

- 1 **Title: The Influence of Sitting, Standing and Stepping Bouts on Cardiometabolic Health Markers in**
- 2 **Older Adults.**

1 **Abstract**

2 Aside from total time spent in (PA) physical activity behaviours, how time is accumulated is important
3 for health. This study examined associations between sitting, standing, and stepping bouts, with
4 cardiometabolic health markers in older adults. Participants from the Mitchelstown Cohort Rescreen
5 Study (n=221) provided cross-sectional data on activity behaviours (assessed via an activPAL3 Micro) and
6 cardiometabolic health. Bouts of ≥ 10 , ≥ 30 and ≥ 60 -minutes sitting, standing, and stepping were
7 calculated. Linear regression models were fitted to examine the associations between bouts and
8 cardiometabolic health markers. Sitting (≥ 10 , ≥ 30 and ≥ 60 minutes) and standing (≥ 10 and ≥ 30 minutes)
9 bouts were detrimentally associated with body composition measures, lipid markers and fasting glucose.
10 The effect for time spent in ≥ 60 -minute sitting, and ≥ 30 -minute standing bouts, were larger than shorter
11 bouts. Fragmenting sitting with bouts of stepping may be targeted to benefit cardiometabolic health.
12 Further insights for the role of standing need to be elicited.

13

14 **Keywords:** *Sedentary behaviour; physical activity; bouts; older adults; cardiometabolic health.*

1 **Introduction**

2 It is well established that regular engagement in physical activity (PA) is a prerequisite for both
3 the development and maintenance of health across the lifespan (Warburton, Nicol, & Bredin, 2006),
4 with age-specific PA guidelines outlining the frequency, intensity and type of PA that individuals should
5 engage in (Bull et al., 2020; U.S. Department of Health and Human Services, 2018; WHO, 2010). In the
6 recent 2020 guidelines on PA and sedentary behaviour (Bull et al., 2020), the World Health Organization
7 (WHO) recommends that older adults (65+ years) should accumulate at least 150 minutes of moderate-
8 intensity aerobic PA, or at least 75 minutes of vigorous-intensity aerobic PA throughout the week to
9 benefit health. Despite this, levels of physical inactivity (i.e. not meeting the PA guidelines (Mark S.
10 Tremblay et al., 2017)) continue to rise, with physical inactivity being prevalent amongst older adults
11 (Gomes et al., 2016; Sun, Norman, & While, 2013). Using data from Wave 4 of the Survey of Health,
12 Ageing, and Retirement in Europe (SHARE) database, Gomes et al. reported that the prevalence of
13 physical inactivity amongst adults aged 55 and over was 12.5%, but that there was a range of 4.9% to
14 29% for the countries involved (Gomes et al., 2016). The authors noted that increasing age was a
15 significant variable associated with physical inactivity (Gomes et al., 2016). As ageing is commonly
16 associated with an increase in one, or more chronic health conditions, and with physical inactivity
17 constituting a major health risk (Australian Institute of Health and Welfare, 2014; Blair, 2009), this
18 population should be a priority for health behaviour interventions.

19 To compound this physical inactivity conundrum, technological advancements in modern society
20 have resulted in an increase in the amount of time spent sedentary for people living in the developed
21 world (Archer et al., 2013; Ng & Popkin, 2012). Older adults have been reported to spend approximately
22 5-9 hours/day being sedentary, depending on how the behaviour is defined and assessed (Harvey,
23 Chastin, & Skelton, 2015). Increased sedentary time is associated with increased adiposity,
24 cardiovascular disease and all-cause mortality (Ekelund et al., 2019; Elhakeem et al., 2018; C. Powell et

1 al., 2020; C. Powell, Herring, Dowd, Donnelly, & Carson, 2018). In addition, previous work by Powell et
2 al. highlighted that older adults spend a considerable proportion of time in PA behaviours of low
3 intensity, including standing (5.3 hours/day) and light-intensity physical activity (LIPA) (1.7 hours/day) (C.
4 Powell et al., 2020). As all PA behaviours and sedentary behaviour occur on a continuum (M. S.
5 Tremblay, Colley, Saunders, Healy, & Owen, 2010), it is important to assess the entire continuum across
6 the 24-h day, rather than focus on one individual behaviour and/or intensity.

