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ORIGINAL ARTICLE

Patterns of sedentary behavior and physical function in older adults

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Abstract
Background/aims The purposes of this study were to examine the relationship between various objectively measured sedentary behavior (SB) variables and physical function in older adults, examine the measurement properties of an SB questionnaire, and describe the domains of SB in our sample.
Methods Forty-four older adults (70 ± 8 years, 64 % female) had their SB measured via activPAL activity monitor and SB questionnaire for 1 week followed by performance-based tests of physical function.
Results The pattern of SB was more important than total SB time. Where a gender by SB interaction was found, increasing time in SB and fewer breaks were associated with worse function in the males only. The SB questionnaire had acceptable test–retest reliability but poor validity compared to activPAL-measured SB. The majority of SB time was spent watching television, using the computer and reading.
Discussion/conclusions This study provides further evidence for the association between SB and physical function and describes where older adults are spending their sedentary time. This information can be used in the design of future intervention to reduce sedentary time and improve function in older adults.

Keywords Sedentary lifestyle · Functionally impaired elderly · Aging · Physical activity · Activities of daily living

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Introduction

Sedentary behavior (SB), defined as waking behaviors characterized by an energy expenditure ≤1.5 METs while in a sitting or reclining posture [1], has increasingly been the subject of physical activity and health-related research. There has been a recent push to examine various properties of SB, such as its determinants and the context in which is it accumulated, as well its relationship with a host of different health outcomes. The majority of the work done in this area, to date, has been limited to children and adults aged 18 to 65. This is despite evidence to suggest older adults represent the most sedentary age group, spending approximately 60 to 70 % of their waking hours in SB [2]. Relatively little is known about the relationship between SB and health outcomes in older adults, and even less work has been done to identify the domains that account for the majority of SB in the 65-year and older population.

A significant health concern for older adults is reductions in their physical function. Approximately 66 % of adults aged 65 years or older and 87 % aged 85 or older report some sort of limitation in the performance of specific physical tasks required for activities of daily living, such as reaching over one's head or being able to grasp small items [3]. As these data suggest, two-thirds of older adults currently have a functional limitation that could eventually lead to difficulty in performing ADLs. One logical contributor to losses in function is prolonged sedentarism, though studies examining this association in free-living conditions are sparse. Longitudinal data for approximately 62,000 women aged 50–79 years from the Women's Health Initiative showed that those with the highest self-reported sitting and total sedentary time at baseline had significantly greater reductions in self-reported physical function after 12.3 years of follow-up [4], and Gennuso et al. [5] used accelerometer data from 1914 older adults from the 2003–2006 NHANES cycles to show that those with higher amounts of daily SB reported more functional limitations compared to those with lesser SB. However, neither of these studies was designed specifically to examine the association between SB and physical function, and, thus, did not use the best available methods to do so.

Both of the previous studies were limited to the use of self-reported measures of physical function and functional limitations, rather than performance-based tests, by their large size and overall scope. In addition, while a methodological strength of the latter study was the use of an objective method to measure activity instead of self-report, the hip-worn accelerometer, like the one used by NHANES, is best at detecting ambulatory activity of higher intensities [6] and is not the ideal tool to measure free-living SB. Better technology now exists to measure SB, like the activPAL (PAL Technologies, Glasgow, Scotland), which has the ability to detect changes in posture and can, therefore, more accurately differentiate time spent sitting from standing and stepping [7]. Therefore, the primary purpose of this study was to take a dedicated look at the relationship between SB and physical function in older adults using activPAL-measured SB and performance-based tests of physical function in older adults. We anticipated that increasing time in SB and less breaks in SB would be negatively associated with objectively measured and self-reported physical function. A secondary purpose of this study was to examine the measurement properties of a self-report SB questionnaire and use it to describe the domains in which older adults accumulated SB most frequently.

Methods

Study population and experimental design

Fifty community-dwelling adults aged 65 years and older with the ability to walk unaided were recruited from the Madison, WI area to participate in this study between October 2012 and May 2013. Participants were required to attend two study visits separated by 1 week of objective activity monitoring. During the initial visit, a demographics and health history questionnaire was completed and anthropometric measurements were taken. This was followed by the administration of a self-reported SB questionnaire and orientation to the proper wear and care of the activity monitors. During the second visit, participants were re-administered the SB questionnaire and underwent physical function testing. The study protocol was approved by the University of Wisconsin Health Sciences Institutional Review Board, and all subjects provided written informed consent before study initiation.

