Segregating the distinct effects of sedentary behaviour and physical activity on older adults' cardiovascular structure and function: Part 1- Linear regression analysis approach.

Physical behaviour and older adults' vasculature.

Original Research

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Abstract

Background – Physical behaviour (PB, physical activity [PA] and sedentary behaviour [SB]) can adjust cardiovascular mortality risk in older adults. The aim was to predict cardiovascular parameters (CVP) using 21 parameters of PB.

Methods - Participants (*n*=93, 73.8 ± 6.23 years) wore a thigh-mounted accelerometer for seven days. Phenotype of the carotid, brachial, and popliteal artery was conducted using ultrasound.

Results - SB was associated with one of 19 CVP. Standing and light intensity PA (LIPA) was associated with three and one CVP, respectively. Our prediction model suggested that an hourly increase in LIPA would be negatively associated with popliteal intima-media thickness (IMT) (0.09 mm [95%CI 0.15, 0.03]). sMVPA (moderate-vigorous PA [MVPA], accumulated in bouts <10 mins) was associated with one CVP. $_{10}$MVPA (MVPA accumulated in bouts ≥10 mins) had no associations. W50% had associations with three CVP. SB%, alpha, true mean PA bout, daily sum of PA bout time, and total week $_{10}$MVPA each were associated with two CVP.

Conclusions - PB patterns are more robust predictors of CVP than hrs·day^{-1} PB. The prediction that popliteal IMT would be negatively associated with increased standing and LIPA engagement suggests that older adults could obtain health benefits without MVPA engagement.

Introduction

Cardiovascular related deaths in the UK increase ~1.8 fold per decade between the ages of 55 – 85+ years[1]. This dramatic increase is likely to augment the socioeconomic burden as people over the age of 60 are forecast to account for 25% of the population by the year 2035[2]. Physical activity (PA), more specifically moderate-vigorous PA (MVPA), has been shown to be successful in the risk reduction and treatment of cardiovascular diseases (CVD) and therefore, is recommended in government policies. It is recommended by the UK government that older adults engage in bouts of at least 10 continuous minutes of MVPA that accumulate to a minimum of 150 minutes over a seven-day week[3] MVPA engagement has shown reductions in cardiovascular parameters that can be a precursor of CVD, such as intima-media thickness (IMT)[4] and artery diameter[5]. At multiple locations, e.g. carotid and popliteal arteries, an increase in IMT and artery diameter can be a sign of increased stiffness[6], and CVD risk[7-9]. In 2011, the government PA recommendations were updated to highlight the need to also avoid bouts of prolonged sedentary behavior (SB), in light of the increased awareness of the independent effects of SB on health[10-12]. However, this initial recommendation was made in the absence of any clear evidence of the metabolic and/or circulatory consequences of prolonged SB in older adults. As such, it was not possible to provide a quantitative recommendation for SB time. Timely and recent evidence highlights the degree to which increments in SB time and other SB measures (e.g. breaks in SB) affect cardiovascular health independent of MVPA engagement[11-16]. Thus, it has been proposed that low intensity PA (standing, and light intensity PA [LIPA]) could be used to reduce SB time and improve heath[17,18], either directly or indirectly.

With technological improvements, it is now possible to accurately quantify physical behaviour levels (SB and PA time). Thigh-mounted triaxial accelerometers are considered the

gold standard for SB time quantification as posture can be determined through recognising the positional orientation of the upper leg relative to the Earth's surface and monitoring can be carried out in real-time over a number of days. However, few key cardiovascular parameters have been mapped against this gold standard method of SB quantification[19,20]. In addition to the accurate quantification of physical behaviour, the patterns in which physical behaviour(s) is accumulated have become a focus of studies due to their associations with health in younger populations [16,21-23]. Furthermore, newly formulated physical behaviour quantifier, such as W50%, which represents a specific SB bout duration, where the sum of SB bouts of that length or greater would accumulate 50% of total SB time. Another physical behaviour quantifier, alpha, represents the decrease in average SB bout length as the number of SB bouts increase[24,25]. Associations with health have been found in the few studies that have measured W50% and alpha[22,26]. However, further analysis of W50% and alpha are needed to strengthen relevance to physiological, health, and well-being outcomes.

Therefore, the goal of the first part of this two part series was to determine the degree of association between thigh-mounted accelerometer measures of habitual physical behaviour and key cardiovascular parameters in older adults. The objectives of this study were threefold: 1) determine whether measures of daily physical behaviour predict older adults' cardiovascular profile; 2) determine which measures of physical behaviour patterns are better predictors of cardiovascular health; 3) highlight any effects of SB on cardiovascular health that are independent of MVPA and vice versa. The aim of this study was to provide an evidence-based recommendation for the parameter of physical behaviour that is the most prolific predictor of cardiovascular profile. It was broadly hypothesised that SB would be independently associated with certain cardiovascular parameters of older adults, as has been shown in previous studies of middle aged adults[16,27], so that an objective lifestyle recommendation of SB may have to consider both amount and pattern of SB accumulation to

ultimately improve health. In addition given that a number of PA are significantly associated with cardiovascular health, this would tend to strengthen the evidence-base for government PA guidelines. Specifically, our data would suggested that government PA guidelines aimed at older adults, should be inclusive of recommendations for every PA intensity, not just MVPA as is currently the norm.

