Introduction
A predominant independent risk factor for peripheral artery disease is the excessive engagement in sedentary behaviours, defined as any waking behaviour characterized by an energy expenditure ≤ 1.5 metabolic equivalents while in a sitting, reclining, or lying posture. Existing research in laboratory settings have demonstrated that acute bouts of prolonged sitting (i.e., ≥3 h) attenuate lower-limb FMD responses. Furthermore, Thosar et al. demonstrated that periodically breaking up an uninterrupted bout of sitting preserved FMD. Although these studies have provided insight into the vascular implications following a single bout of sitting in lab-based settings only, our understanding regarding the impact of habitual sedentary time or patterns on popliteal endothelial health in a free-living environment is unknown. The purpose of this study was to determine whether sedentary time and patterns measured in a free-living environment were associated with popliteal endothelial-dependent vasodilatory function. It was hypothesized that greater total habitual sedentary time, an increased number of prolonged sedentary bouts, and fewer sedentary breaks would be associated with worse popliteal flow-mediated dilation (FMD) responses. Methods: This cross-sectional study used 98 healthy participants (19–77 years, 53 females) that wore an activPAL monitor on the thigh for 6.4 ± 0.8 days to objectively measure sedentary activity and completed a popliteal ultrasound assessment to determine FMD. Both relative (%baseline diameter) and absolute (mm) FMD were calculated. Using bivariate correlation and multiple regression analyses, we examined if there were relationships between sedentary outcomes and FMD while statistically controlling for any potential confounders. Results: In the multiple regression model, age (β = −0.030, 95% CI = −0.051, −0.009) and total time in sedentary bouts >1 hour (β = −0.005, 95% CI = −0.009, −0.001) were independent predictors of relative FMD. Age (β = −0.002, 95% CI = −0.003, −0.001), mean blood flow (β = 0.013, 95% CI = 0.002, 0.024), moderate-intensity physical activity (β = 155.9E–5, 95% CI = 22.4E–5, 289.4E–5), sedentary breaks (β = 0.036, 95% CI = 0.007, 0.066), and total time spent in sedentary bouts >1 hour (β = −25.02E–5, 95% CI = −47.67E–5, −2.378E–5) were predictors of absolute FMD (all, p < 0.047). All independent outcomes remained significant after partially controlling for all other predictor variables (all, p < 0.031). Conclusions: Habitual prolonged sedentary bouts and sedentary breaks, but not total sedentary time, were predictors of popliteal endothelial-dependent vasodilatory function. The patterns by which sedentary time is accumulated may be more important than the total sedentary time on lower-limb arterial health.

Keywords
endothelial function, flow-mediated vasodilation (FMD), sedentary breaks, sedentary lifestyle

Abstract
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Impact of habitual sedentary patterns on popliteal artery endothelial-dependent vasodilation in healthy adults

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Abstract
Introduction: Acute, laboratory-based bouts of prolonged sitting attenuate lower-limb arterial endothelial-dependent vasodilation. However, the impact of habitual sedentary patterns on popliteal artery endothelial health is unclear. We tested the hypothesis that greater habitual total sedentary time, more time spent in prolonged sedentary bouts, and fewer sedentary breaks would be associated with worse popliteal flow-mediated dilation (FMD) responses. Methods: This cross-sectional study used 98 healthy participants (19–77 years, 53 females) that wore an activPAL monitor on the thigh for 6.4 ± 0.8 days to objectively measure sedentary activity and completed a popliteal ultrasound assessment to determine FMD. Both relative (%baseline diameter) and absolute (mm) FMD were calculated. Using bivariate correlation and multiple regression analyses, we examined if there were relationships between sedentary outcomes and FMD while statistically controlling for any potential confounders. Results: In the multiple regression model, age (β = −0.006, 95% CI = −0.051, −0.009) and total time in sedentary bouts >1 hour (β = −0.005, 95% CI = −0.009, −0.001) were independent predictors of relative FMD. Age (β = −0.002, 95% CI = −0.003, −0.001), mean blood flow (β = 0.013, 95% CI = 0.002, 0.024), moderate-intensity physical activity (β = 155.9E–5, 95% CI = 22.4E–5, 289.4E–5), sedentary breaks (β = 0.036, 95% CI = 0.007, 0.066), and total time spent in sedentary bouts >1 hour (β = −25.02E–5, 95% CI = −47.67E–5, −2.378E–5) were predictors of absolute FMD (all, p < 0.047). All independent outcomes remained significant after partially controlling for all other predictor variables (all, p < 0.031). Conclusions: Habitual prolonged sedentary bouts and sedentary breaks, but not total sedentary time, were predictors of popliteal endothelial-dependent vasodilatory function. The patterns by which sedentary time is accumulated may be more important than the total sedentary time on lower-limb arterial health.

