Title: Arterial Stiffness, Total Sedentary Behavior, and Fragmentation of Sedentary Behavior in Physically Active Individuals

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Running Head: Arterial Stiffness in Physically Active Individuals

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Abstract

It has been documented that levels of sedentary behavior (SB) are related to elevated cardiovascular disease (CVD) risk. However, details regarding the association between arterial stiffness (AS) and SB, along with breaking and prolonging of SB, remain unclear among individuals meeting physical activity (PA) guidelines. **PURPOSE:** To determine the relationship between total amount of SB and fragmentation (FI) of SB per day and AS in individuals who meet the PA guidelines as established by the ACSM. **SUBJECTS:** A total of 54 (32 females, 22 males) participants with an average age of 43 ± 7 years, meeting physical activity guidelines**. METHODS:** An ActivPal accelerometer was worn for 7 days to monitor SB. SB is defined as sitting or reclined position ≤1.5 METs during waking hours. FI is defined as the average number of sit-to-stand transitions completed during sedentary periods, when total hours of SB are controlled for. Measurement of pulse wave velocity (PWV) was completed as an estimation of AS. **RESULTS:** Participants averaged 8.6 ± 1.6 h/day of SB, and 3.7 ± 1.2 sit-stand-transitions/seated hour, and PWV of 7.9 ± 1.4 m/s. SB explained 4.5% of the variance in AS. With covariates of age and gender controlled for, the explained variance of FI by the regression equation was 1.1% and was not a significant predictor. **CONCLUSION:** SB is a significant predictor of AS, a preclinical marker of CVD. Individuals who meet PA guidelines should make a conscious effort to reduce SB to further reduce their risk of developing premature CVD.

Keywords: physical activity, exercise, vascular health, arterial stiffness, sedentary behavior

New & Noteworthy: To our knowledge, this is the first objective assessment of sedentary behaviors and patterning of sedentary behaviors as they relate to pulse wave velocity in individuals meeting the physical activity guidelines. We believe that the current findings give insight from a free-living perspective and allow for important insights into future guidelines for sedentary behavior participation.

Introduction:

Cardiovascular disease (CVD) is a leading cause of death across the globe with a multimodal array of causes and risk factors (1). One of the lifestyle precursors to the development of CVD is sedentary behavior (SB) (2, 3). Sedentary behavior is defined as any waking behavior characterized by an energy expenditure of ≤ 1.5 METs while sitting, reclining or lying (4). There is a link between negative consequences to vascular health, such as elevated oxidative stress, endothelial dysfunction, and increased vessel stiffness in individuals who participate in many hours of sitting throughout their day (1, 5, 6). Encouragement to participate in physical activity (PA) has been the focus for reducing the risk of lifestyle related chronic

disease (4). However, researchers have suggested that minimization of time spent sitting may be just as important to negate effects on vascular and metabolic health (5, 7, 9). Researchers have identified SB is an independent risk factor in the development of cardiovascular disease and as sitting time increases, so does the risk of all-cause mortality (5, 7, 9). Therefore, individuals who meet the PA guidelines, but participate in high amounts of SB, may still be at higher risk for CVD development, with Arterial Stiffness (AS) as the precursor (4, 8,10).

Currently, Americans are sedentary for 9+ hours per day, and less than 27% are meeting the PA guidelines (1, 4). Sedentary behavior is not limited to watching television or other forms of screen time, but also an individual's occupation. Sedentary occupations currently account for 80% of all U.S. jobs (11, 12). The PA guidelines have been well established as a means to provide health-enhancing benefits, reduction of the development of CVD, and reduced mortality risk (4, 13). However, it remains unclear whether healthy individuals who meet the PA guidelines will negate the negative vascular health consequences of participating in an otherwise sedentary lifestyle.

Additionally, how SB is fragmented with sitting-to-standing/movement has been associated with vascular health changes. A recent systematic review conducted by Carter and colleagues (2017) highlighted that more frequent breaks from sitting are likely to be associated with higher endothelial function and less stiff vasculature in sedentary or recreationally active individuals across studies which utilized various measures of vessel stiffness and function at the brachial or femoral arteries including mean arterial pressure, FMD, or pulse wave velocity (PWV). However, it remains unclear whether fragmentation of SB and total average SB per day are able to predict levels of AS in individuals who are meeting or exceeding the PA guidelines.