Methods

Ninety-three older participants (73.8 ± 6.23 years, 60 – 89 years, 55% female) whose screening questionnaire revealed as independently mobile (did not require a wheelchair or Zimmer frame), did not suffer from an untreated cardiovascular disease, had not sustained an injury within the preceding three months, had not ever/recently suffered from a neurological disease that impaired motor control or academic ability, and were not diabetic, were recruited for the study. These participants were specifically recruited for the study through convenience sampling in the local area. Participants were contacted through older adult community groups (ranging from recreation sports to luncheon groups), to maximize the catchment area and footfall of the study, within 29 miles of the local University Campus. The local University (Manchester Metropolitan University) Ethics Sub-Committee granted ethical approval. Participant approval was acquired through written informed consent. Data collection took place between January 2015 and June 2016. Participants visited the laboratory on two occasions separated by a minimum of seven days, the details of which are outlined below.

First Laboratory Visit

On the first visit participant demographics were collected (Supplemental Material Table 2) and test protocols familiarisation was conducted. Participants were fitted with a commercially available thigh mounted (anterior aspect, at 50% of greater trochanter to

femoral condyle distance) triaxial accelerometer (GENEA, GENEActiv Original, Activinsights Ltd, Kimbolton, UK) using a waterproof adhesive patch (3M Tegaderm Film, North Ryde, Australia) on their dominant leg, which remained in place for seven consecutive, free-living days. Standing leg preference during a single leg balance exercise, determined leg dominance. GENEA data (60.0 Hz frequency) were smoothed using 10 s epochs. Residual G was selected as the GENEA output, (Residual G = √[[standard deviation x]^2 + [standard deviation y]^2 + [standard deviation z]^2]), adapted from our previous work on total movement analysis in older persons[28] and termed the Cheshire Algorithm for Sedentarism (CAS).

The SB-LIPA (1.50 Metabolic Equivalent Tasks [METs]) cut-off point was 0.057 Residual G and the LIPA-MVPA (3.00 METs) cut-off point was 0.216 Residual G. Physical activity was classified as Standing if posture orientation was PA but the Residual G was below the SB-LIPA cut-off point. To obtain these cut-off points, a systematic validation of the GENEA against expired gas during a laboratory-based activity calibration protocol, was carried out in a sub-sample of older adults (n = 20). Thus, ten ambulatory functions (i.e. 1-lying down, 2-sitting, 3-standing quietly, 4-repeated side-stepping 5-self-selected speed ground walking, 6-3.5 km·hr^{-1} walk on treadmill, 7-self-selected speed walk on treadmill, 8-self-selected speed weighted-vest treadmill walking (at 15% of body weight), 9-self-selected speed brisk walk on treadmill, 10-cycling) were monitored with concurrent gas analyses, heart rate, motion analysis and accelerometer output. The scatter plot exhibited a strong explained variance between Residual G (GENEA) and METs (expired gas) (r^2 = 0.89, p < 0.001). Postural identification using accelerometer axes orientation, similar to that developed by Rowlands, Olds, Hillsdon, Pulsford, Hurst, Eston, Gomersall, Johnston, Langford [29], showed perfect agreement with known time spent in SB (6.00 mins) and PA (21.0 mins) postures during the laboratory-based activity calibration protocol (Cohen's kappa = 1.00 [95% confidence interval (CI) 1.0, 1.0], p < 0.001). Residual G cut-off points and MET

thresholds had a strong agreement for physical behaviour intensity identification (Cohen's kappa = 0.81 [95%CI 0.49, 1.31], $p < 0.001$). To help account for individual differences in physical fitness, one MET was equal to the resting metabolic rate (RMR) of the participant.

Participants were provided with a self-report sleep diary (wake-up time, lights-off, go to sleep time, naps not included) and were requested to complete it throughout the GENEA data collection week. GENEA outcome variables and definitions are provided in Supplemental File Table 3. Three participants were removed from the analyses for not having sufficient accelerometer data (≥ 6 days).

Hydration guidance was provided as participants were asked to arrive at the second laboratory visit in a fasted state (> 8.00 hrs) but also hydrated as this could influence vascular parameters.

Second Laboratory Visit

Participants arrived for the second laboratory visit in a fasted, hydrated state. Where appropriate, participants were asked to refrain from taking medication until testing had been completed. All participants refrained from taking medication prior to the completion of the laboratory tests. A standardised meal (43.0% carbohydrate, 43.0% protein, 14.0% fat) was provided to participants before commencing the testing session.

A three lead electrocardiogram (ECG) was fitted to participants to allow for R-gated artery analysis and resting heart rate measures. The skin was cleaned with an alcohol wipe prior to electrode (BlueSensor M, Ambu, Copenhagen, Denmark) placement. Participants began testing by resting in the supine position for 15.0 mins to minimise any impact of orthostatic changes. Room temperature and light intensity were maintained at 22.0°C and 20.0 $lm \cdot ft^2$ (Sekonic Studio Deluxe III L-398A Light Meter, Sekonic, Newcastle Under Lyme, UK), respectively, in order to minimise any impact of environmental ambience variations. Supine blood pressure (BP) (M2 HEM-7121-F, OMRON, Hoofddorp, The

Netherlands) was assessed three times to obtain an average systolic BP, diastolic BP, and pulse pressure.

Hydration was assessed using bioelectrical impedance analysis (BIA) (BodyStat 1500, BodyStat, Douglas, UK). The BIA assessed total body water as a percentage of total body mass using the manufacturer's own algorithms that accounted for sex, age (yrs), height (cm), and body mass (kg). BIA has been shown to be a reliable[30] and valid[31] method for hydration assessment. Participants were hydrated if total body water as a percentage of total body mass was 55.0% - 65.0% for males or 50.0% - 60.0% for females.

