**SUPPLEMENTAL CONTENT**

**Associations Between Habitual Sedentary Behavior and Endothelial Cell Health**

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**Supplemental Methods**

**Endothelium-dependent Vasodilation:** Endothelial-dependent vasodilation (EDV) was determined using the reactive hyperemia index (RHI), which is measured as the transient increase in blood flow following a brief period of arterial occlusion. RHI was assessed using the EndoPAT2000™, a validated peripheral arterial tonometry (PAT) device (1-4). A finger probe for the EndoPAT2000 device was placed on the first digit of each hand. A blood pressure (BP) cuff was placed on the non-dominant forearm for inducing reactive hyperemia. After instrumentation, the participant relaxed for 30 min. Following this rest, EDV assessment was completed. To induce reactive hyperemia, the BP cuff was inflated to 200 mmHg or 60 mmHg plus systolic BP (i.e., whichever occlusion pressure was higher); the pressure was maintained for 5 min, and then the cuff was deflated (1, 3, 5). RHI was calculated as the ratio of the average amplitude of the PAT signal through the range of a 90–120 s period post deflation, divided by the average amplitude of the PAT signal of a 2 min period before cuff inflation (i.e., resting period) (6). RHI values were then normalized to the concurrent signal from the contralateral, control arm, and corrected for baseline vascular tone (1, 5, 7) to control for fluctuations in sympathetic nerve outflow that may induce changes in peripheral arterial tone, superimposed on the hyperemic response (8). RHI moderately correlates with endothelial vasodilator function in the coronary arteries (1), and with brachial flow-mediated dilation (7).

**Endothelial-cell Derived Microparticles:** Endothelial-cell Derived Microparticles (EMPs) were measured using flow cytometry as previously described (9-11). Citrated blood was centrifuged at 160×g for 10 min to prepare platelet-rich plasma (PRP), and the PRP was further centrifuged for 6 min at 1500×g to obtain platelet-poor plasma (PPP). Fifty microliters of PPP were incubated with two sets: (a) 4 µL of phycoerythrin (PE)-conjugated monoclonal antibody to CD31 (BD) and 4 µL of fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody to CD42b (BD); and (b) 5 µL of PE-conjugated monoclonal antibody to CD62E (BD). EMPs were defined as the number of particles with a size <1.5 µm and that were positively labelled by CD62E+ (EMPs expressing CD62E), and positively labelled by CD31 and negatively labelled by CD42 (CD31+/CD42 EMPs). Appropriate FITC-labelled and PE-labelled isotype-matched IgG were used as negative controls. Using standard beads (Bang Laboratories), total flow cytometry counts for each experiment were converted to the number of EMPs per microliter.

**Endothelial Progenitor Cells:** Endothelial Progenitor Cells (EPCs) were prepared and processed using flow cytometry (BD FACS Calibur) and analyzed using previously published protocols (12-16). Mononuclear cells in EDTA-anticoagulated blood were isolated by density-gradient centrifugation with Ficoll (Sigma) and counted using a Coulter Counter (Abx Pentra 60, Horiba). One million mononuclear cells were first aliquoted and incubated with 15 µL mouse serum (Sigma) to block non-specific binding of antibodies, followed by an incubation with monoclonal antibodies against human KDR (PE-labelled) (10 µL; R&D Systems), CD34 (FITC-labelled) (20 µL; BD) and CD133 (APC-labelled) (20 µL; Miltenyi Biotec). Isotype-identical antibodies IgG1-PE (BD), IgG-FITC (BD) and IgG2b-APC (eBioscience) served as negative controls. Data were gated on the mononuclear lymphocytic population, and 500, 000 events were collected in the gated region for each sample. Data for the two EPCs measures were expressed as percentages of the mononuclear lymphocytic populations that consist of CD34+/KDR+ cells, the primary EPC outcome and CD34+/CD133+/KDR+ cells, a secondary EPC outcome. A reduced number of EPCs expressing CD34+/KDR+ and CD34+/CD133+/KDR+ have been associated with increased risk of subclinical atherosclerosis, ischemic stroke, and future vascular events (14, 15, 17-20).