Study measures

Activity monitors

Participants were asked to wear two activity monitors during all waking hours except during situations where they might get wet. The activPAL was affixed directly to the midline of thigh of the participant with the use of Medipore soft cloth surgical tape (3 M, St. Paul, MN). This device was used to measure time spent sitting or lying and the number of times this behavior was disrupted (i.e., breaks in SB) with a change in posture. These data were used to calculate average daily SB (h/day), SB bout length, break rate (breaks/sedentary hour), and time spent in sedentary bouts of varying lengths (i.e., $\geq$20, $\geq$40, and $\geq$60 min). The ActiGraph GT3X was worn on an elastic belt over the right hip. Data from this monitor were scored to indicate the average time (min/day) spent in moderate-to-vigorous-intensity physical activity (MVPA). Count thresholds were used to differentiate light intensity activity (100–760 counts/min) from moderate (760–5725 counts/min) and vigorous (counts/min $\geq$5725) intensity activity. Wear time of both monitors was determined by subtracting non-wear time from total daily observation time using a combination of ActiGraph data and wear-time logs completed by participants. Non-wear from the ActiGraph was defined as periods of at least 60 consecutive minutes of no activity with an allowance for two consecutive minutes of observations between 1 and 100 counts. A valid day was considered a day with at least 600 min (10 h) of wear without excessive counts (>20,000 counts). At least three valid days were required to be included in the current analysis, and all valid days were averaged to calculate the summary variables.

Physical function

Participants completed the Short Physical Performance Battery (SPPB), hand grip dynamometry, long distance corridor walk (LDCW), dynamic balance testing, and the MOS 36-Item Short Form Health Survey (SF-36). The SPPB consists of three standing balance tasks, a 4-m walk for usual gait speed, and chair stands [8]. Collectively, times from the three tests were used to create a summary linear score that ranges from 0 to 12. The LDCW is a two-part test of mobility, consisting first of a self-paced 2-min walk, followed by a timed 400-m walk [9]. A Biodex Balance System SD (Biodex, Shirley, New York) was used to assess dynamic balance by quantifying the ability to appropriately shift body weight and maintain postural stability on stable and unstable surface conditions. A screen providing visual feedback guided participants through proprietary testing protocols while calculating overall postural stability and fall risk scores. This task has been safely used in studies with older adult populations to examine balance deficits as a determinant of falls [10] and to measure change in balance in response to a motor skill training intervention [11]. Although objective measures of function were the primary outcome of interest, we also employed the physical function subscale of the SF-36 [12]. This 10-item subscale assesses activities of daily living (e.g., bathing and dressing) and instrumental activities of daily living (e.g., walking several blocks, lifting/carrying groceries).

Sedentary behavior questionnaire

The questionnaire used to measure time spent in different domains of SB was adapted from a self-report survey of older adults' sedentary time [13]. Information about SB in leisure and transportation is collected by determining the total time spent during the past week sitting or lying down and (1) watching television, (2) using the computer, (3) reading, (4) socializing, (5) driving or in public transportation, (6) doing hobbies, and (7) any other activities. We added a question concerning occupational SB by asking about time spent sitting in paid or volunteer work.

Statistical considerations

All analyses were conducted using SAS v9.3 software (SAS Institute Inc., Cary, NC). Multiple linear regression models were used to address the primary purpose of examining the relationships between the various physical function measures and SB. Potential covariates assessed for confounding were gender, BMI, age, minutes of monitor wear time, and MVPA (measured by ActiGraph). Those found to be significant confounders and included in the models were age, minutes of monitor wear time, and MVPA. In addition, effect modification by gender and level of MVPA (sufficient to meet the public health guidelines of ≥150 min/week or insufficient) on the SB and function relationships was explored. No effect modification by MVPA was found. There was evidence of modification by gender, so separate analyses were performed for males and females. Where the gender by SB interaction was not statistically significant, estimates for men and women combined are presented. Gender was additionally controlled for in those models. Accordingly, a minimum sample size of 49 participants was determined to be required to detect an effect size of 0.30 or greater in a model with five predictors at 80 % power. As secondary analyses, we also explored the test–retest reliability, using intraclass correlations, and the comparative validity, using Lin's concordance correlation [14], Spearman's rank-order correlation, and a Bland–Altman analysis, of the SB questionnaire and used it to describe the domains in which our sample spent the most time. The following descriptive scales were used to determine the strength of the validity/reliability: Lin's coefficient (poor, <0.90; moderate, 0.90–0.95; substantial, 0.96–0.99; almost perfect, >0.99) [15], Spearman's and ICC (poor, <0.30; moderate, 0.30–0.70; substantial, 0.71–0.80; almost perfect, >0.80).