Thus, the purpose of the current study was to examine the extent to which AS can be predicted by SB (average hours per day) in individuals who meet PA guidelines. Additionally, we aimed to examine the extent to which AS can be predicted by the fragmentation of SB (FI); i.e. the number of sit-to-stand transitions an individual completes during their sedentary bouts, whilst controlling for total time spent being sedentary. It was hypothesized that a positive linear prediction between average hours of SB and arterial stiffness would be present, and in contrast, a fragmentation of SB would positively impact arterial stiffness among healthy individuals who meet PA guidelines.

Methods

The linear predictions hypothesized by the researchers were analyzed using two multiple regression analyses which are non-experimental, and correlational, in design.

Subjects

Both males (n = 22) and females (n = 32) ages 43.4 ± 7.5 years were included in the current study. Additional demographic information can be found in Table 1. Subjects were recruited from the Northeastern region of the United States via volunteer sampling using recruitment flyers. Approval was gained from local organizations, such as fitness centers and gyms, to speak to employees about volunteering for this study. Additionally, emails were sent to local establishments seeking volunteers.

All study procedures were reviewed and approved by the Springfield College Institutional Review Board and subjects were fully informed of the purpose of the study and of potential risks before giving written informed consent. Subjects were then asked to complete a health history questionnaire and the International Physical Activity Questionnaire (IPAQ) as inclusion criteria. Both the health history and IPAQ were used for pre-screening purposes in order to subjectively determine whether or not the subject was healthy and met PA guidelines of

150 minutes or more of moderate to vigorous physical activity per week (17). Subjects who met the PA criteria, were healthy, not being treated for any chronic cardiovascular or metabolic disease, non-smokers, normotensive, were confirmed for participation. Participants were excluded if they met any of the following criteria: current smoker, history of heart attack, chest pain, or blood vessel surgery, have high cholesterol, high blood pressure, or heart disease, or are taking medications for treating or preventing high cholesterol, high blood pressure, or heart disease, have diabetes, cancer or experience blood clots, or have acute live or gallbladder disease.

Measures

International Physical Activity Questionnaire (IPAQ). Participants were pre-screened for meeting PA guidelines with the use of the IPAQ (18). This recall PA questionnaire has the ability to assess both PA and inactivity in regard to activity dimensions and domains (17). Dimensions of PA include the mode or type, frequency, duration, and intensity of the activity, along with energy expenditure. Previous work has shown that the IPAQ questionnaires is a valid and reliable tool to assess levels of physical activity (17).

Body composition and weight. Body fat percentage and weight were estimated using BOD POD® (CosMed, Rome, Italy). The BOD POD® has been determined to be a valid and reliable means of estimating body fat percentage (19).

Heart rate and blood pressure. Both resting heart rate (HR) and blood pressure (BP) were assessed after 10 minutes of seated rest. During this period of time, participants were asked to refrain from using any electronic devices or participating in conversation. Resting heart rate was measured with the use of a polar heart rate monitor and chest strap (Polar USA). Blood pressure was measured manually while seated and at least two measures were averaged with 1

minute separating each measure. A third measure of resting blood pressure was completed if the first two assessments were greater than 6 mmHg different for systolic BP (SBP) or diastolic BP (DBP) (20, 21).

Arterial stiffness. The Sphygmocor XCEL system (AtCor Medical, Sydney, NSW, Australia) was used to estimate AS by measurement of cfPWV, or the velocity at which the pressure waveform moves forward through the circulatory system. The Sphygmocor XCEL system is a highly reliable and valid tool for assessing pulse pressure, augmentation index, and cfPWV (22). This system has been utilized in other published studies which have been conducted at Springfield College (23, 24, 25). PWV was conducted after 15 minutes of lying rest where subjects were not permitted to utilized electronic devices or participate in conversation. Subjects were asked to lay facing up with legs flat on the assessment table. Between the 3 trials of PWV, there were 2 minutes of a wash out period. A trial was only included in data analysis if it was within 0.5 m/s of the former trial and if it was quality checked or "QC". If a trial was not within 0.5 m/s or not "QC", the trial was completed again after another 2-minute wash out period. The Sphygmocor device also measures pulse pressure and augmentation index (AIx), both of which were included in the analysis.

