 averaged data.
468 *P<0.05 vs. pre.

469

470 **Figure 3.** Mean shear rate measured before (pre-heating) and during one
471 session of the experimental protocol in which one leg was submerged in ~42ºC
472 water (i.e., heated leg) and the contralateral leg remained dry (i.e., nonheated
473 leg).
474 *P<0.05 vs. rest. $^{\dagger}P$<0.05 vs. heated leg.

475

476 **Figure 4.** Popliteal artery flow-mediated dilation in the heated and nonheated
477 legs during baseline (pre) and after five days of reduced daily physical activity
478 (post).
479 *P<0.05 vs. pre. $^{\dagger}P$<0.05 vs. heated leg.

480 **Table 1.** Physiological variables before (pre-heating) and during heating

481 protocol at ~42°C.

	Pre-heating	42°C water
Popliteal artery		
Diameter, mm		
Heated leg	5.76 ± 0.22	5.78 ± 0.27
Nonheated leg	5.79 ± 0.16	5.78 ± 0.20
Velocity, cm/s		
Heated leg	7.45 ± 0.99	14.35 ± 1.82*†
Nonheated leg	7.76 ± 1.02	5.60 ± 0.89*
Blood flow, ml/min		
Heated leg	123.3 ± 19.3	224.2 ± 33.8*†
Nonheated leg	128.3 ± 20.2	86.2 ± 11.5*
Shear rate, s^{-1}		
Heated leg	47.64 ± 5.40	104.32 ± 15.53*†
Nonheated leg	49.68 ± 5.54	40.11 ± 7.46*
Hemodynamics		
Heart rate, bpm	70 ± 2	71 ± 3
Systolic BP, mmHg	120 ± 3	118 ± 3
Diastolic BP, mmHg	66 ± 2	68 ± 2
Mean BP, mmHg	84 ± 2	84 ± 2

Data are presented as means ± SE. BP, blood pressure; *P <0.05 vs. pre-heating. †P <0.05 vs. nonheated leg.

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483

484

485 **Table 2.** Popliteal artery vascular measures at baseline and during FMD before
486 (pre) and after (post) five days of reduced daily physical activity in the heated
487 and nonheated legs.

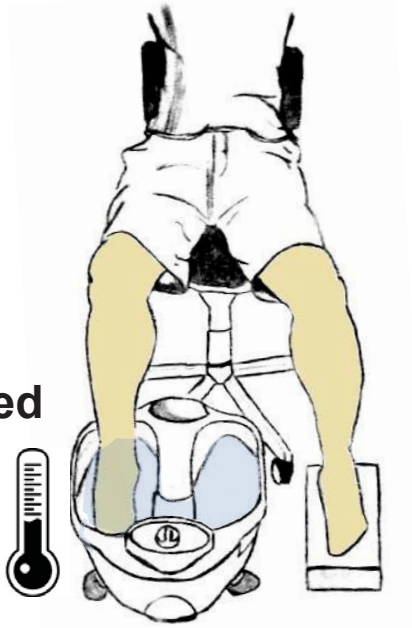
	Baseline diameter, mm	Peak diameter, mm	Shear, AUC	Peak FMD:SR_{AUC} Ratio, a.u.	Time to peak, s
Heated leg					
Pre	5.78 ± 0.21	6.17 ± 0.22	22262.91 ± 2616.98	0.37 ± 0.06	98.69 ± 7.67
Post	5.66 ± 0.22	6.02 ± 0.24	22254.92 ± 3126.64	0.35 ± 0.05	78.77 ± 6.43
Nonheated leg					
Pre	5.81 ± 0.23	6.20 ± 0.24	20628.46 ± 2963.32	0.38 ± 0.05	85.62 ± 9.87
Post	5.77 ± 0.22	5.94 ± 0.23	19825.03 ± 2296.18	0.18 ± 0.03*†	84.15 ± 6.03

488

489 *P<0.05 vs. pre. †P<0.05 vs. heated leg.

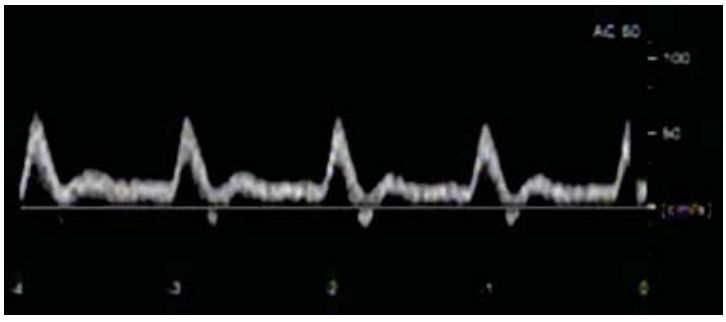
490

A



B

Heated



Nonheated