Results

Participant summary

Of the 50 older adults recruited to participate in this study, two voluntarily withdrew before their second visit. An additional four participants are missing SB data; one from a lost activPal and the other three from errors during data downloading. For the 44 participants with complete data, the median (25–75 %) age and BMI were 70 (68–76) years and 27.7 (24.2–30.6) kg/m^2, respectively. The sample was mostly female (64 %) and white (91 %) with a high socioeconomic status. Participant characteristics by gender can be found in Table 1. On average, the males tended to be older and have a higher BMI than the females. Both genders had similarly high physical function (Table 2), with 64 % of the sample being classified as high functioning by the SPPB (having a total score of ≥10). Information regarding the participants' objectively measured SB and MVPA can be found in Table 3. The median (25–75 %) time spent in objectively measured SB and MVPA for the entire sample was 9.4 (8.2–10.5) h/day and 129.0 (46.3–223.2) min/week, respectively, with 47.6 (40.6–58.5) breaks per day. Males tended to be sedentary longer with less breaks, resulting in a longer average SB bout length. However, males also accumulated more time spent in MVPA and were more likely to meet physical activity guidelines.

Sedentary behavior with physical function

The primary purpose of the study was to examine the relationship between various activPAL-measured SB variables and performance-based tests of physical function in our sample of older adults. A number of the function measures lacked a statistically significant relationship with SB. This was the case for handgrip strength, postural stability and fall risk measured by the Biodex, the physical function subscale of the SF-36, and the balance and gait subscales of the SPPB. All comparisons for these outcomes were $P > 0.05$ (results not shown). Results for associations between SB and the remaining physical function measures can be found in Table 4.

Table 1 Participant characteristics ($n = 44$)

	Male ($n = 16$)	Female ($n = 28$)
Age (years)[a]	71 (69–74)	70 (67–78)
BMI (kg/m^2)[a]	29.2 (27.2–32.5)	26.8 (23.8–29.2)
Race (*n*)		
White	16	24
Asian	0	2
Hispanic	0	1
Black/African American	0	1
Marital status (*n*)		
Single	2	2
Married	10	8
Divorced	3	12
Widowed	1	6
Income (*n*)		
Declined	1	0
<$10,000	0	1
$10,000–20,000	5	9
$20,000–30,000	2	4
>$40,000	8	14
Education completed (*n*)		
High school	1	4
College	5	12
Graduate school	10	12

BMI body mass index

[a] Values are expressed as median (25–75 %)

Overall, we found support of our hypothesis for a negative association between SB and physical function in the remaining function measures that were significantly associated with SB. In general, where a gender by SB interaction was found, statistically significant associations between SB and physical function were found in the males only. For the SPPB-chair stand subscore, significant associations between SB and physical function were found in males and females combined where the gender by SB interactions were non-significant. This is in contrast to the SPPB-total score, where no significant associations were found in the combined analysis.

When looking at the various SB variables, there seemed to be little to no relationship between total daily SB time and the physical function outcomes; however, the pattern with which it was accumulated seemed to matter. For instance, the number of breaks per day and break rate were associated with all three outcomes in males only. Also, average SB bout length and increasing time spent in longer bouts of SB were associated with SPPB-chair stand subscore in males and females combined and with 400-m walk gait speed in males only.