7 Sedentary behaviour and PA are often reported as total daily durations spent in an intensity,
8 typically minutes or hours per day. While the volume of these behaviours are important for health
9 (Maddison et al., 2015; C. Powell et al., 2020; Santos et al., 2013; van der Ploeg et al., 2014), some
10 recent evidence also suggests that the manner in which these are accumulated, such as the number and
11 duration of bouts, may be of public health importance (Bailey & Locke, 2015; Bankoski et al., 2011;
12 Judice, Silva, & Sardinha, 2015; Stephens, Granados, Zderic, Hamilton, & Braun, 2011). In contrast to
13 previous iterations of the PA guidelines (WHO, 2010), the most recent guidelines do not have a bout
14 length requirement (Bull et al., 2020). Sedentary behaviour research suggests that frequently breaking
15 up sedentary bouts may be beneficial for acute and long-term health (Bailey & Locke, 2015; Bankoski et
16 al., 2011; Judice et al., 2015; Stephens et al., 2011). Using the 2003-2006 NHANES data, Bankoski et al.
17 found that older adults who had a greater number of sedentary breaks were less likely to suffer from
18 the metabolic syndrome (Bankoski et al., 2011). In addition, Bailey and Locke showed that interrupting
19 sitting time with frequent bouts of LIPA was beneficial for postprandial plasma glucose levels, compared
20 to standing breaks and prolonged sitting (Bailey & Locke, 2015), while Stephens and colleagues reported
21 that one day of prolonged sitting (i.e. a lack of sedentary breaks) considerably reduced insulin activity
22 (Stephens et al., 2011). However, both studies only included younger adults, highlighting a need for
23 research to examine associations between transitions and cardiometabolic health in older adults,

1 particularly because older adults engage in repeated and prolonged bouts of sedentary time on a regular
2 basis (Diaz et al., 2016; Haga, Vrotsou, & Bredland, 2018; Schlaff, Baruth, Boggs, & Hutto, 2017).

3 For example, Schlaff et al. reported that older adults engage in 73.3 sedentary bouts per day,
4 with a bout lasting an average of 7.8 minutes (Schlaff et al., 2017). Similar findings were reported by
5 Diaz and colleagues for sedentary bouts (68.3 sedentary bouts per day), but for a longer average
6 sedentary bout length (11.4 minutes) (Diaz et al., 2016). This study additionally reported a positive
7 relationship between age and the odds of engaging in more prolonged bouts (≥ 30 minutes) (Diaz et al.,
8 2016). An increase in either the amount, or duration, of such sedentary bouts can be deleterious for
9 health, with Júdice and colleagues reporting that for each additional sedentary bout of 10-20 minutes,
10 the odds of being abdominally obese increased by 6.8% (Judice et al., 2015). These behaviours are
11 common in older adults, and potentially detrimental for health, yet little research has examined bout
12 lengths of other PA behaviours (i.e. standing and stepping) and associations between a range of
13 cardiometabolic health markers. A greater understanding of these relationships is needed, to help
14 identify potential intervention targets.

15 Whilst these previously conducted studies provide important insights into how activity is
16 accumulated, it typically has focused on only one behaviour/intensity (e.g. sedentary behaviour), and/or
17 ignored the older population. In addition, count-based PA monitors (e.g. accelerometers) have been
18 predominantly used, which lack the sensitivity to accurately differentiate between specific behaviours
19 (e.g. standing from LIPA) (Lyden, Kozey Keadle, Staudenmayer, & Freedson, 2012; Wijndaele & Healy,
20 2016). To overcome these limitations, the use of monitors that can accurately detect changes in posture
21 (e.g. activPAL devices) should be considered (Lyden et al., 2012; Wijndaele & Healy, 2016). Overall, there
22 is a dearth of research around the accumulation of both sedentary time and PA in older adults, using
23 posture-based devices, and the subsequent associations between different accumulation patterns (e.g.
24 bouts) with cardiometabolic health. Research on the accumulation of PA and sedentary behaviour,

1 including potential associations of different bout durations and health, is warranted to incorporate
2 recommendations on how to accumulate movement behaviours in future guidelines. Therefore, the
3 primary aim of this study was to examine associations between the duration of sitting, standing and
4 stepping bouts with cardiometabolic health markers in older adults.

1 **Methods**

2 ***Study Participants***

3 Participants were recruited to the Mitchelstown Cohort Rescreen (MCR) Study, which has been
4 described previously (C. Powell et al., 2020). Briefly, the MCR Study was a follow-up study to the 2010
5 Cork and Kerry Diabetes and Heart Disease Study (Kearney, Harrington, Mc Carthy, Fitzgerald, & Perry,
6 2013), in which 1,378 participants returned for a rescreen (of an initial 2,047 participants) between
7 November 2015 and May 2017. All participants were between the ages of 55 and 74 years. The
8 participants who attended the clinic on days three, four and five of the week (i.e. the days that the
9 Research Assistant was at the Clinic), were asked to wear an activPAL3 Micro (PAL Technologies,
10 Glasgow, Scotland) for seven consecutive days. Three hundred and ninety-nine participants participated
11 in the monitor wear protocol (29% of those who returned for the rescreen between 2015-2017). Ethics
12 committee approval conforming to the Declaration of Helsinki was obtained from an institutional Clinical
13 Research Ethics Committee (ethics approval code: ECM 4 (nnn) 07/07/15). All participants signed
14 informed consent, including permission to use their data for research purposes. All procedures and
15 measurements were conducted by trained research staff, according to study-specific standardised
16 operating procedures.