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breaks would be associated with lower popliteal FMD responses.

Methods

Participants

Based on a moderate effect size ($\beta = 0.2$) and eight predictor variables, a sample size calculation estimated that 84 participants were needed using a multiple regression model assuming a two-tailed, $\alpha = 0.05$ and $\beta = 80\%$ power (G*Power, v3.1). To minimize the effects of fluctuating hormonal levels, naturally menstruating younger females were tested 1–5 days following the start of menstruation, and those using oral contraceptives were assessed during the placebo pill phase. Postmenopausal females were not using hormonal replacement therapy. Participants’ activPAL data have been previously presented. However, the current purpose and statistical analyses were independent from this previous report, which did not examine peripheral vascular function. Prior to testing, verbal and written informed consent were acquired. All protocols and procedures conformed to the Declaration of Helsinki and were approved by the Dalhousie University Health Sciences Research Ethics Board.

Habitual activity monitoring

The FMD assessments (see below) were completed in a thermoneutral environment (21°C), 6 hours postprandially, after participants had refrained from vigorous physical activity for 24 hours and caffeine and alcohol for 12 hours. Participants were equipped with an activPAL inclinometer (Pal Technologies Ltd, Glasgow, UK), a valid and reliable measure of habitual sedentary patterns and physical activity. Using standardized procedures, the activPAL was waterproofed and secured using Tegaderm™ transparent medical dressing (3M, London, ON, Canada) to the midline of their right anterior thigh. Participants wore the activPAL 24 hours per day for a minimum of 5 days (6.4 ± 0.8 d).

The activPAL data were analyzed using a customized LabVIEW program (LabVIEW 2018; National Instruments, Austin, TX, USA) that estimated waking hours and summarized daily averages of waking hours spent in sedentary postures. Since FMD responses are attenuated following 1 hour of sitting, we defined prolonged sedentary bouts as ≥ 1 hour in duration. Participants self-reported their waking hours to accommodate activPAL analysis. Sedentary breaks were calculated per waking hour. Physical activity intensity was determined using step rate thresholds for younger and older adults. All habitual sedentary and physical activity data were analyzed by a researcher blinded to participant popliteal outcomes. Our analysis program has previously demonstrated excellent inter-observer reliability.

Systemic hemodynamics

Heart rate (HR) was determined from a lead II electrocardiogram configuration. Beat-by-beat systolic (SBP) and diastolic blood pressure (DBP) were measured via finger photoplethysmography (Portapres; Finapres Medical Systems, Amsterdam, The Netherlands). The Portapres height correction unit accounted for deviations in the vertical distance between the heart and the pressure cuff. Intermittent brachial SBP and DBP were determined using an automated vital signs monitor (Carescape V100; General Electric Healthcare, Mississauga, ON, Canada) to calibrate the Portapres waveform (Figure 1). The electrocardiogram and Portapres waveforms were sampled at 1000 Hz and 200 Hz, respectively, using a PowerLab data acquisition system (PL3508 PowerLab 8/53; ADInstruments, Sydney, Australia). LabChart software (Version 8; ADInstruments) was used to view recorded signals in real-time and for offline analysis. At least 5 minutes of supine data were averaged to represent resting systemic hemodynamic outcomes. Mean arterial pressure (MAP) was calculated using the equation: $\frac{1}{3} \text{SBP} + \frac{2}{3} \text{DBP}$.