Baseline Vascular Assessment

Ultrasound assessments were performed using an echo Doppler ultrasound machine (model AU5; Esaote, Genova, Italy) with a 7.50 MHz broadband linear array transducer in brightness or B-mode with an angle of insonnation of 60.0°[32] (B gain: 75.0, Doppler gain: 49.0, CFM gain: 47.0, depth of penetration: 49.3 mm, depth of focus: 27.0 – 31.0). Live streaming of all assessments were collected on a Hewlett-Packard computer running video capture software (Premier 6.0, Adobe Systems, San Jose, USA) through an analogue to digital converter (Pinnacle, Corel Inc., Ottawa, Canada) at 25.0 Hz. The depth of the transducer penetration was noted to allow for video scaling during off-line analyses using Brachial Analyzer (no Bland-Altman bias in reliability, and low pixel error in synthetic data analysis[33]) and Carotid Analyzer (Medical Imaging Application LLC, Iowa, USA), which has shown excellent validity compared to previous methods ($r^2 = 0.98$, $p < 0.001$, Bland-Altman bias 0.04, $p = 0.82$)[34]. Participants were supine for left common carotid artery, right brachial artery, and prone for left popliteal artery baseline assessments. Video recordings were collected over ten cardiac cycles[32,35] for the assessment of systemic peak blood velocity, IMT, artery diameter, calculation of shear rate, and resistance index (RI) (Supplemental File Table 4). All structural measures were obtained in a 10 mm region of interest (ROI), which

was 10 mm distal to the carotid bulb in the anterior (AL) and posterior longitudinal (PL) plane and 10 mm distal to the superior medial genicular bifurcation for the popliteal artery[36]. Artery diameter measures were filtered using automated R-gating to ensure artery diameter was measured during the end-diastolic phase. Remaining frame-to-frame measurements were filtered from final analysis if they did not use at least 70% of the ROI to measure artery diameter and/or were more than one SD from the mean artery diameter. All automated measures were assessed for errors by one researcher. Measurement of carotid, popliteal and brachial IMT was performed on the far wall as this is shown to truly reflect anatomic intima-media layer[37,38]. Previous validation of ultrasound showed 6.52% underestimation of carotid far wall IMT compared to histological measures whereas near wall IMT had a 25.3% overestimation in autopsies of 36 males (69.0 ± 8.00 years) with an intraobserver error of 5.40 ± 4.3%[37].

Intra-day and inter-day coefficient of variation (CV) were calculated from seven participants. Inter-day CV were 4.47%, 1.57%, and 5.33% for brachial, carotid, and popliteal artery diameter respectively, whilst intra-day CV were 4.97%, 2.34%, and 4.03% for brachial, carotid, and popliteal artery diameter, respectively. Inter-day CV were 1.45%, 7.91%, and 11.3% for brachial, carotid, and popliteal IMT respectively, whilst intra-day CV were 3.04%, 3.40%, and 7.04% for brachial, carotid, and popliteal IMT, respectively. Artery diameter and IMT CV should be sensitive enough to detect PA related changes as three months of aerobic leg exercise caused a 9.00% increase in diameter and a 16.0% reduction in IMT[5]. Blood Velocity CV was below 20.0% for inter-day and intra-day measures of all three arteries. Baseline Shear Rate CV was below 16.0% for inter-day and intra-day measures off all three arteries. Both blood velocity and shear rate CV should be sensitive enough to detect changes caused by PA as MVPA has been shown to increase blood velocity and shear rate by 39.8% and 43.7%, respectively[39]. Inter-day and intra-day CV was 5.75% and 11.1% for

carotid RI, respectively. RI CV could be sensitive enough to detect PA related changes as exercised individuals display a 6.94% lower RI compared to sedentary individuals[40].

Statistical Analyses

SPSS version 22 (IBM, New York, USA) was used for statistical analyses. Firstly, bivariate linear regression models were used to examine any association between physical behaviour (measured in hrs·day^{-1} only), covariables (including hydration status, amount of prescribed medication that primarily targets cardiovascular disease, total of prescribed medication that could influence cardiovascular profile), and cardiovascular parameters. Age is a frequently used covariate within epidemiology however, it is suggested that ageing has a minimal effect on cardiovascular profile in strictly older adults populations[41,42]. In our study sample bivariate linear regressions were used to verify the presence or otherwise, of any associations between age and cardiovascular (Supplemental File Table 1). Age was only used as a covariate for the single cardiovascular parameters it showed an association with (i.e. brachial artery diameter). If two or more physical behaviour or covariate parameters showed predictive qualities for a cardiovascular profile, a stepwise multivariate linear regression was used to assess the association between multiple physical behaviours (measured in hrs·day^{-1} only) and/or covariate parameters and their combined association with cardiovascular parameters. If SB was a predictor of a specific cardiovascular marker, whilst MVPA (sMVPA or $_{10}$MVPA) was not, or vice versa, within bivariate or multivariate models then it was determined that, the predictive qualities of SB or MVPA were likely independent of one another. Bivariate linear regression models were also used to examine the associations between patterns of physical behaviour and cardiovascular parameters. Cardiovascular variables were natural LOG transformed if they were non-normally distributed (Kolmogorov-Smirnov or Shapiro-Wilk, $p \leq 0.05$).

GENEA outliers of daily averages were identified using box and whisker plots and subsequently removed from statistical analysis of the respective GENEA variable. The data outside of the group range (higher or lower), would be considered outliers even if they were biologically possible. The aforementioned statistical tests were then re-performed to determine whether the GENEA outliers were influencing the statistical outcomes.

Statistical significance was set at $p \leq 0.05$. Data are presented as Mean (Standard Deviation [SD]) or Median (Interquartile Range [IR]) if parametricity was violated, unless stated otherwise.

Results

GENEA

After discounting the participants with insufficient accelerometer data (< 6 days), the remaining participants' physical behaviour and patterns of physical behaviour parameters are outlined in Supplemental File Table 5. Of the identified outliers, there were only 5 cases where the outlier data was lower than the mean for the respective GENEA variable, suggesting that those with the greatest amount of PA and SB (the extremes) were removed from the statistical analysis. Therefore, the remaining data was representative of an average older adult population within the local area. It is unlikely these outliers were a source of measurement error as the data within the current study fell within the ranges of previous studies using similar participants and accelerometer placement[43-45].