17

18 ***Measurements***

19 *Cardiometabolic Health Markers*

20 Stature (cm) and body mass (kg) were measured without footwear, heavy outer clothes and
21 headgear, using a portable stadiometer (Seca, Leicester, United Kingdom) and portable electronic scales
22 (Tanita, Amsterdam, Netherlands). Subsequently, body mass index (BMI) was calculated (kg/m^2).
23 Percentage body fat, fat mass and fat-free mass were measured via bioelectrical impedance (BIA)
24 (Tanita MC 780_MA Body Composition Analyser, Tanita, Amsterdam, Netherlands). For the BIA

1 measurements, participants were measured after either an overnight fast, or a three-hour fast,
2 depending on their appointment time, and informed to not engage in any meaningful exercise 12 hours
3 before their appointment. All participants provided overnight fasted blood samples via venepuncture
4 on the morning of their appointment day, that were subsequently analysed for glycated haemoglobin
5 (HbA1c) (mmol/mol), glucose (mmol/L), triglycerides (mmol/L), total cholesterol (mmol/L), high- (HDL-C;
6 mmol/L), low- (LDL-C; mmol/L), and very-low-density lipoprotein cholesterol (VLDL-C; mmol/L), at an
7 accredited hospital laboratory. Blood pressure (systolic [SBP] and diastolic [DBP]) was measured using
8 an OMRON M7 Digital Blood Pressure Monitor (OMRON Healthcare, Hoofddorp, Netherlands) on the
9 right arm, after a five-minute rest period in the seated position. The average of the second and third
10 measurements was used for analyses. All cardiometabolic health measurements were taken prior to the
11 participant being provided with the activPAL3 Micro.

12

13 *Habitual sitting, standing and stepping behaviours*

14 Habitual sitting, standing and stepping behaviours were assessed using the activPAL3 Micro.
15 Participants wore the monitor for 24 hours per day, for seven consecutive days. Participants were
16 instructed to remove the monitor if they were going to be submerged in water for a prolonged period of
17 time (e.g. bathing, swimming, etc.) to limit any potential device damage or loss but could leave it on for
18 showering. The activPAL3 Micro was set to record at 20Hz, and to start recording at 22:00 on the day
19 the participant received the monitor. The monitor was attached to the anterior aspect of the midline of
20 the right thigh, using a nitrile sleeve and waterproof Tegaderm dressing. The aforementioned wear
21 protocol has been used previously (C. Powell et al., 2020). Raw data were downloaded using the
22 activPAL software into event files (PAL Technologies; www.palt.com). These were then processed using
23 the ProcessingPAL software (ProcessingPAL, software v.1.3, University of Leicester, UK) with algorithms
24 for adults outlined by Winkler et al. (Winkler et al., 2016) to determine time spent sitting, standing, and

1 stepping. Sleep time and non-wear time were excluded using previously validated activPAL wear criteria
2 (Winkler et al., 2016). Consequently, the obtained 24-hour heat-maps (see example in the
3 **Supplementary Materials, Figure S1**) using these criteria were visually inspected by two researchers
4 (S.E.C. and S.J.J.M.V.), and manual adjustments were made whenever identified sleep and/or non-wear
5 times seemed implausible, based on existing recommendations (Winkler et al., 2016). The time spent in
6 ≥ 10 , ≥ 30 and ≥ 60 -minute sitting, standing and stepping bouts (with no allowance for a proportion of the
7 bout time in other intensities incorporated) were also calculated. No upper limits for bouts were used,
8 so the bouts were not mutually exclusive (e.g. sitting bouts of ≥ 10 minutes also contribute to ≥ 30 and
9 ≥ 60 -minute bouts). These bout lengths were based on previous experimental studies that investigated
10 effects of breaking up prolonged sitting time (e.g. hourly or 30 minute interruptions in sitting) (Benatti &
11 Ried-Larsen, 2015). The number of sit-to-upright transitions were also calculated. To be included in the
12 statistical analyses, participants had to provide ≥ 4 days of valid activity data (≥ 10 hours of waking
13 data/day) (Edwardson et al., 2016), and include at least one week day and one weekend day. This
14 inclusion criteria has been used in a similar cohort previously (C. Powell et al., 2020). All activity
15 variables were averaged across all valid wear days and standardized using the residuals obtained by
16 regressing the activity variables on wear time, to adjust for wear time (Willett & Stampfer, 1986).