Popliteal artery endothelial-dependent assessments

Popliteal assessments were conducted following published guidelines. With participants in the prone position, a pressure cuff was secured around the widest circumference of the calf and attached to a rapid inflation system (E20 and AG101; Hokanson, Bellevue, WA, USA). The cuff was inflated to the supra-systolic pressure of 250 mmHg for 5 minutes to ensure distal ischemia was maintained. Endothelial-dependent vasodilatory function was assessed via duplex ultrasonography using a 12-MHz multifrequency linear array probe (Vivid i; General Electric Healthcare). The left popliteal artery was imaged proximal to the bifurcation at, or slightly above, the popliteal fossa by experienced operators (MWO, JAJ, JLP). Our lab has demonstrated intra-tester coefficients of variation of 2.2% and 4.2% for baseline diameter and relative popliteal FMD, respectively. Specifically, we blindly analyzed the same ultrasound recording for 20 participants on two different occasions, and the variation was calculated for each recording as: $\frac{((\text{difference between 2 measurements/average value}) \times 100\%)}{}$. Red blood cell velocity (RBCv) was continuously recorded using a pulsed frequency of 5 MHz and an insonation angle of 60° that was maintained across all participants. Superior and inferior edges of the pulsed-wave sample volume were adjusted to encompass the entire arterial lumen, as recommended in published guidelines.

Artery lumen diameter, RBCv, and shear rate (SR) were analyzed using an automated, commercial edge-detection and wall-tracking software (FMD Studio, Cardiovascular Suite; Quipu srl, Pisa, Italy) that has been previously demonstrated to have high reproducibility with the analyses of popliteal FMD using this software. Measures of popliteal blood flow, SR, shear rate area under curve (SR_AUC), and absolute and relative FMD were determined using standardized calculations. The statistical assumptions required to conduct allometric scaling or SR_AUC-normalization of FMD were not met. Specifically, the regression between relative FMD ($\beta = 8.33E^{-5}$, 95% CI = 1.81E^{-5}, 14.85E^{-5}; y-intercept = 3.566, 95% CI =
2.821, 4.311) and absolute FMD ($\beta = 4.19E-6, 95\% CI = -0.01E-6, 8.38E-6$; y-intercept $= 0.175, 95\% CI = 0.175, 0.271$), with $S_{RAUC}$ did not have an intercept of zero.

**Statistical analysis**

All participant descriptive characteristics (sex via independent samples t-test), habitual physical activity and sedentary patterns, systemic hemodynamic, and popliteal data were entered into a bivariate correlation to determine if they were univariately related to relative or absolute FMD. All univariately related ($p < 0.05$) predictor variables were entered into a multiple regression model. Measures exhibiting high multicollinearity (variance inflation factor $\geq 2.5$ and condition index $> 15$) were removed from the model. The primary model was selected based on the strongest $R^2$ value. Alternate models are presented in online Supplemental Table 1. The predicted residuals of all models were confirmed normal via a Shapiro–Wilk test. Partial correlations were conducted for all significantly related predictor variables identified from the multiple regressions. All statistical analyses were completed in IBM SPSS, Version 26.0 (IBM Corp., Armonk, NY, USA). Statistical significance was accepted as $p < 0.05$. All data are presented as means ± SD.

**Results**

Ninety-eight healthy, normotensive adults (53 females) were included in the present study ($n = 34$ older than 55 years). Table 1 includes the descriptive characteristics, systemic and popliteal hemodynamics, and physical activity