Cardiovascular Profile

Cardiovascular characteristics are outlined in Supplemental File Table 2. Measurement of carotid PL and popliteal variables was performed on a sub-population (n = 45, age: 73.6 (7.17) yrs, male: 22, female: 23).

Bivariate Linear Physical Behaviour Regressions (measured in hrs·day^{-1} only)

SB showed no predictive qualities for cardiovascular parameters. Meanwhile PA variables showed a number of associations (4 out of 19) with cardiovascular parameters (Table 1). Notably, our prediction model suggested an hour per day increase in low intensity PA (Standing, and LIPA) would be negatively associated with popliteal artery diameter (Standing -0.75 [95%CI -1.41, -0.09] mm) and IMT (LIPA -0.09 [95%CI -0.15, -0.03] mm). In addition, an hour increase in sMVPA was also negatively associated with popliteal IMT (-0.06 [95%CI -0.12, 0.002] mm) and resting heart rate (-3.36 [95%CI -5.67, -1.05] bpm).

When GENEA outliers were removed from the data (Supplemental File Table 6), SB was found to be a predictor of heart rate (1.58 [95%CI 0.17, 2.99] bpm). Standing was also found to be a predictor of popliteal IMT (-0.13 [95%CI -0.22, -0.03] mm) with the removal of outliers (thus predicting 3 out of 19 cardiovascular parameters). LIPA as a predictor had no outliers (13 out of 19 predictions for cardiovascular parameters). sMVPA was no longer a predictor of popliteal IMT when outliers were removed (thus now predicting 1 out of 19 cardiovascular parameters). $_{10}$MVPA was no longer a predictor of popliteal shear rate following the removal of outliers.

Multivariate Stepwise Physical Behaviour Regressions (measured in hrs·day^{-1} only)

Heart rate had the most physical behaviour predictors, excluding only LIPA from our prediction model. Standing explained 12.2% of the variance in heart rate whilst controlling for the other physical behaviour parameters in the prediction model. This was the largest partial correlation of the physical behaviour parameters included in the prediction of heart rate. There were no other cardiovascular parameters that could be predicted using multiple physical behaviour parameters (Table 1).

With the removal of GENEA outliers, SB and $_{10}$MVPA were removed from the heart rate regression model (Supplemental File Table 6). SB and sMVPA (as well as age) became associated with brachial artery diameter, following the removal of GENEA outliers

(Supplemental File Table 6). The regression suggested that brachial artery diameter was associated with a 0.55 (95%CI 0.19, 0.92) mm and 0.20 (95%CI 0.02, 0.39) mm increase per hour·day^{-1} increase in sMVPA and SB, respectively.

Bivariate Linear Patterns of Physical Behaviour Regressions

The predictive quality of cardiovascular parameters using patterns of physical behaviour is displayed in Supplemental File Table 8. W50%, SB%, and alpha appear to be the most common predictors within the SB category, showing predictive qualities for three, two, and two cardiovascular parameters (out of a possible 19), respectively (Table 2). Within the PA category, daily sum of PA bout time showed predictive qualities for two cardiovascular markers (out of 19), with PA bouts, true mean PA bout, Standing%, LIPA%, sMVPA%, and $_{10}$MVPA% all showing predictive qualities for one cardiovascular parameter (out of 19) (Table 2).

After the removal of outliers (Supplemental File Table 7), W50%, SB%, and alpha remained the best predictors of cardiovascular parameters within the SB category (three, two, and two out of 19, respectively), showing no change in prediction quality. Within the PA category, true mean PA bout, daily sum of PA bout time, and total week $_{10}$MVPA showed the most predictive qualities for cardiovascular parameters (two each), followed by PA bouts, $_{10}$MVPA bouts, Standing%, LIPA%, and sMVPA% (one each) (Supplemental File Table 7).

Please note that only significant associations are displayed in table 2 to reduce its size. The complete results tables can be found in Supplemental File Table 8.

Discussion

The purpose of the current study was to address four objectives: 1) determine whether measures of daily physical behaviour predict older adults' cardiovascular profile; 2) determine which measures of physical behaviour patterns are better predictors of cardiovascular profile; 3) highlight any effects of SB on cardiovascular health that are

independent of MVPA and vice versa. It was broadly hypothesized that a number of cardiovascular parameters will be uniquely sensitive to SB, and others to MVPA.

Physical Behaviour Predicts Cardiovascular Parameters

A lack of predictive qualities of physical behaviour relative to brachial (one association) or carotid parameters (no association) would tend to suggest that the effects of physical behaviour may be site specific given the number of associations elsewhere. Alternatively, our observations on the brachial and carotid arteries may be indicative of an ongoing local remodelling process, which may have masked any sensitivity to physical behaviour. This 'masking' hypothesis could explain why brachial artery diameter was not associated with SB or sMVPA in isolation (owing to small individual effects), but was reliably predicted in a regression model that took both SB and sMVPA into account. Interestingly, predictive qualities relative to popliteal parameters were seen with low intensity PA (Standing and LIPA) within our models, suggesting that low intensity physical activity could reduce popliteal parameters that are associated with CVD[9]. Within our bivariate regression models both an hour increase in standing and LIPA would be negatively associated with a 0.14 and 0.09 mm reduction in popliteal IMT, respectively, whilst LIPA was the only physical behaviour variable included in the multivariate stepwise regression model that associated popliteal IMT with a 0.11 mm decrease per hour increase in LIPA. This finding is consistent with a training study that found popliteal IMT decreased by 0.038 mm over 12 weeks (18 hours) of LIPA (30% heart rate reserve), which equated to a 0.002 mm reduction in popliteal IMT per hour of LIPA[46]. The results of the current study would have strong implications in older adults who struggle to accumulate sufficient $_{10}$MVPA, as they may find it easier to accumulate LIPA.