17

18 *Covariates*

19 All participants completed a clinical report form and a computer-assisted personal interview
20 general health questionnaire (with a trained Researcher). This allowed for demographic covariates (age
21 (years), sex (male/female) and employment status (employed/not employed)), as well as disease status
22 (reported heart conditions (yes/no)), medication use (reported blood pressure medication (yes/no),
23 cholesterol medication (yes/no) and diabetes medication (yes/no)), and lifestyle factors (diet (quality),
24 smoking status (yes/no) and alcohol consumption (yes/no)) to be obtained. For diet quality, participants

1 completed a standard validated food frequency questionnaire (FFQ), thus allowing for a Dietary
2 Approaches to Stop Hypertension (DASH) score to be derived (Harrington et al., 2011). The DASH score
3 is a composite score derived from standard food groups within the FFQ. An overall DASH score was
4 calculated for each participant, with a lower score indicating poorer diet quality.

5

6 ***Statistical Analysis***

7 For the analyses performed in the current study, additional Ethics Committee approval was
8 obtained for the secondary analysis (HEAG-H 170_2019). Only participants with complete
9 cardiometabolic health marker, confounder, and valid accelerometry data were included in the analyses.
10 Data inspection showed that only low proportions of participants engaged in some of the PA behaviours;
11 7% spent time in ≥ 60 -minute standing bouts; 8% spent time in ≥ 30 -minute stepping bouts; and 0% spent
12 time in ≥ 60 -minute stepping bouts. Consequently, these variables were excluded from analyses. The
13 final included variables were observed in at least 30% of the sample, however, as variables are averaged
14 across all valid wear days, the observed averages may be lower than the defined bout duration (e.g.,
15 mean time in 30 minute bouts is 5.26 min). All other identified pattern variables (i.e., minutes in bouts
16 and number of sit-to-upright transitions) were included as continuous exposure variables.

17 Linear regression models were fitted to obtain β regression coefficients and 95% confidence
18 intervals (CIs) for the associations between each of the remaining accelerometry exposures (continuous)
19 and cardiometabolic health markers (continuous). After the wear-time residual adjustment of the
20 activity variables (Willett & Stampfer, 1986), all other assumptions for linear regression were met.

21 Three models were considered to assess the specific effect of covariate adjustment for
22 demographic covariates, separate from adjustments for disease status, medication use, and lifestyle
23 factors. Model 1 only adjusted for wear time using the residual adjustment method (Willett & Stampfer,
24 1986). Model 2 additionally adjusted for age (continuous), and sex and employment status (both

1 binary). Model 3 further adjusted for reported heart conditions, blood pressure medication use,
2 cholesterol medication use, diabetes medication use (all binary), and lifestyle factors including diet
3 (continuous), smoking status and alcohol consumption (both binary). Fat mass was included into the
4 final Model 3 as a covariate in models of non-weight related cardiometabolic risk markers (i.e. HbA1c,
5 fasting glucose, triglycerides, total cholesterol, HDL-C, LDL-C, VLDL-C, SBP, and DBP).

6 Associations of the total times (i.e. volume) spent in sleep, sitting, standing and stepping with
7 cardiometabolic health markers have been previously reported (C. Powell et al., 2020). Therefore, the
8 current study reports associations between the duration of sitting, standing and stepping bouts and sit-
9 to-upright transitions, with the same cardiometabolic health markers, in a sub-sample of the population
10 reported previously (C. Powell et al., 2020). All statistical analyses were performed using Stata v16.0
11 (StataCorp, College Station, TX, USA).

1 in ≥ 10 , ≥ 30 , and ≥ 60 -minute sitting bouts was also positively associated with fat-free mass ($\beta=0.012$,
2 $\beta=0.014$ and $\beta=0.020$). Additionally, time spent in ≥ 10 -minute sitting bouts (but not ≥ 30 -minute bouts)
3 were detrimentally associated with a number of blood lipid markers, including triglycerides ($\beta=0.001$),
4 HDL-C ($\beta=-0.001$), and VLDL-C ($\beta=0.000$), with time spent in ≥ 60 -minute sitting bouts detrimentally
5 associated with glucose ($\beta=0.003$) and HDL-C ($\beta=-0.001$). Time spent in ≥ 10 and ≥ 30 -minute standing
6 bouts were detrimentally associated with both percentage body fat ($\beta=0.045$ and $\beta=0.087$) and fat mass
7 ($\beta=0.060$ and $\beta=0.131$), while time spent in ≥ 10 -minute standing bouts (but not ≥ 30 -minute bouts) were
8 detrimentally associated with BMI ($\beta=0.040$). Finally, the number of sit-to-upright transitions was
9 beneficially associated with SBP only ($\beta=-0.201$). No statistically significant associations between
10 stepping bouts and any of the cardiometabolic health markers were observed.

11

12

Insert Table 2 here

13

14 Sitting and standing, but not stepping bouts, seemed to be associated with a range of
15 cardiometabolic health markers. To facilitate comparisons of the observed effect sizes for sitting and
16 standing bouts, regression coefficients and 95% CIs were plotted in **Figure 1**. Plots were only included
17 for those health markers that indicated significance at $p < 0.05$, as reported in **Table 2**. The plots in **Figure**
18 **1** show that the observed β -coefficients were greatest for time spent in ≥ 60 -minute sitting bouts
19 compared to shorter sitting bouts (≥ 10 - and 30-minute bouts). Whilst β -coefficients for time spent in the
20 sitting bouts were typically smaller compared to those for standing bouts, these had narrower CIs. This
21 indicates less variability in sitting bouts compared to standing bouts.