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**Figure 1.** Hemodynamic data collection was performed in the supine position and featured a lead II electrocardiogram configuration, finger photoplethysmography (Portapres; Finapres Medical Systems, Amsterdam, The Netherlands), and automated brachial blood pressure assessments (CareScape V100; General Electric Healthcare, Mississauga, ON, Canada) **(A)**. With participants in the prone position, a pressure cuff was secured around the widest circumference of the calf. The endothelial-dependent vasodilatory function was assessed via duplex ultrasonography using a 12-MHz multifrequency linear array probe (Vivid i, General Electric Healthcare) **(B)**. Participants were equipped with a waterproofed activPAL inclinometer on the midline of their right anterior thigh using Tegaderm medical dressing to quantify sedentary and physical activity patterns **(C)**.
monitoring outcomes. All predictor variables in Table 2 were univariately associated with popliteal FMD (all, \( p < 0.047 \)). For absolute popliteal FMD, there was no univariate relationship with total sedentary time (Figure 2A). The final multiple regression model accounted for ~44% of the variance in absolute FMD (\( R^2 = 0.666, p < 0.001 \)). Age, peak diameter, MPA, RBCv, total time in sedentary bouts > 1 hour (Figure 2B), and sedentary breaks (Figure 2C) remained as the only independent predictors. Partially controlling for all independent predictors simultaneously did not alter this relationship (all, \( p < 0.031 \)). Sedentary breaks were univariately (positively) correlated with relative FMD (Figure 2F).

**Discussion**

The purpose of this study was to test the hypothesis that higher total habitual sedentary time, fewer breaks in prolonged sedentary bouts, and an increase in the number of prolonged sedentary bouts would be associated with worse (i.e., lower) popliteal FMD responses. Our results support that increased engagement in habitual sedentary bouts > 1 hour and fewer sedentary breaks were associated with

| Table 1. | Participant descriptive characteristics, systemic hemodynamics, and habitual and sedentary activity. |
|-----------|------------------------------------------------------|
| **Sample (n = 98; 53 females)** | |
| **Descriptive characteristics** | Mean ± SD | Range |
| Age (years) | 39 ± 21 | 19–77 |
| Height (m) | 1.71 ± 0.10 | 1.46–1.93 |
| Body mass (kg) | 74 ± 13 | 41–105 |
| Body mass index (kg·m–2) | 25.3 ± 3.7 | 17.7–40.6 |
| **Systemic hemodynamics** | | |
| Resting heart rate (beats·min–1) | 67 ± 10 | 44–92 |
| Systolic blood pressure (mmHg) | 118 ± 11 | 90–140 |
| Diastolic blood pressure (mmHg) | 66 ± 10 | 32–89 |
| Mean arterial pressure (mmHg) | 84 ± 8 | 65–102 |
| **Popliteal hemodynamics** | | |
| Baseline diameter (mm) | 6.14 ± 1.11 | 4.01–10.77 |
| Red blood cell velocity (cm·s–1) | 4.7 ± 1.8 | 1.6–9.8 |
| Blood flow (mL·min–1) | 88 ± 43 | 22–215 |
| Shear rate (s–1) | 54 ± 30 | 7–152 |
| **Flow-mediated dilation** | | |
| Peak diameter (mm) | 6.41 ± 1.13 | 4.01–10.81 |
| Absolute FMD (Δmm) | 0.26 ± 0.12 | 0.03–0.55 |
| Shear rate area under curve (a.u.) | 9739 ± 5981 | 2100–37,270 |
| Time to peak diameter (s) | 96 ± 27 | 40–166 |
| **Habitual activity** | | |
| Standing time (min·day–1) | 360 ± 99 | 152–733 |
| Waking time (h·day–1) | 17.3 ± 1.18 | 14.8–20.2 |
| Sleeping time (h·day–1) | 6.9 ± 1.8 | 3.8–9.2 |
| **Habitual physical activity** | | |
| Step count (steps·day–1) | 9700 ± 3131 | 4409–18,259 |
| LPA (min·day–1) | 67 ± 22 | 11–145 |
| MPA (min·day–1) | 32 ± 17 | 5–89 |
| VPA (min·day–1) | 4 ± 5 | 0–34 |
| MVPA (min·day–1) | 37 ± 20 | 6–108 |
| **Habitual sedentary activity** | | |
| Total time (min·day–1) | 505 ± 124 | 256–782 |
| Number bouts < 1 h (bouts·day–1) | 46 ± 12 | 21–85 |
| Total time < 1 h bouts (min·day–1) | 337 ± 78 | 209–592 |
| Number bouts > 1 h (bouts·day–1) | 1.9 ± 0.9 | 0.3–5.2 |
| Total time > 1 h bouts (min·day–1) | 170 ± 92 | 28–519 |
| Breaks (breaks·waking h–1) | 2.8 ± 0.7 | 1.4–4.8 |