Which Physical Behaviour Pattern is the Best Predictor of Cardiovascular Health?

With improvements in the objective measurement of SB, the focus of research has shifted to the patterns in which SB is accumulated rather than total SB time per se. In particular, the number of SB breaks has become a heavily researched parameter[21,47] especially in acute interventions[17,18,48,49]. Within the current data, SB breaks only had predictive qualities for resting heart rate, whilst other patterns of SB, W50% and alpha showed more predictive qualities for cardiovascular parameters (three and two, respectively). W50% and alpha were first introduced by Chastin, Granat [24] to create a more sensitive measure of change in SB accumulation as SB breaks can be similar when W50% and alpha are significantly different between the pre and post phases of an intervention, or between groups[25,50]. W50% is the usual SB bout length that would accumulate 50.0% of total SB time if all of the SB bouts of that length and shorter/greater were accumulated[24]. W50% had predictive qualities for popliteal IMT as a minute increase in W50%, was positively associated with a 0.003 mm increase in popliteal IMT, which may have CVD implications as those with a history of CVD have exhibited a 0.04 (95%CI 0.03, 0.04) mm increase in popliteal IMT than those with no history[9]. Thus, as little as a ten-minute increase in W50% could lead to CVD complications.

Alpha is a unit-less power-law distribution that displays the increase in SB bouts as SB bout duration decreases[25]. Diastolic BP and carotid PL IMT showed predictive associations with alpha while W50% did not in the current study. The direction of these predictive models suggested that an increase in alpha (more SB bouts, shorter duration) would be associated with an increase in diastolic BP and carotid PL IMT. This is opposite to what would be intuited as brachial diastolic BP has been found to be similar between supine and orthostatic postures[51]. On the other hand, orthostatic posture increases carotid circumferential wall tension, compared to supine posture, and is associated with an increase in carotid plaque formation, which can be expressed as an increased IMT. This suggests that

the association between alpha and carotid PL IMT may be a result of more PA (orthostatic posture) due to a reduction SB bout length. The large 95%CI (0.29, 2.90 mm) in the current data may highlight the need for further data to confirm or otherwise, the reported association between alpha and carotid PL IMT, since a 0.10 mm increase in IMT can increase the relative risk of stroke by 18.0%[52]. The association of an increase in diastolic BP with increasing alpha may hold true as more bouts of a shorter duration would indicate that the older person causes this offset by engaging in more PA, where the arms are likely hanging by their side (as may be the case in gentle strolling or even chair-based exercise). In other words, there may have been PA not captured by the thigh-mounted accelerometer in the current study. This may cause a hydrostatic pressure that would subsequently increase blood pressure[53]. However, the lack of association between standing and diastolic BP within the current data does not support this idea.

The current findings suggest that W50% should be the preferred SB pattern of physical behaviour parameters for predicating cardiovascular risk because it had the most predictive qualities (three). In addition, W50% is presented in minutes, which can be easily understood and explained in a 'real-world' therapeutic (clinical or lifestyle) intervention settings.

Within PA patterns of physical behaviour, true mean PA bout length[24], showed the most predictive qualities for cardiovascular parameters (two), where 'true mean' refers to the mean duration of a PA bout succeeding anti-log transformations of previously LOG transformed non-normally distributed PA bout lengths[24]. This adds strength to the argument that it is not the number of SB breaks that is the most important, but the complex interaction between those SB breaks and the duration of individual SB breaks. The predictive qualities of total week $_{10}$MVPA (two) and the number of $_{10}$MVPA bouts (one) within the current study

support the government's use of a total $_{10}$MVPA recommendation accumulated in, at least, ten minute bouts within their PA guidelines for older adults[3].

Independence of SB and MVPA Physiological Effects

The basis of SB physiology stems from the apparently independent effects of SB and MVPA on health status[10,11]. Prior to the removal of outliers, MVPA (sMVPA or $_{10}$MVPA) showed predictive qualities for resting heart rate, popliteal IMT, and popliteal LOG shear rate, whereas SB did not within bivariate regression models. After the removal of outliers, sMVPA only showed predictive qualities for heart rate however, SB now displayed predictive qualities for heart rate too. This could infer that the effects of SB and MVPA on heart rate may not be independent. Furthermore, SB is excluded whilst sMVPA is included in the multivariate predictive model for heart rate suggesting SB does not add any further strength to the predictive model for heart rate, this could infer that SB and sMVPA use the same mechanistic pathways to affect heart rate. MVPA is known to reduce resting heart rate through increases in stroke volume[54] and reduction in peripheral resistance[55] whilst SB does the opposite[56,57]. The results of the current study support the idea that SB and MVPA effect resting heart rate indirectly by impacting on stroke volume and total peripheral resistance.

The predictive qualities of W50% and alpha were just as prevalent as those of true mean PA bout length, daily sum of PA bout time, and total week $_{10}$MVPA. Of the six cardiovascular markers these patterns of physical behaviour parameters predicted, three of them were only associated with one pattern of physical behaviour variable. This may infer that SB and PA parameters are physiologically independent and as such, warrants the need for future studies to include multiple physical behaviour parameters to be able to fully assess the effect of physical behaviour on health.