Sedentary behavior. All activity over the course of a seven-day wear time was measured with the use of an ActivPAL accelerometer (Fitness Instrument Technologies, Quogue, NY). This accelerometer has the ability to obtain outcome measures of sitting/lying, standing time, steps, step rate, number of posture changes (fragmentation, FI), MET hours, and physical activity level. Kim, Barry, and Kang (2015) observed ActivPal to be an accurate monitoring device for the assessment of SB parameters in a free-living environment when compared to other activity monitors including the ActiGraph.

Procedures

Subjects were fully informed of the purpose of the study and of potential risks before giving written informed consent. Once the researcher received verbal or written confirmation from the participant, the IPAQ was sent via email to the potential participant. Upon completion of the IPAQ with the confirmation of the subject meeting PA guidelines and the health history questionnaire regarding inclusion/exclusion criteria, the subject was scheduled for both Sessions 1 and 2.

Session 1. For this session, the subjects arrived at the Springfield College Human Performance Lab between the hours of 6:00am and 12:00 noon. The subjects did not arrive at the lab in any particular condition (i.e. fasted, without caffeine) for this session as it was administrative and paperwork heavy. The subjects first completed paperwork including the informed consent and demographic information. After completion of proper paperwork, the subjects were fitted and administered the ActivPal accelerometer device. The ActivPal was applied to the thigh of the subject with the use of a medical grade skin adhesive and double-sided adhesive tape which has been specially designed for this device. Instructions were provided the to the subject for the use of the ActivPal in both verbal and written form. This completed the first session, and subjects left the Human Performance Lab to resume normal daily activities while wearing the ActivPal for the next seven days.

Intersession adherence. At day three of wear time, the subjects were contacted by the researcher to discuss any questions or adherence issues between days passed. The subjects were contacted via phone, and the researcher asked for confirmation of wear time and for insight into any issues with the ActivPal accelerometer. The subjects were contacted again on day six of wear time to discuss arrival protocol for the following morning.

Session 2. At the completion of seven full days, subjects returned to the Springfield College Human Performance Lab between the hours of 6:00am and 8:30am; complete with the visit by 9:00am. The subjects arrived at least 8 hours fasted, and were asked to refrain from use of any substances containing caffeine, alcohol, or any vasoactive substances for at least 8 hours before their scheduled arrival (27). Subjects were encouraged to continue to drink water throughout the fasted time period. Upon arrival, the ActivPal accelerometer was removed from the subjects' thigh. Next, subjects' demographic information such as height, weight, and percent body fat were collected. Both body weight and percent body fat measurements were completed via the BOD POD. Before entering the BOD POD, subjects were asked to change into a tight fitted swim suit and were given a swim cap to contain their hair. Calibration procedures, as described by the manufacturer of the BOD POD, were completed each morning prior to subjects' arrival.

Next, the subjects were instructed to stay seated, feet flat on the floor, back against the chair for 10 minutes of rest prior to resting heart rate and blood pressure measurements. Blood pressures were manually taken by the same researcher across the study. Two blood pressure measures, separated by a 1-minute wash-out period, were taken, and a third was completed if the two measures were > 4 mmHg different. Heart rate was measured via a polar heart rate monitor and chest strap. Resting heart rate was recorded as the heart rate after the 10 minutes of seated rest (20).

After completion of the resting blood pressure and heart rate, participants were moved to an athletic training table, where they laid supine for 15 minutes before PWA and PWV measurements were recorded with the use of the Sphygmocor XCEL. PWA was conducted first after the 15-minute laying rest period and was followed by the cfPWV measurements.

Measurements required for cfPWV were conducted twice to confirm distances. Researchers conducted 3 trials of cfPWV for each participant with 2 minutes of wash out between each. PWV measurement needed to be quality control/check (QC) confirmed, and be ≤0.5m/s of one another in order to be recorded. An average of the 3 QC'd, consistent cfPWVs was used in analyses.

Data from the ActivPal wearable accelerometer was analyzed for total wear time, average time spent in SB, and FI. Subjects kept a diary of sleep habits each night in order to exclude this information from the SB analysis. Activity under 50 counts/minute, as detected by the accelerometer was considered SB as determined by Campbell and associates (2017).