22

23

Insert Figure 1 here

1 Discussion

2 This study provides a comprehensive picture of how older adults accumulate their daily sitting,
3 standing and stepping, specifically with regards to prolonged bouts. The average amount of time spent
4 daily in sitting bouts of different durations were 376.48 minutes (≥ 10 -minute bouts), 231.22 minutes
5 (≥ 30 -minute bouts) and 117.27 minutes (≥ 60 -minute bouts). While the largest amount of time was spent
6 in bouts ≥ 10 minutes, a considerable amount of time was also spent in bouts of longer durations (i.e.
7 ≥ 30 and ≥ 60 minutes). Average daily time spent in standing and stepping bouts were relatively low, in
8 comparison to sitting bouts of the same durations.

9 The primary aim of the current study was to examine associations between sitting, standing and
10 stepping bouts with cardiometabolic health markers in older adults. The strongest associations were
11 seen for body composition measures. Increased time spent in sitting bouts, regardless of bout duration,
12 were detrimentally associated with a range of body composition markers, including BMI, percentage
13 body fat and fat mass, with a greater effect seen for bouts of increased duration (e.g. percentage body
14 fat: $\beta=0.015$ (≥ 10 -minute bouts) vs. $\beta=0.019$ (≥ 60 -minute bouts)). In addition to body composition
15 measures, prolonged sitting bouts were also associated with increased fasting glucose and decreased
16 HDL-C. These findings are consistent with previous research, whereby sedentary bouts of longer
17 durations are adversely associated with cardiometabolic health in adults (Bellettiere et al., 2019; Owen,
18 Healy, Matthews, & Dunstan, 2010; Parry, Straker, Gilson, & Smith, 2013). The findings not only justify
19 that prolonged sedentary behaviour should continue to be a focus in behavioural interventions, but also
20 suggests that even sitting bouts as short as ≥ 10 minutes should be targeted in older populations.

21 Increased time spent in standing bouts of both ≥ 10 and ≥ 30 minutes were also shown to be
22 detrimentally associated with percentage body fat and fat mass, again with a greater effect seen for
23 bouts of a longer duration (e.g. percentage body fat: $\beta=0.045$ (≥ 10 -minute bouts) vs. $\beta=0.087$ (≥ 30 -
24 minute bouts)). The detrimental relationship between time spent in standing bouts of ≥ 10 and ≥ 30

1 minutes, and measures of body composition (percentage body fat and fat mass) is a curious finding. The
2 role of standing as a PA behaviour that may improve cardiometabolic health is an ambiguous one.
3 Perhaps the time spent standing may negatively impact on how much time is spent stepping, rather
4 than time spent sitting. While some evidence exists to suggest the benefits of increased standing time
5 on markers of cardiometabolic health (Danquah et al., 2018; Healy, Winkler, Owen, Anuradha, &
6 Dunstan, 2015; Van Der Berg et al., 2017; van der Ploeg et al., 2014), more recent acute interventional
7 data suggests that using standing as a means for breaking up sedentary time on an hourly basis has no
8 significant effect on postprandial cardiometabolic biomarkers, including glucose metabolism and lipids
9 (Altenburg, Rotteveel, Serné, & Chinapaw, 2019). Additionally, previous research by Carr et al. (Carr,
10 Swift, Ferrer, & Benzo, 2016) and Chaput et al. (Chaput et al., 2015) found no changes in BMI, body
11 mass, or percentage body fat with increased standing time. In fact, depending on the context relating to
12 the standing behaviour, increased standing time may have a deleterious impact on health. Over a 12
13 year follow-up, Smith and colleagues (Smith, Ma, Glazier, Gilbert-Ouimet, & Mustard, 2017) tracked the
14 incidence of heart disease for 7,320 Canadian participants. Occupations involving predominantly
15 standing were associated with almost a 2-fold risk of heart disease, compared to occupations that
16 predominantly involved sitting (Smith et al., 2017). This is in line with the PA paradox proposed by
17 Holtermann and colleagues (Holtermann, Hansen, Burr, Sjøgaard, & Sjøgaard, 2012; Holtermann, Krause,
18 van der Beek, & Straker, 2018), where occupational PA can lead to detrimental health outcomes.
19 Approximately half of the present study sample reported to be currently employed. Although the
20 specific occupation types of the included participants are unknown, it is possible that the working
21 proportion of the included sample were predominantly employed in standing occupations, potentially
22 explaining the unfavourable body composition outcomes.