The main strength of this study is the use of a thigh-mounted accelerometer, which allows for accurate posture classification. However, one physical behaviour variable this

study did not measure is seated/reclined physical behaviour eliciting >1.50 METs (as would occur in seated exercise training programs for instance, a modality of exercise of particular prevalence in frail older persons[58]. Arguably, it is unlikely that this classification of physical behaviour would be prevalent within independent living older adults and therefore its absence from our current physical behaviour stratification would be minimal in this type of population. It is however, notable that owing to the age group of our study participants, the sample was skewed towards low adherence to $_{10}$MVPA and hence any relationship assessment up to that level of PA intensity would be incomplete. Arguably also, whilst we have been able to identify the best predictors from 21 physical behaviour parameters, given that the highest explained variance was up to 22% it would appear that the cumulative effect of factors, other than physical behaviour, have a more substantial effect on the cardiovascular parameters of interest within the current study. A limitation of the current study modelling approach was that owing to the study being under powered to examine sub-groups, models did not adjust for potential other confounding variables such co-morbidities and physical function. Future, large studies should aim to account for at least these 3 key variables in their estimations of the effects of physical behaviour on cardiovascular health outcomes.

Conclusions

The purpose of this study was to determine which measures of physical behaviour display predictive qualities for cardiovascular variables so future research could justify the use of specific physical behaviour parameters as dependent variables within intervention studies. The main strength of this study is the use of a thigh-mounted accelerometer, which allows for accurate posture classification. Overall, the present study displayed that all physical behaviour measures (hrs·day^{-1}), excluding $_{10}$MVPA, showed predictive qualities for at least one cardiovascular variable. Within patterns of physical behaviour, W50% and total week MVPA, daily sum of PA time, and true mean PA bout length were the best predictors

of cardiovascular parameters. The results suggest patterns of physical behaviour are more prolific predictors of cardiovascular health status than total physical behaviour measured in hours per day. SB and MVPA physical behaviour measures showed different and unique predicative qualities for cardiovascular parameters. This observation further supports the notion that both SB and MVPA engagements need to be considered in future physical behaviour research and/or lifestyle recommendations. Finally, increasing standing and LIPA engagement showed predictive qualities for popliteal IMT reduction. We propose this to be one of the most clinically relevant findings from our current work as it suggests that older adults do not have to engage in MVPA (which they have, in any case, shown poor long-term compliance to), in order to gain health benefits.

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Table 1 Bivariate and multivariate stepwise linear regressions between physical behaviour, covariates and cardiovascular parameters.

Variable	Model	b	-95% CI	+95% CI	*p*	r^2	r^2 adj.	P. Corr.
Systolic	SB[a]	-0.39	-2.85	2.06	0.75	0.001	-0.01	
BP	Standing[b]	-1.27	-9.38	6.82	0.75	0.001	-0.01	
	LIPA[c]	-0.75	-6.45	4.94	0.79	0.001	-0.01	
	sMVPA[d]	1.02	-3.58	5.64	0.65	0.002	-0.01	
	$_{10}$MVPA[e]	1.44	-11.7	14.6	0.82	0.001	-0.01	
	Hydration[h]	**-0.65**	**-1.14**	**-0.17**	**0.01**	**0.07**	**0.06**	
LOG	SB[a]	0.001	-0.01	0.01	0.93	0.00	-0.01	
Diastolic	Standing[b]	-0.03	-0.09	0.01	0.15	0.02	0.01	
BP	LIPA[c]	-0.01	-0.05	0.02	0.39	0.01	-0.003	
	sMVPA[d]	0.01	-0.01	0.04	0.37	0.01	-0.002	
	$_{10}$MVPA[e]	-0.01	-0.10	0.08	0.81	0.001	-0.01	
	Hydration[h]	**-0.004**	**-0.007**	**0.00**	**0.03**	**0.05**	**0.04**	
Pulse	SB[a]	-0.45	-2.31	1.39	0.62	0.003	-0.01	
Pressure	Standing[b]	1.54	-4.56	7.64	0.61	0.003	-0.01	
	LIPA[c]	0.61	-3.68	4.91	0.77	0.001	-0.01	
	sMVPA[d]	0.17	-3.30	3.65	0.92	0.00	-0.01	
	$_{10}$MVPA[e]	2.48	-7.44	12.4	0.62	0.003	-0.01	
	Hydration[h]	**-0.39**	**-0.75**	**-0.02**	**0.03**	**0.04**	**0.03**	
Heart Rate	SB[a]	1.10	-0.16	2.37	0.08	0.03	0.02	
	Standing[b]	**-5.59**	**-9.67**	**-1.52**	**0.01**	**0.07**	**0.06**	

	LIPA[c]	-2.74	-5.67	0.18	0.06	0.03	0.02	
	sMVPA[d]	**-3.36**	**-5.67**	**-1.05**	**0.01**	**0.08**	**0.07**	
	$_{10}$MVPA[e]	-6.66	-13.4	0.10	0.05	0.04	0.03	
	MR[dbae]	**-6.02[d]**	**-9.54[d]**	**-2.50[d]**	**0.001[d]**	**0.22**	**0.19**	**-0.34[d]**
		-8.50[b]	**-13.4[b]**	**-3.57[b]**	**0.001[b]**			**-0.34[b]**
		-3.01[a]	**-5.23[a]**	**-0.78[a]**	**0.01[a]**			**-0.28[a]**
		-6.84[e]	**-13.1[e]**	**-0.53[e]**	**0.03[e]**			**-0.22[e]**
					0.00[db]			
					[ae]			
Brachial								
Artery	SB[a]	0.002	-0.10	0.10	0.97	0.00	-0.01	
Diameter	Standing[b]	0.08	-0.26	0.43	0.63	0.003	-0.01	
	LIPA[c]	0.02	-0.22	0.26	0.88	0.00	-0.01	
	sMVPA[d]	0.05	-0.14	0.25	0.59	0.003	-0.01	
	$_{10}$MVPA[e]	0.02	-0.54	0.58	0.94	0.00	-0.01	
	Primary CVD Meds[f]	**0.09**	**0.01**	**0.19**	**0.03**	**0.04**	**0.03**	
	Hydration[h]	**0.02**	**0.003**	**0.04**	**0.02**	**0.05**	**0.04**	
	MR[ih]	**0.03[i]**	**0.00[i]**	**0.05[i]**	**0.02[i]**	**0.12**	**0.10**	**0.25[i]**
		0.02[h]	**0.00[h]**	**0.05[h]**	**0.03[h]**			**0.24[h]**
					0.01[ih]			
LOG	SB[a]	-0.01	-0.04	0.03	0.80	0.001	-0.01	
Blood	Standing[b]	-0.06	-0.14	0.12	0.92	0.00	-0.01	
Velocity	LIPA[c]	-0.03	-0.12	0.06	0.50	0.01	-0.01	