FMD, flow-mediated dilation; LPA, light-intensity physical activity; MPA, moderate-intensity physical activity; MVPA, moderate–vigorous intensity physical activity; VPA, vigorous-intensity physical activity.
Table 2. Univariate and multivariate regression analyses examining the determinants of popliteal relative and absolute FMD.

| Variable | Univariate analysis | Multiple regression analysis |
|----------|---------------------|-----------------------------|
|          | R (p-value)         | Unstandardized β (95% CI)   | SE | t-value | Significant predictor (p-value) |
| **Relative FMD** |                     |                             |    |         |                                 |
| Age (years) | -0.524 (< 0.001) | -0.030 (-0.051, -0.009) | 0.011 | -2.837 | YES (0.006)                 |
| SBP (mmHg) | -0.246 (0.015) | -0.013 (-0.053, 0.028) | 0.020 | -0.618 | NO (0.538)                  |
| Mean RBCv (cm·s⁻¹) | 0.211 (0.037) | 0.013 (-0.059, 0.053) | 0.013 | -0.109 | NO (0.913)                 |
| Mean RBCv (cm·s⁻¹) | 0.251 (0.013) | 1.38* (-5.10*, 7.90*) | 3.30* | 0.423 | NO (0.673)                 |
| MPA (min·day⁻¹) | 0.390 (< 0.001) | 0.021 (-0.001, 0.043) | 0.011 | 1.906 | NO (0.060)                 |
| Sedentary breaks (breaks·waking h⁻¹) | 0.214 (0.037) | 0.425 (-0.066, 0.916) | 0.247 | 1.719 | NO (0.089)                 |
| Sedentary bouts > 1 h (min·day⁻¹) | -0.304 (0.002) | -0.005 (-0.009, -0.001) | 0.002 | -2.606 | YES (0.011)                |
| Intercept | – | 5.409 (0.829, 9.990) | 2.305 | 2.347 | YES (0.021)                |
| **Absolute FMD** |                     |                             |    |         |                                 |
| Age (years) | -0.403 (< 0.001) | -0.002 (-0.003, -0.001) | 0.001 | -0.322 | YES (0.001)                |
| Peak diameter (mm) | 0.278 (0.006) | 0.050 (0.033, 0.068) | 0.009 | 5.495 | YES (< 0.001)           |
| Mean RBCv (cm·s⁻¹) | 0.203 (0.045) | 0.013 (0.002, 0.024) | 0.006 | 2.356 | YES (0.021)                |
| MPA (min·day⁻¹) | 0.315 (0.002) | 155.9* (22.4*, 289.4*) | 0.001 | 2.320 | YES (0.023)                |
| Sedentary breaks (breaks·waking h⁻¹) | 0.201 (0.047) | 0.036 (0.007, 0.066) | 0.015 | 2.452 | YES (0.016)                |
| Sedentary bouts > 1 h (min·day⁻¹) | -0.223 (0.028) | -25.02* (-47.67*, -2.378*) | 11.40* | -2.195 | YES (0.031)                |
| Intercept | – | -0.157 (-0.340, 0.025) | 0.092 | -1.712 | NO (0.090)                |

Univariate analysis represents the variables that exhibited a significant (p < 0.05) relationship with relative/absolute popliteal FMD. Multiple regression analyses were conducted by simultaneously entering all significant univariate variables as predictors of relative/absolute popliteal FMD as the outcome. The relative FMD multiple regression model including all predictors had an R = 0.634 (R² = 0.402, p < 0.001). The absolute FMD multiple regression model including all predictors had an R = 0.666 (R² = 0.443, p < 0.001).

* Multiplied by 10⁻⁵.

a.u., arbitrary units; β, unstandardized beta; FMD, flow-mediated dilation; MAP, mean arterial pressure; MPA, moderate-intensity physical activity; RBCv, red blood cell velocity; SBP, systolic blood pressure; SR_AUC, shear rate area under curve (reactive hyperemia).