Statistical Analysis

An a priori power analysis was conducted using G*Power 3.1 (14). The power analysis aiming to meet 80% power was conducted using an effect size ($F = 0.33$) from a previously conducted study on physically active individuals who sit for the majority of their waking hours attempting to determine the relationship between SB and vascular and metabolic function (16). Total number of subjects required to meet 80% power was estimated to be 60. (Figure 1).

Prior to the running of the desired statistical analyses, the data were screened for missing values, normality, outliers, and the meeting of basic assumptions. Descriptive statistics were calculated for all demographics and for SB and FI (Table 1). Two multiple regression analyses were conducted. One, to examine the extent to which SB can predict PWV in individuals who meet PA guidelines and when both age and gender were controlled for by including them as covariates in the analyses. Two, to examine the extent to which FI can predict PWV in the same individuals. The predictor variables of interest were average time spent in SB per day and fragmentation index. The two covariates were age and gender. Gender was dummy coded to

emulate a continuous variable in the regression analysis. The criterion variable was PWV. The alpha level was set to 0.05 and all analyses were conducted with SPSS 21.0.

Results

A total of 54 subjects (59% female) ages 43 ± 7 years were included in the current study. Subjects averaged a PWV of 7.9 ± 1.4 m/s, 8.6 ± 1.6 hours of SB per day across one week, and a fragmentation index of 3.7 ± 1.2. The purpose of the first regression analysis was to determine whether or not average hours of SB per week could predict an individual's arterial stiffness via pulse wave velocity (PWV) when controlling for age and gender. No significant linear relationship existed between the covariates of age and gender (r_{pb} = .176, p = .102). Significant linear relationships existed between age and PWV (r = .563, p =.000), and gender and PWV (r_{pb} = .525, p =.000). No significant linear relationship existed between the covariate age and predictor SB (r = -.116, p =.135), or the covariate gender and SB (r_{pb} = .033, p = .089). As for FI, no significant linear relationship existed with age (r = -.222 p = .053), but a significant linear relationship existed between gender (r_{pb} = -.259 p =.029). The predictor SB had no significant linear relationship with PWV (r = .167, p =.063). However, FI and PWV had a significant linear relationship (r = -.321, p = .009). When data from the ActivPAL were insufficient, the entirety of the subject's data was not included in the analysis. ActivPAL data was insufficient in 4 cases where the monitor itself drained battery very quickly, unknown by the researcher and therefore did not record at least 3 days of activity.

Average Sedentary Behavior and Arterial Stiffness

SB was also used to predict AS, as measured by PWV, when controlling for age and gender. The SB variable was normally mesokurtic and normally distributed. AS was leptokurtic and positively skewed, but no other assumptions were violated.

The regression equation was significant, $F(2, 51) = 20.244$, $p = .000$. Gender [$t(51) = 4.426$, $p = .000$] and age [$t(51) = 5.265$, $p = .000$] were significant predictors and contributed to 50.4% ($R^2 = 0.504$) of the explained variance in AS and are pictured in Figure 2, panels B and C. Results are included in Table 2. SB [$t(51) = 2.223$, $p = .031$] was also a significant predictor and when both age and gender were controlled for and explained 4.5% ($R^2 = 0.045$) of the variance of AS. This model is pictured in Figure 2, panel A. AS, as measured via PWV, increased by .185 m/s for every average hour increase of SB.

Pulse Pressure and AIx were also analyzed to determine if other markers of vascular function can be predicted by SB. SB did not significantly predict pulse pressure ($F(1,51) = 2.81$, $p = 0.10$), AIx ($F(1,51) = .27$, $p = 0.60$), or AIx75 ($F(1,51) = 0.32$, $p = 0.57$).

Fragmentation Index and Arterial Stiffness

With age and gender controlled for, FI was used in a multiple regression to predict AS. Both FI and AS were leptokurtic and positively skewed but no other assumptions were violated.