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| **Supplemental Table 1.** Characteristics of PUME study participants who were included or excluded from the present analyses. | | | |
| **Participant Characteristics** | **Included**  *(n=83)* | **Excluded**  *(n=197)* | **P-Value** |
| Sociodemographic |  |  |  |
| Age (yrs) | 25.5 (5.8) | 26.5 (7.9) | 0.29 |
| Men (%) | 43.4 | 50.25 | 0.29 |
| Black Race (%) | 4.82 | 17.26 | <0.01 |
| Hispanic Ethnicity (%) | 25.3 | 29.95 | 0.19 |
| Education |  |  | 0.46 |
| ≤ High School Graduate (%) | 6.0 | 8.1 | 0.01 |
| Some College (%) | 18.1 | 23.4 |  |
| College Graduate (%) | 39.8 | 49.8 |  |
| Graduate/Professional School (%) | 36.1 | 18.3 |  |
| Body Mass Index (kg/m2) | 24.1 (4.0) | 24.9 (4.3) | 0.15 |
| Endothelial Cell Variables |  |  |  |
| Endothelial-dependent vasodilationa | 2.42 (0.85) | 2.31 (0.80) | 0.29 |
| Endothelial Microparticlesb |  |  |  |
| CD62E+ (counts/μl) | 6.63 (0.42) | 6.69 (0.48) | 0.31 |
| CD31+/CD42- (counts/μl) | 6.23 (0.47) | 6.25 (0.48) | 0.85 |
| Endothelial Progenitor Cellsc |  |  |  |
| CD34+/KDR+ (%) | 13.43 (7.36) x 10-2 | 13.83 (6.25) x 10-3 | 0.65 |
| CD34+/CD133+/KDR+ (%) | 2.03 (1.50) x 10-2 | 2.86 (1.99) x 10-2 | <0.01 |
| Data are presented as mean (standard deviation) or frequency.  adata represent reactive hyperemia index.  bdata are natural log transformed.  cdata are square root transformed. | | | |

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| **Supplemental Table 2.** Characteristics of participants in the high and low mean sedentary bout duration groups (n=83). | | | |
| **Variable** | **Low**  *(n=41)* | **High**  *(n=42)* | **P-Value** |
| Participant Characteristics |  |  |  |
| Age (years) | 26.7 (7.0) | 24.4 (3.9) | 0.07 |
| Men (%) | 31.7 | 54.8 | 0.03 |
| Black Race (%) | 4.9 | 4.8 | 0.98 |
| Hispanic Ethnicity (%) | 26.8 | 23.8 | 0.75 |
| Education |  |  | 0.19 |
| ≤ High School Graduate (%) | 7.3 | 4.8 |  |
| Some College (%) | 12.2 | 23.8 |  |
| College Graduate (%) | 34.2 | 45.2 |  |
| Graduate/Professional School (%) | 46.3 | 26.2 |  |
| Body Mass Index (kg/m2) | 24.3 (3.8) | 23.8 (4.1) | 0.57 |
| Accelerometer Characteristics |  |  |  |
| Total Sedentary Time (mins/day) | 546.5 (80.6) | 638.2 (95.4) | <0.01 |
| Mean Sedentary Bout Duration (mins/bout) | 13.8 (2.3) | 23.6 (4.1) | <0.01 |
| Standing Time (mins/day) | 292.2 (72.4) | 226.3 (86.9) | <0.01 |
| LIPA (mins/day) | 346.5 (79.0) | 267.6 (95.5) | <0.01 |
| MVPA (mins/day) | 69.7 (25.6) | 59.4 (29.5) | 0.30 |
| MVPA Bouts (mins/day) | 12.4 (11.9) | 9.8 (10.8) | 0.09 |
| Wear Time (mins/day) | 964.8 (57.5) | 969.0 (61.9) | 0.76 |
| Valid Wear Days |  |  | 0.62 |
| 3-5 days (%) | 7.3 | 4.8 |  |
| 6-7 days (%) | 92.7 | 95.2 |  |
| Data are presented as mean (standard deviation) or frequency | | | |
| LIPA= light intensity physical activity; MVPA= moderate-vigorous physical activity.  MVPA Bouts= total minutes of MVPA accrued in bouts ≥10 min; defined as any period of ≥10 minutes for which each consecutive 15-sec epoch had an activity intensity was ≥3 METs.  Median split cut-point was 17.2 min/bout for mean sedentary bout duration. | | | |