Our results also align with laboratory-based studies that observed that breaking up prolonged sitting bouts mitigated robust declines in FMD. Carter et al. and Thosar et al. recommend that 0.5 breaks/waking hour and one break/waking hour, respectively, was effective in preventing reduction in superficial femoral FMD. In contrast, this study demonstrated that participants engaged in 2.8 breaks/waking hour and that each additional break was associated with a 0.43% increase in popliteal relative FMD (Table 2). However, in comparison to the superficial femoral artery, the popliteal artery is subject to a robust distal deformation (i.e., kinked) in a knee-bent sitting posture, which may further attenuate blood flow/shear stress and endothelial function. Sedentary breaks were an independent (positive) predictor of absolute FMD (Table 2), suggesting that breaking up sedentary bouts may offer protective benefits to popliteal health. This suggests that sedentary breaks may be important to include in a future version of the World Health Organization’s sedentary behaviour guidelines.

**Study strengths and limitations**

The present study is strengthened by the use of thigh-worn inclinometry rather than subjective questionnaires or nonhigh worn accelerometers that cannot distinguish standing time from sedentary time and thus cannot truly quantify sedentary time or patterns. Examination of sedentary breaks and time spent engaged in prolonged bouts,
versus only total sedentary time, in a relatively large heterogeneous sample also adds to the impact of our findings. However, we acknowledge that this study is limited by its cross-sectional design and is unable to establish cause and effect. Lastly, our pooled sample was composed of mostly healthy younger (<30 years: \( n = 64 \)) and older adults (>55 years: \( n = 34 \)). As such, our findings cannot be extrapolated to middle-aged adults or persons with vascular diseases.

**Conclusion**

In conclusion, more time spent engaged in prolonged sedentary time, but not total sedentary time, was associated with worse popliteal endothelial-dependent vasodilator function. This provides support for the current sedentary behaviour guidelines from the World Health Organization that recommend minimizing the amount of time spent in prolonged sitting.\(^\text{27}\) Also, it presents evidence for the addition of recommending breaking up long periods of sitting as often as possible to the existing guidelines. Decreasing habitual time spent engaged in prolonged sedentary bouts and implementing more sedentary breaks could be an effective strategy to promote better lower-limb arterial health.

**Declaration of conflicting interests**

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**Supplementary material**

The supplementary material is available online with the article.