23 Powell et al. (C. Powell et al., 2020) recently reported on the theoretical effects (through
24 compositional data analysis) of replacing sedentary time with standing time, using the same dataset

1 used in the current study. Results suggested that increased standing time related to an increase in BMI,
2 percentage body fat and fat mass, with limited theoretical benefits of reallocating time from sedentary
3 time to standing time. The current study provides further insights into why these findings may have
4 been observed, as it suggests that prolonged standing bouts may be detrimental for health (Garcia,
5 Läubli, & Martin, 2018). This is in line with previous literature, for example, by Garcia et al. (Garcia et al.,
6 2018), which showed that lower-leg volume significantly increased after five hours of prolonged
7 standing work, indicating worsened vascular functioning. Given the increased interest in standing as a
8 viable behaviour for health change/improvement (Buckley et al., 2015), a greater understanding of the
9 context, and in particular the sustained duration in which standing is occurring, is warranted. It is
10 possible that time spent standing may be beneficial for some cardiometabolic health markers and/or
11 population and age groups, but that it may be detrimental for others highlighting the complex nature of
12 targeting standing in interventional strategies for health improvement. This information will be
13 important for the development of future guidelines that include guidance on activity accumulation in
14 older adults, to benefit health beyond the effects of increased physical activity and reduced sedentary
15 time.

16 It is necessary to address some of the unexpected results from the current study. Regardless of
17 the length of the sitting bout, there appeared to be a beneficial association between this behaviour and
18 fat-free mass. It is noted that this was not observed for the relative fat-free mass measure (i.e., fat-free
19 mass percentage of total mass), hence this may have been caused due to the total mass not being taken
20 into account. Another potential explanation for this finding is the association between increasing fat
21 mass and fat-free mass (Gray & Bauer, 1991). Put simply, there is a positive relationship between fat
22 mass and fat-free mass, which could be due to several factors, including increased organ size (Naeye &
23 Roode, 1970) and blood volume (Messerli, 1982) (both which would be classified under fat-free mass) of
24 obese individuals, compared to their non-obese counterparts. Prolonged sitting is not something that

1 the authors would endorse, given the extensive literature around the detrimental association between
2 sitting/sedentary time, and a whole host of cardiometabolic health markers (Bellettiere et al., 2017;
3 Carson et al., 2014; Gennuso, Gangnon, Thraen-Borowski, & Colbert, 2015; Healy et al., 2015; C. Powell
4 et al., 2020; C. Powell et al., 2018). This may be something that was specific and unique to the included
5 sample. The lack of an association between stepping (which would include both LIPA and MVPA), and
6 any of the included cardiometabolic health markers could be partly explained by the low level and/or
7 homogeneity of the time participants spent stepping. Given the decade's worth of evidence showing the
8 benefits of PA (Bucksch, 2005; K. E. Powell, Paluch, & Blair, 2011; Warburton et al., 2006), the authors
9 would categorically support this as a behaviour for both the development and maintenance of health,
10 and that any public health recommendations on sitting, standing and LIPA should only supplement the
11 current MVPA guidelines. Additionally, where there were no associations between ≥ 30 -minute bouts
12 and some health markers (where an association for ≥ 10 -minute bouts with a cardiometabolic health
13 marker), could be partially explained by the higher number of ≥ 10 -minute bouts, and the total time
14 spent in these bouts, making it more likely that a significant association would be found.

15

16 ***Strengths and Limitations***

17 The strengths of the current study include the use of an activPAL3 Micro to accurately classify
18 the behaviours of interest (i.e. sitting, standing and stepping) (Edwardson et al., 2016; Kozey-Keadle,
19 Libertine, Lyden, Staudenmayer, & Freedson, 2011), and the analytical methods used to determine the
20 length of the bouts of interest. In addition, the extensive health profile gathered for all participants
21 allowed for a comprehensive insight into cardiometabolic health to be examined.

22 The limitations of this study also need to be recognised. Due to the cross-sectional nature of the
23 study, causation cannot be determined. Another limitation is participants' potential reactivity to wearing
24 the monitor. However, previous evidence highlighted that no differences were observed between weeks

1 one and week two in sedentary time, standing time and PA for participants who wore an activPAL3
2 Micro for 14 consecutive days (C. Powell et al., 2020), suggesting that the potential of this reactivity to
3 effect findings was minimal. In addition, no feedback was given to participants with regards to their PA
4 behaviours while wearing the activPAL, so any reactivity effects were likely to be short-lived. With
5 regards to socioeconomic status (SES), a direct measure was not available; however, employment status
6 was assessed, which may be considered as a gauge of SES status, as distinction between occupations
7 may be drawn from portions of the day spent sitting, standing and stepping. However, given the lack of
8 indicators regarding occupation, we were unable to test whether specific types of employment would
9 influence findings.