	sMVPA[d]	0.04	-0.03	0.11	0.28	0.01	0.002
	$_{10}$MVPA[e]	0.03	-0.18	0.25	0.75	0.03	0.001
LOG	SB[a]	-0.002	-0.05	0.04	0.94	0.00	-0.01
Shear Rate	Standing[b]	-0.04	-0.20	0.11	0.58	0.003	-0.01
	LIPA[c]	-0.04	-0.15	0.06	0.41	0.01	-0.004
	sMVPA[d]	0.02	-0.06	0.11	0.57	0.004	-0.01
	$_{10}$MVPA[e]	0.01	-0.24	0.27	0.91	0.00	-0.01
LOG IMT	SB[a]	0.01	-0.02	0.03	0.77	0.001	-0.01
	Standing[b]	0.04	-0.06	0.16	0.37	0.01	-0.002
	LIPA[c]	0.01	-0.06	0.09	0.68	0.002	-0.01
	sMVPA[d]	0.003	0.06	0.06	0.92	0.00	-0.01
	$_{10}$MVPA[e]	-0.06	-0.24	0.11	0.48	0.01	-0.01
Carotid							
AL Artery	SB[a]	0.07	-0.05	0.19	0.26	0.01	0.003
Diameter	Standing[b]	-0.12	-0.54	0.28	0.53	0.01	-0.01
	LIPA[c]	-0.22	-0.50	0.06	0.12	0.02	0.01
	sMVPA[d]	0.01	-0.22	0.23	0.96	0.00	-0.01
	$_{10}$MVPA[e]	0.33	-0.33	1.00	0.32	0.01	0.00
	Primary CVD Meds[f]	**0.11**	**0.003**	**0.22**	**0.04**	**0.04**	**0.03**
LOG AL	SB[a]	0.001	-0.02	0.02	0.91	0.00	-0.01
IMT	Standing[b]	0.05	-0.02	0.12	0.17	0.02	0.01
	LIPA[c]	0.01	-0.03	0.07	0.50	0.01	-0.01
	sMVPA[d]	0.002	-0.04	0.04	0.91	0.00	-0.01

	$_{10}$MVPA[e]	0.06	-0.06	0.18	0.32	0.01	0.00
LOG AL	SB[a]	-0.02	-0.05	0.02	0.32	0.01	0.00
Blood	Standing[b]	-0.003	-0.13	0.12	0.95	0.00	-0.01
Velocity	LIPA[c]	0.03	-0.05	0.12	0.47	0.01	-0.01
	sMVPA[d]	0.04	-0.03	0.11	0.25	0.01	0.004
	$_{10}$MVPA[e]	0.11	-0.10	0.32	0.30	0.01	0.001
AL Shear	SB[a]	-9.64	-27.4	8.16	0.28	0.01	0.002
Rate	Standing[b]	2.95	-56.4	62.3	0.92	0.000	-0.01
	LIPA[c]	21.8	-19.8	63.5	0.30	0.01	0.001
	sMVPA[d]	14.5	-18.9	48.1	0.38	0.01	-0.003
	$_{10}$MVPA[e]	20.7	-76.2	117	0.67	0.002	-0.01
LOG AL	SB[a]	0.001	-0.01	0.01	0.90	0.01	0.00
RI	Standing[b]	0.02	-0.03	0.07	0.42	0.01	-0.004
	LIPA[c]	0.01	-0.03	0.04	0.74	0.001	-0.01
	sMVPA[d]	-0.01	-0.04	0.02	0.45	0.01	-0.01
	$_{10}$MVPA[e]	-0.05	-0.14	0.03	0.24	0.01	0.004
PL Artery	SB[a]	0.07	-0.06	0.20	0.28	0.03	0.01
Diameter	Standing[b]	-0.07	-0.42	0.27	0.65	0.01	-0.02
	LIPA[c]	-0.17	-0.46	0.13	0.25	0.04	0.01
	sMVPA[d]	-0.19	-0.48	0.09	0.17	0.06	0.03
	$_{10}$MVPA[e]	0.27	-0.77	1.32	0.59	0.01	-0.02
PL IMT	SB[a]	-0.01	-0.05	0.01	0.24	0.04	0.01
	Standing[b]	-0.01	-0.09	0.07	0.88	0.001	-0.03
	LIPA[c]	0.02	-0.05	0.09	0.52	0.01	-0.02
	sMVPA[d]	0.01	-0.05	0.08	0.64	0.01	-0.02