**References**

1. Després JP. Physical activity, sedentary behaviours, and cardiovascular health: When will cardiorespiratory fitness become a vital sign? Can J Cardiol 2016; 32: 505–513.
2. Pandey A, Salahuddin U, Garg S, et al. Continuous dose-response association between sedentary time and risk for cardiovascular disease a meta-analysis. *JAMA Cardiol* 2016; 1: 575–583.
3. Tremblay MS, Aubert S, Barnes JD, et al. Sedentary behavior research network (SBRN) - terminology consensus project process and outcome. *Int J Behav Nutr Phys Act* 2017; 14: 75.
4. O’Brien MW, Johns JA, Williams TD, et al. Sex does not influence impairments in popliteal endothelial-dependent vasodilator or vasoconstrictor responses following prolonged sitting. *J Appl Physiol* 2019; 127: 679–687.
5. Thosar SS, Bielko SL, Mather KJ, et al. Effect of prolonged sitting and breaks in sitting time on endothelial function. *Med Sci Sports Exerc* 2015; 47: 843–849.
6. Restaino RM, Holwerda SW, Credeur DP, et al. Impact of prolonged sitting on lower on upper limb micro- and macrovascular dilator function. *Exp Physiol* 2015; 100: 829–838.
7. Faubert TD, Buchner A, et al. Statistical power analyses using O’Power 3.1: Tests for correlation and regression analyses. *Behav Res Methods* 2009; 41: 1149–1160.
8. O’Brien MW, Al-Hinnawi A, Wu Y, et al. The influence of habitual breaks in sedentary time on cardiovascular baroreflex function. *Appl Physiol Nutr Metab* 2021; 46: 1143–1146.
9. Kozey-Keadle S, Liberine A, Lyden K, et al. Validation of wearable monitors for assessing sedentary behavior. *Med Sci Sports Exerc* 2011; 43: 1561–1567.
10. Edwardson CL, Winkler EAH, Bodicoat DH, et al. Considerations when using the activPAL monitor in field-based research with adult populations. *J Sport Heal Sci* 2017; 6: 162–178.
11. Hart TL, Swartz AM, Cashin SE, et al. How many days of monitoring predict physical activity and sedentary behaviour in older adults? *Int J Behav Nutr Phys Act* 2011; 8: 62.
12. Aguilar-Farias N, Martino-Fuentela P, Salom-Diaz N, et al. How many days are enough for measuring weekly activity behaviours with the ActivPAL in adults? *J Sci Med Sport* 2019; 22: 684–688.
13. Wu Y, Johns JA, Poitras J, et al. Improving the criterion validity of the activPAL in determining physical activity intensity during laboratory and free-living conditions. *J Sports Sci* 2021; 39: 826–834.
14. O’Brien MW, Kivell MJ, Wojcik WR, et al. Influence of anthropometrics on step-rate thresholds for moderate and vigorous physical activity in older adults: Scientific modeling study. *JMIR Aging* 2018; 1: e12363.
15. Thijsen DHJ, Bruno RM, van Mil ACCM, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J* 2019; 40: 2534–2547.
16. O’Brien MW, Johns JA, Robinson SA, et al. Relationship between brachial and popliteal artery low-flow-mediated constriction in older adults: Impact of aerobic fitness on vascular endothelial function. *J Appl Physiol* 2019; 127: 134–142.
17. O’Brien MW, Mekary S, Robinson SA, et al. The relationship between aerobic fitness and low-flow-mediated constriction in older adults. *Eur J Appl Physiol* 2019; 119: 351–359.
18. Johns JA, O’Brien MW, Bungay A, et al. Sex and light physical activity impact popliteal, but not brachial artery flow-mediated dilation in physically active young adults. *Appl Physiol Nutr Metab* 2020; 45: 1387–1395.
19. O’Brien MW, Johns JA, Al-Hinnawi A, et al. Popliteal flow-mediated dilatory responses to an acute bout of prolonged sitting between earlier and later phases of natural menstrual and oral contraceptive pill cycles. *J Appl Physiol* 2020; 129: 637–645.
20. O’Brien MW, Johns JA, Petterson JL, et al. The impact of age and sex on popliteal artery endothelial-dependent vasodilator and vasoconstrictor function. *Exp Gerontol* 2021; 145: 111221.
21. Carter SE, Draijer R, Holder SM, et al. Effect of different walking break strategies on superficial femoral artery endothelial function. *Physiol Rep*. 7. Epub ahead of print 18 August 2019. DOI: 10.14814/phy2.14190.
22. Padilla J, Sheldon RD, Sitar DM, et al. Impact of acute exposure to increased hydrostatic pressure and reduced shear rate on conduit artery endothelial function: A limb-specific response. *Am J Physiol Circ Physiol* 2009; 297: H1103–H1108.
23. Jadidi M, Razian SA, Anttila E, et al. Comparison of morphometric, structural, mechanical, and physiologic characteristics of human superficial femoral and popliteal arteries. *Acta Biomater* 2021; 121: 431–443.
24. Restaino RM, Walsh LK, Morishima T, et al. Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear stress. *Am J Physiol Circ Physiol* 2016; 310: H648–H653.
25. Cox DA, Vita JA, Treasure CB, et al. Atherosclerosis impairs flow-mediated dilation of coronary arteries in humans. *Circulation* 1989; 80: 458–465.
26. Walsh LK, Restaino RM, Martinez-Lemus LA, et al. Prolonged leg bending impairs endothelial function in the popliteal artery. *Physiol Rep* 2017; 5: e13478.
27. Bull FC, Al-Ansari SS, Biddle S, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *Br J Sports Med* 2020; 54: 1451–1462.