1 **Conclusion**

2 Findings from the current study provide detailed and novel insights into the PA behaviour
3 patterns of older adults, and the subsequent relationships between these patterns and cardiometabolic
4 health markers. Sitting bouts as short as ≥ 10 minutes appear to be associated with increased BMI,
5 percentage body fat, fat mass, triglycerides, and VLDL-C, as well as decreased HDL-C. Additionally, short
6 standing bouts (≥ 10 minutes) also appear to be associated with increased BMI, percentage body fat and
7 fat mass. Overall, these findings suggest that for older adults, repeated short sedentary bouts may have
8 detrimental health effects, and that standing may not be a viable PA behaviour to induce changes in
9 cardiometabolic health. Whilst these findings need to be confirmed in other samples, this suggests that
10 future interventions may focus on reducing prolonged sedentary bouts (even those as short as 10
11 minutes) and use a PA behaviour that has a stepping element to it (i.e., not prolonged standing) to
12 fragment sedentary behaviour.

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32

1 **Tables**

Table 1. Participant characteristics (n=221)

Demographic characteristics	Mean (SD)
Age (years)	65.05 (5.14)
Sex (% female)	48.8 %
Employment status (% yes)	47.5%
Cardiometabolic health markers	Mean (SD)
BMI (kg/m ²)	28.13 (5.99)
Body fat (%)	29.68 (8.20)
Fat mass (kg)	23.23 (8.78)
Fat-free mass (kg)	54.40 (11.36)
HbA1c (mmol/mol)	39.76 (6.77)
Fasting glucose (mmol/L)	5.27 (1.28)
Total cholesterol (mmol/L)	5.22 (1.02)
Triglycerides (mmol/L)	1.17 (0.56)
HDL-C (mmol/L)	1.47 (0.39)
LDL-C (mmol/L)	3.22 (0.92)
VLDL-C (mmol/L)	0.54 (0.26)
Systolic blood pressure (mmHg)	128.71 (19.49)
Diastolic blood pressure (mmHg)	75.90 (10.41)
Covariates	
Reported heart condition (% yes)	80.5%
Blood pressure medication (% yes)	29.4%
Cholesterol medication (% yes)	41.2%
Diabetes medication (% yes)	5.0%
DASH Score (Mean (SD) score)	24.38 (5.32)
Smoking status (% yes)	6.8%
Alcohol consumption (% yes)	74.7%
Activity variables	Mean (SD)
Waking wear time (min)	934.02 (64.26)
Total sitting time (min)	479.02 (105.17)
Time in ≥10-min sitting bouts	376.48 (106.01)
Time in ≥30-min sitting bouts	231.22 (91.79)
Time in ≥60-min sitting bouts	117.27 (67.52)
Total standing time (min)	314.43 (94.19)
Time in ≥10-min standing bouts	28.04 (31.10)
Time in ≥30-min standing bouts	5.26 (10.37)
Total stepping time (min)	140.57 (57.01)
Time in ≥10-min stepping bouts	3.87 (8.33)
Sit-to-upright transitions (no.)	49.28 (14.07)

- 2 All variables are expressed as a mean (standard deviation) unless stated otherwise.
3 SD: standard deviation, BMI: body mass index, HbA1c: glycated haemoglobin, HDL-C: high-density lipoprotein cholesterol, LDL-
4 C: low-density lipoprotein cholesterol, VLDL-C: very-low-density lipoprotein cholesterol.
5 Diet was measured using the Dietary Approaches to Stop Hypertension (DASH) diet quality score derived from the standard
6 validated Food Frequency Questionnaire (FFQ) (Harrington et al., 2011). A lower DASH score indicates poorer diet quality.
7 All sitting, stepping and standing exposure variables were standardized for wear time by using the residuals method.
8

Table 2. Fully adjusted results of the association between sitting, stepping, and standing bouts and cardiometabolic health markers (Model 3; n=221)