	$_{10}$MVPA[e]	0.10	-0.15	0.35	0.41	0.02	-0.01
Popliteal							
Artery	SB[a]	0.14	-0.10	0.40	0.24	0.03	0.01
Diameter	Standing[b]	**-0.75**	**-1.41**	**-0.08**	**0.02**	**0.10**	**0.08**
	LIPA[c]	-0.35	-0.91	0.20	0.21	0.03	0.01
	sMVPA[d]	0.18	-0.37	0.74	0.51	0.01	-0.01
	$_{10}$MVPA[e]	0.89	-1.19	2.98	0.39	0.01	-0.01
IMT	SB[a]	0.02	-0.003	0.05	0.07	0.07	0.05
	Standing[b]	-0.06	-0.15	0.01	0.11	0.06	0.03
	LIPA[c]	**-0.09**	**-0.15**	**-0.03**	**0.004**	**0.17**	**0.15**
	sMVPA[d]	**-0.06**	**-0.12**	**-0.002**	**0.04**	**0.09**	**0.07**
	$_{10}$MVPA[e]	-0.07	-0.32	0.17	0.54	0.01	-0.01
	MR[c]	**-0.09**	**-0.15**	**-0.03**	**0.004**	**0.17**	**0.15**
LOG	SB[a]	0.03	-0.03	0.10	0.36	0.01	-0.004
Blood	Standing[b]	-0.12	-0.31	0.06	0.18	0.04	0.01
Velocity	LIPA[c]	-0.06	-0.22	0.08	0.37	0.01	-0.01
	sMVPA[d]	-0.08	-0.23	0.06	0.25	0.03	0.01
	$_{10}$MVPA[e]	-0.51	-1.06	0.03	0.06	0.07	0.05
	Hydration[h]	**-0.01**	**-0.02**	**-0.01**	**0.002**	**0.20**	**0.19**
LOG	SB[a]	0.01	-0.07	0.08	0.90	0.00	-0.02
Shear Rate	Standing[b]	0.01	-0.21	0.22	0.96	0.00	-0.02
	LIPA[c]	-0.01	-0.18	0.17	0.95	0.00	-0.02
	sMVPA[d]	-0.11	-0.28	0.06	0.20	0.03	0.01
	$_{10}$MVPA[e]	**-0.66**	**-1.29**	**-0.03**	**0.04**	**0.09**	**0.07**
	Hydration[h]	**-0.01**	**-0.03**	**-0.004**	**0.01**	**0.14**	**0.12**

MR[h]	**-0.01**	**-0.03**	**-0.004**	**0.01**	**0.14**	**0.12**

Bold font highlights significant ($p \leq 0.05$) bivariate and multivariate stepwise linear regression models. Hydration – Change per percent increase in total body water. Primary CVD Meds – Change per one unit increase in the number of medications directly targeting CVD risk. CVD Meds – Change per one unit increase in the number of medications (in)directly targeting CVD risk.

MR Multivariate stepwise linear regression model. Superscript letters represent which, and what order physical behaviour variables are included in the multivariate model. [i] adjusted for age.

b Change in cardiovascular variable per unit increase in GENEA variable.

-95%CI Negative 95% confidence interval.

+95%CI Positive 95% confidence interval.

p Significance value.

r^2 Explained variance.

r^2 adj. Adjusted explained variance.

P. Corr. Partial Correlation.

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Table 2 Bivariate linear regressions models between patterns of physical behaviour and cardiovascular parameters. Note that neither Systolic BP, Pulse Pressure, Brachial; Artery Diameter, LOG Blood Velocity, LOG Shear Rate, LOG IMT, Carotid; AL Artery Diameter, LOG AL IMT, LOG AL Blood Velocity, LOG AL Shear Rate, LOG AL RI, and PL Artery Diameter showed any significant model with the 16 patterns of physical behaviour of interest and hence these models are not shown. Note that only significant models between patterns of physical behaviour and cardiovascular variables are shown.

Variable	Model	b	-95% CI	+95% CI	*p*	r^2	r^2 adj.
LOG Diastolic BP	Alpha	0.73	0.10	1.37	0.02	0.05	0.04
Heart Rate	SB Breaks	-0.63	-1.13	-0.13	0.01	0.06	0.05
	<5min SB Bout	-1.34	-2.27	-0.40	0.01	0.08	0.07
	True Mean SB Bout	0.17	0.01	0.35	0.04	0.04	0.03
	W50%	0.19	0.17	0.31	0.001	0.11	0.10
	PA Bouts	-0.63	-1.13	-0.13	0.01	0.06	0.05
	Daily Sum of PA Bout Time	-0.03	-0.05	-0.01	0.001	0.11	0.10
	SB%	0.27	0.08	0.46	0.01	0.08	0.07
	Standing%	-0.80	-1.45	-0.15	0.01	0.06	0.05

	sMVPA%	-0.41	-0.78	-0.04	0.02	0.05	0.04
Carotid							
PL IMT	Alpha	1.60	0.29	2.90	0.01	0.17	0.14
Popliteal							
Artery	Standing%	-0.12	-0.22	-0.01	0.02	0.11	0.09
Diameter							
IMT	W50%	0.003	0.00	0.01	0.04	0.09	0.07
	Daily Sum of PA Bout Time	-0.001	-0.001	0.00	0.001	0.15	0.13
	True Mean PA Bout	-0.01	-0.01	-0.001	0.02	0.11	0.09
	SB%	0.01	0.001	0.01	0.01	0.13	0.11
	LIPA%	-0.01	-0.02	-0.004	0.01	0.15	0.13
LOG Blood	W50%	0.01	0.00	0.01	0.03	0.09	0.07
Velocity							
LOG Shear	$_{10}$MVPA%	-0.10	-0.20	-0.001	0.04	0.08	0.06
Rate							

Significant bivariate linear regressions ($p \leq 0.05$).

b Change in cardiovascular variable per unit increase in GENEA variable.

-95%CI Negative 95% confidence interval.

+95%CI Positive 95% confidence interval.

p Significance value.

r^2 Explained variance.

r^2 adj. Adjusted explained variance.

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