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| **Supplemental Table 3.** Association of total sedentary time (expressed continuously) with endothelial measures (n=83). | | | |
| **Endothelial Cell Variable** | ***β*** | ***95% CI*** | **P-value** |
| Endothelial-dependent Vasodilationa |  |  |  |
| Unadjusted | -0.019 | (-0.133 - 0.094) | 0.736 |
| Model 1 | 0.043 | (-0.083 – 0.168) | 0.502 |
| Model 2 | 0.091 | (-0.058 - 0.239) | 0.227 |
| Model 3 | 0.062 | (-0.090 – 0.215) | 0.417 |
| Endothelial Microparticlesb |  |  |  |
| CD62E+ (counts/μl) |  |  |  |
| Unadjusted | -0.035 | (-0.091 – 0.021) | 0.220 |
| Model 1 | -0.048 | (-0.110 – 0.013) | 0.121 |
| Model 2 | -0.033 | (-0.105 – 0.040) | 0.375 |
| Model 3 | -0.033 | (-0.107 – 0.042) | 0.386 |
| CD31+/CD42- (counts/μl) |  |  |  |
| Unadjusted | 0.009 | (-0.054 - 0.072) | 0.774 |
| Model 1 | 0.012 | (-0.057 - 0.082) | 0.725 |
| Model 2 | -0.010 | (-0.092 - 0.072) | 0.805 |
| Model 3 | -0.002 | (-0.085 - 0.081) | 0.965 |
| Endothelial Progenitor Cellsc |  |  |  |
| CD34+/KDR+ (%) |  |  |  |
| Unadjusted | 0.149 x 10-3 | (-9.841 – 10.139) x 10-3 | 0.976 |
| Model 1 | -3.721 x 10-3 | (-14.834 – 7.392) x 10-3 | 0.507 |
| Model 2 | 1.148 x 10-3 | (-11.512 – 13.808) x 10-3 | 0.857 |
| Model 3 | 2.582 x 10-3 | (-10.579 – 15.743) x 10-3 | 0.697 |
| CD34+/CD133+/KDR+ (%) |  |  |  |
| Unadjusted | -0.398 x 10-3 | (-2.430 – 1.635) x 10-3 | 0.698 |
| Model 1 | -0.035 x 10-3 | (-2.402 – 2.332) x 10-3 | 0.976 |
| Model 2 | -0.515 x 10-3 | (-3.246 - 2.217) x 10-3 | 0.708 |
| Model 3 | -0.462 x 10-3 | (-3.268 – 2.344) x 10-3 | 0.744 |
| Data are presented as unadjusted/adjusted parameter estimate and 95% confidence interval; sedentary time was converted to hours per day for analyses.  adata represent reactive hyperemia index.  bdata are natural log transformed.  cdata are square root transformed. | | | |
| Model 1: Adjusted for age, sex, race, ethnicity and education | | | |
| Model 2: Adjusted for covariates in model 1 plus moderate-vigorous physical activity. | | | |
| Model 3: Adjusted for covariates in model 2 plus body mass index | | | |
| **Supplemental Table 4.** Association of mean sedentary bout duration (expressed continuously) with endothelial measures (n=83). | | | |
| **Endothelial Cell Variable** | ***β*** | ***95% CI*** | **P-value** |
| Endothelial-dependent Vasodilationa |  |  |  |
| Unadjusted | 0.346 | (-1.186 – 1.877) | 0.655 |
| Model 1 | 0.637 | (-0.896 - 2.170) | 0.411 |
| Model 2 | 0.897 | (-0.725 - 2.520) | 0.274 |
| Model 3 | 0.677 | (-0.970 - 2.323) | 0.415 |
| Endothelial Microparticlesb |  |  |  |
| CD62E+ (counts/μl) |  |  |  |
| Unadjusted | -0.671 | (-1.421 - 0.079) | 0.079 |
| Model 1 | -0.718 | (-1.462 – 0.026) | 0.058 |
| Model 2 | -0.594 | (-1.382 - 0.194) | 0.137 |
| Model 3 | -0.536 | (-1.337 - 0.265) | 0.186 |
| CD31+/CD42- (counts/μl) |  |  |  |
| Unadjusted | -0.326 | (-1.177 - 0.525) | 0.448 |
| Model 1 | -0.234 | (-1.081 – 0.613) | 0.584 |
| Model 2 | -0.425 | (-1.317 - 0.467) | 0.346 |
| Model 3 | -0.336 | (-0.034 - 0.027) | 0.455 |
| Endothelial Progenitor Cellsc |  |  |  |
| CD34+/KDR+ (%) |  |  |  |
| Unadjusted | -3.333 x 10-2 | (-16.665 - 9.999) x 10-2 | 0.620 |
| Model 1 | -3.914 x 10-2 | (-17.154 - 9.325) x 10-2 | 0.558 |
| Model 2 | -0.139 x 10-2 | (-13.941 - 14.219) x 10-2 | 0.984 |
| Model 3 | -1.008 x 10-2 | (-13.508 - 15.524) x 10-2 | 0.890 |
| CD34+/CD133+/KDR+ (%) |  |  |  |
| Unadjusted | -0.972 x 10-2 | (-3.682 - 1.738) x 10-2 | 0.477 |
| Model 1 | -0.546 x 10-2 | (-3.361 - 2.269) x 10-2 | 0.700 |
| Model 2 | -0.989 x 10-2 | (-4.020 - 2.043) x 10-2 | 0.518 |
| Model 3 | -1.136 x 10-2 | (-4.218 - 1.947) x 10-2 | 0.465 |
| Data are presented as unadjusted/adjusted parameter estimate and 95% confidence interval; WBC=white blood cells; sedentary bout duration was converted to hours per day for analyses.  adata represent reactive hyperemia index.  bdata are natural log transformed.  cdata are square root transformed. | | | |
| Model 1: Adjusted for age, sex, race, ethnicity and education | | | |
| Model 2: Adjusted for covariates in model 1 plus moderate-vigorous physical activity. | | | |
| Model 3: Adjusted for covariates in model 2 plus body mass index | | | |

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