Cardiometabolic health markers	Time in sitting bouts (min)			Time in standing bouts (min)		Time in stepping bouts (min)	Sit-to-upright Transitions (no.)
	≥10-min bouts β (95% CI)	≥30-min bouts β (95% CI)	≥60-min bouts β (95% CI)	≥10-min bouts β (95% CI)	≥30-min bouts β (95% CI)	≥10-min bouts β (95% CI)	β (95% CI)
BMI (kg/m ²)	0.011 (0.003, 0.019)**	0.012 (0.003, 0.021)**	0.017 (0.005, 0.029)**	0.040 (0.014, 0.065)**	0.072 (-0.006, 0.149)	-0.062 (-0.159, 0.035)	-0.029 (-0.090, 0.031)
Body fat (%)	0.015 (0.007, 0.023)**	0.016 (0.007, 0.025)**	0.019 (0.007, 0.031)**	0.045 (0.019, 0.072)**	0.087 (0.007, 0.166)*	-0.033 (-0.132, 0.067)	-0.036 (-0.098, 0.026)
Fat mass (kg)	0.023 (0.012, 0.034)**	0.026 (0.013, 0.038)**	0.032 (0.015, 0.049)**	0.060 (0.023, 0.096)**	0.131 (0.020, 0.241)*	-0.077 (-0.215, 0.062)	-0.049 (-0.136, 0.038)
Fat-free mass (kg)	0.012 (0.004, 0.020)**	0.014 (0.005, 0.023)**	0.020 (0.008, 0.032)**	0.005 (-0.022, 0.031)	0.037 (-0.043, 0.116)	-0.065 (-0.164, 0.033)	-0.001 (-0.063, 0.061)
Fat-free mass (%)	-0.000 (-0.000, -0.000)*	-0.000 (-0.000, -0.000)*	-0.000 (-0.001, -0.000)*	-0.000 (-0.001, 0.000)	-0.002 (-0.003, 0.000)	-0.001 (-0.002, 0.002)	0.000 (-0.001, 0.002)
HbA1c (mmol/mol)	0.002 (-0.006, 0.009)	0.005 (-0.004, 0.014)	0.010 (-0.002, 0.022)	-0.007 (-0.033, 0.018)	-0.006 (-0.080, 0.068)	-0.036 (-0.128, 0.055)	-0.034 (-0.091, 0.023)
Fasting glucose (mmol/L)	0.001 (-0.001, 0.002)	0.001 (0.000, 0.003)	0.003 (0.000, 0.005)*	-0.002 (-0.006, 0.003)	-0.005 (-0.019, 0.010)	-0.009 (-0.026, 0.009)	-0.005 (-0.016, 0.006)
Total cholesterol (mmol/L)	0.001 (-0.001, 0.002)	0.001 (-0.001, 0.002)	0.000 (-0.002, 0.002)	0.000 (-0.004, 0.004)	0.001 (-0.010, 0.012)	-0.005 (-0.019, 0.008)	0.003 (-0.005, 0.011)
Triglycerides (mmol/L)	0.001 (0.000, 0.002)*	0.000 (0.000, 0.001)	0.000 (-0.001, 0.001)	0.001 (-0.001, 0.004)	0.006 (-0.001, 0.013)	-0.006 (-0.015, 0.003)	0.003 (-0.003, 0.008)
HDL-C (mmol/L)	-0.001 (-0.001, 0.000)**	-0.001 (-0.001, 0.000)	-0.001 (-0.001, 0.000)*	0.001 (-0.001, 0.002)	0.000 (-0.004, 0.004)	0.000 (-0.005, 0.005)	-0.001 (-0.004, 0.002)
LDL-C (mmol/L)	0.001 (0.000, 0.002)	0.001 (0.000, 0.002)	0.001 (-0.001, 0.002)	-0.002 (-0.005, 0.002)	-0.002 (-0.012, 0.008)	-0.002 (-0.015, 0.010)	0.003 (-0.005, 0.011)
VLDL-C (mmol/L)	0.000 (0.000, 0.001)*	0.000 (0.000, 0.001)	0.000 (-0.001, 0.001)	0.001 (-0.001, 0.002)	0.003 (0.000, 0.006)	-0.003 (-0.007, 0.001)	0.001 (-0.001, 0.004)
SBP (mmHg)	0.020 (-0.007, 0.047)	0.026 (-0.004, 0.057)	0.036 (-0.005, 0.077)	0.084 (-0.003, 0.171)	0.197 (-0.058, 0.453)	-0.107 (-0.423, 0.209)	-0.201 (-0.397-, 0.005)*
DBP (mmHg)	0.010 (-0.004, 0.024)	0.011 (-0.004, 0.027)	0.015 (-0.006, 0.037)	0.009 (-0.036, 0.055)	0.028 (-0.106, 0.161)	-0.105 (-0.268, 0.059)	0.031 (-0.072, 0.134)

- 1 All results are expressed as β-coefficient (95% CIs). **Bold values** indicate significance at *p<0.05 and **p<0.01.
- 2 BMI: body mass index, HbA1c: glycated haemoglobin, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, VLDL-C: very-low-density lipoprotein cholesterol, SBP: systolic blood pressure, DBP: Diastolic blood pressure.
- 3 All sitting, stepping and standing exposure variables were standardized for wear time by using the residuals method. Model 3 additionally adjusted for demographic characteristics, including age (continuous), and sex and employment status (binary), reported heart conditions, blood pressure medication use, cholesterol medication use, diabetes medication use (binary), and lifestyle factors, including diet (continuous), smoking status and alcohol consumption (binary). Fat mass was included as a covariate in models of non-weight related cardiometabolic risk markers (HbA1c, fasting glucose, triglycerides, total cholesterol, HDL-C, LDL-C, VLDL-C, SBP, and DBP).
- 4 Diet was measured using the Dietary Approaches to Stop Hypertension (DASH) diet quality score derived from the standard validated Food Frequency Questionnaire (FFQ) (Harrington et al., 2011). A lower DASH score indicates poorer diet quality.