The results are included in Table 3. The regression equation was significant, $F(2, 51) = 17.688$, $p = .000$. Gender [$t(51) = 4.031$, $p = .000$] and age [$t(51) = 4.570$, $p = .000$] were significant predictors of AS, but FI [$t(51) = -1.065$, $p = .292$] was not. The covariates of age and gender contributed to 50.4% ($R^2 = 0.504$) of the explained variance in AS. When the influence of the covariates was controlled for, the explained variance of FI by the regression equation was 1.1% ($R^2 = 0.011$).

Discussion

Traditionally, healthy, physically active populations have been shown to be at lower risk for the development of CVD (4). However, epidemiological researchers have claimed individuals may not be safe from the development of vascular dysfunction and disease if they are

sitting for a large majority of their wakeful day, independent of meeting physical activity guidelines (1, 29, 30). An observation of healthy individuals, meeting PA guidelines within a real-world setting was needed in order to substantiate these associations. With the use of objectively measured SB, in healthy, free-living, physically active individuals, this study added to the body of literature examining vascular health and its' relationship with SB, and patterning of SB, within the current sample.

It was demonstrated that fragmentation was not a significant predictor of AS. However, total SB per day was a significant predictor of AS in the physically active population. Therefore, total accumulation of sedentary time per day may be targeted for reduction rather than emphasizing the fragmentation of sedentary time for individuals who meet the current PA guidelines.

Total Sedentary Behavior Per Day and Arterial Stiffness

Subjects of the current cohort averaged a cfPWV of 7.9 ± 1.4 m/s and 8.6 ± 1.6 hours of SB per day. Healthy individuals who are between the ages of 30 – 55 have cfPWV between 5.2 to 9.0m/s (20); identifying that this cohort's mean cfPWV was within normal range. However, SB was able to significantly predict 4.5% of AS within the current study. When age and gender are used as covariates, the higher the average SB per day, the higher AS they can expect to develop even if they meet the PA guidelines.

This finding raises concern that the beneficial effects of PA may be attenuated by sedentary time in terms of vascular stiffness. Baldo et al., (2019) reported that aging increases cfPWV by 0.05m/s per year when all other risk factors were controlled. In the current study, the unstandardized slope of SB indicates that for every 1 hour increase in average SB, there will be

an increase of .185m/s in cfPWV. Therefore, suggesting that SB demonstrates a clinically significant contribution to arterial stiffness.

Along with epidemiological research, others who have directly measured cfPWV and objective measures of PA have observed increases in AS the more time subjects spent being sedentary (33). Bohn et al., (2017) aimed to compare those with and without metabolic syndrome under a number of parameters; including cfPWV, SB, and PA. Researchers found that in healthy individuals (without metabolic syndrome) who spent more time in SB (>7.78 hrs/day) their cfPWV (9.9 ± 1.0 m/s) was higher than those who spent less time in SB (<7.78 hrs/day, cfPWV 8.9 ± 1.0 m/s). Additionally, the less time the healthy individuals spent participating in MVPA (<30 min/day) and the more time spent sedentary, the greater their average cfPWV (9.6 ± 1.0 m/s). Interestingly, those who participated in the lowest amount of total sedentary time per day, regardless of MVPA amount, had the lowest cfPWV (8.9 ± 1.0 m/s).

Mechanistically, the reductions in shear stress at the vascular endothelium, especially at the lower extremities, are thought to result in endothelial dysfunction due to prolonged bouts of impingement at the popliteal artery and of lack of skeletal muscle contraction (34). Excessive sitting, and therefore little muscular contraction, results in a decrease in the shear stress on the endothelium. A reduction in shear stress does not facilitate the endothelium-derived relaxation factor, or NO, to be produced and released to allow for vasodilatation to occur (34). Without changes in vasomotion of these vessels, there is stimulation of inflammation via reactive oxygen species (ROS) such as superoxide (35). Additionally, the action of nitric oxide synthase (eNOS) is reduced along with other important co-factors allowing for the production and release of NO. Reduction and blunting of these processes result in further endothelial dysfunction and structural changes prior to the development of atherosclerosis. Dysfunctional and structurally

311 compromised endothelium can lead to difficulties in the management of blood pressure, the
312 stiffening of arteries, and are an important part of the pro-atherogenic process (34).
313 Individuals who are physically active are thought to produce NO quickly and at an
314 adequate rate, allowing for ease in the change of lumen size resulting in better manipulation of
315 blood pressure and reduced ROS production (36). However, if these individuals are excessively
316 sedentary, presumably greater than 8 hours per day, the rate of AS may still be on the rise even if
317 the individual is meeting physical activity guidelines (29, 30).
318 **Fragmentation of Sedentary Behavior, Arterial Stiffness, and the Impact of Age**
319 Interestingly, FI, or the average number of sit-to-stand transitions per hour of SB, was not
320 observed to have a significant inverse linear relationship to AS; meaning the ability to predict
321 AS. In other words, individuals who broke up their sitting time more frequently did not have
322 more vessel stiffness than those who participated in prolonged bouts of SB when total average
323 SB per day is matched. This information has yet to be explored in healthy men and women
324 meeting PA guidelines
325 In both multiple regression analyses, age and gender were controlled to obtain additional
326 information regarding the relationship between SB, FI and AS. Both gender and age were
327 significant predictors of AS, therefore important to control for in the models. Peripheral artery
328 disease (PAD) is an atherosclerotic disease, leading to obstruction and indicating ischemia of the
329 extremities (37). Being male, having diabetes, hypertension, and/or hyperlipidemia, along with
330 other factors of age, are risk factors for the development of PAD and atherosclerosis acceleration
331 (37). Therefore, the finding of gender as a significant predictor of AS is not surprising.
332 Additionally, several population studies have identified male gender as a risk factor for higher
333 rates of coronary heart disease (CHD) and CHD-related mortality (39). CHD is manifested by

myocardial infarction, angina pectoris, heart failure and coronary death, but atherosclerosis is the underlying cause for almost all cases of CHD (40).

Age was observed to be a significant predictor of AS ($p < .05$). Therefore, age contributes to almost half of the level of AS. One of the major age-related changes in body function lies within the vascular system (32). Increasing age results in losses in arterial distensibility, which is a function of the replacement of elastic fibers by collagen-like tissue, due to the structural remodeling of the vascular wall by an increase in systolic blood pressure. With an increase in arterial stiffening alongside age, there is an increase in the risk of cardiovascular and cerebrovascular disease (32). The current study observed age and gender combined explained 50.4% of AS and this finding aligns with the expected atherosclerotic development and vessel wall stiffening with an increase in age.

Limitations

The limitation of the use of self-reported PA data as a means to determine whether or not the subjects met PA guidelines. The IPAQ is a valid and reliable means to assess weekly amounts of physical activity and has been validated for use within the current population. Due to its acceptance within the field, cost effectiveness, and reduced burden of its use, the IPAQ was chosen to be a more feasible option compared to the use of an additional accelerometer for objective PA assessment. The intention of the researchers in the current study was to use SB as a primary predictor variable, and therefore the objective measurement of SB, using the ActivPal, was more important to the analysis than the use of a PA specific accelerometer. Additionally, the hormonal/menstrual phase and the menopause status of the female participants was not determined in the current study, which could have impacted measures of AS (41, 42, 43).

Conclusion

Meeting the PA guidelines may not negate changes in vascular stiffness as a result of SB participation greater than 8 hours per day. The observations of the current study corroborate the findings of increase risk in CVD, events and all-cause mortality as identified by previous researchers in a free-living, observational setting (1, 4, 30). In healthy individuals meeting PA guidelines, total SB is a significant predictor of AS; a preclinical marker of CVD. Although SB may impact an individual's level of AS, the fragmentation of the total amount of SB they participate in does not predict the degree of vascular stiffness observed. Based upon our findings, individuals who meet PA guidelines should make a conscious effort to reduce SB to further reduce their risk of developing premature CVD.

Future research should aim to develop the dose-response relationship between volume and/or intensity of PA combined with SB and health outcomes. According to the most recent guidelines developed by the World Health Organization in 2020, there is insufficient evidence regarding the quantitative threshold on the amount of SB to negate negative health outcomes (4). With the development of this threshold across healthy or disease populations, health care professionals will be able to provide SB guidance just as PA prescriptions are given. Additionally, deeper work into specific health outcomes, such as vascular changes, with bouts of or habits of SB will allow for the determination of the underlying mechanistic changes leading to these associations.

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Disclosures

None.

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