Sedentary behavior questionnaire

A secondary purpose of this study was to examine the measurement properties of a self-report measure of SB and

Table 2 Physical function outcome scores by gender in a sample of older adults

	Male ($n = 16$)	Female ($n = 28$)
SPPB		
Balance	4.0 (3.5–4.0)	4.0 (4.0–4.0)
Gait	4.0 (4.0–4.0)	4.0 (4.0–4.0)
Chair rise	2.5 (1.0–3.5)	2.5 (1.5–3.0)
Total	10.0 (9.0–11.0)	10.0 (9.0–11.0)
SPPB functional classification[a]		
Low	1 (6)	1 (4)
Medium	5 (31)	9 (32)
High	10 (63)	18 (64)
400-m walk gait speed (m/s)	1.39 (1.20–1.63)	1.30 (1.19–1.50)
Postural stability	0.40 (0.20–0.50)	0.35 (0.20–0.48)
Fall risk	1.00 (0.74–1.20)	0.75 (0.60–0.95)
SF-36 physical function scale (0–100)	90.0 (80.0–95.0)	82.5 (70.0–95.0)

Values expressed as median (25–75 %) unless otherwise noted

SPPB short physical performance battery, *400 MW* 400 meter walk

[a] Functional classification, expressed as *n* (%), is determined by the SPPB based on the following SPPB-total scores; low = 0–6, medium = 6–9, and high = 10–12

Table 3 Sedentary behavior and MVPA by gender in a sample of older adults

	Male (n = 16)	Female (n = 28)
Total daily SB time (h/day)	9.6 (8.7–11.1)	9.3 (7.9–10.3)
Breaks (breaks/day)	45.5 (31.9–52.7)	52.0 (41.2–61.3)
Break rate (breaks/h)	4.7 (3.8–5.6)	5.5 (4.5–6.9)
Average bout length (min)	12.7 (10.7–16.0)	10.7 (8.7–13.4)
20 min bouts[a] (h/day)	6.2 (5.2–7.1)	5.7 (4.7–6.9)
40 min bouts[a] (h/day)	3.7 (3.1–5.0)	3.8 (3.3–4.5)
60 min bouts[a] (h/day)	2.4 (1.8–3.1)	2.4 (1.6–3.3)
MVPA weekly (min/week)	145.4 (34.7–310.4)	124.7 (76.8–192.3)
≥150 min/week MVPA[b]	8 (50)	9 (32)

Values expressed as median (25–75 %) unless otherwise stated

SB sedentary behavior, *MVPA* moderate-to-vigorous physical activity

[a] Denotes the amount of time spent in SB (h/day) in bouts lasting at least as long as the value provided

[b] Expressed as n (%)

Table 4 Associations between sedentary behavior physical function in a sample of older adults

	Male	Female	$P_{interaction}$	Combined
SPPB-total score (0–12)				
Total SB time (h/day)	−0.28 (0.24)	0.10 (0.18)	0.63	−0.09 (0.14)
Breaks (breaks/day)	0.07 (0.02)*	0.001 (0.02)	0.04	
Break rate (breaks/h)	0.79 (0.17)***	−0.05 (0.14)	0.03	
Average bout length (min)	−0.18 (0.05)**	0.03 (0.07)	0.09	−0.07 (0.04)
20 min bouts[a] (h/day)	−0.48 (0.13)**	0.07 (0.13)	0.09	−0.12 (0.10)
40 min bouts[a] (h/day)	−0.53 (0.12)**	0.04 (0.15)	0.10	−0.17 (0.11)
60 min bouts[a] (h/day)	−0.57 (0.15)**	0.009 (0.17)	0.12	−0.22 (0.11)
SPPB-Chair stand subscore (0–4)				
Total SB time[a] (h/day)	−0.28 (0.21)	−0.11 (0.15)	0.64	−0.21 (0.11)
Breaks (breaks/day)	0.06 (0.02)*	0.006 (0.02)	0.03	
Break rate(breaks/h)	0.60 (0.19)**	0.04 (0.12)	0.04	
Av bout length[a] (min)	−0.17 (0.04)**	−0.04 (0.06)	0.16	−0.10 (0.03)**
20 min bouts[a] (h/day)	−0.39 (0.13)**	−0.06 (0.11)	0.13	−0.18 (0.08)*
40 min bouts[a] (h/day)	−0.42 (0.13)**	−0.10 (0.13)	0.18	−0.23 (0.09)*
60 min bouts[a] (h/day)	−0.48 (0.14)**	−0.17 (0.14)	0.25	−0.29 (0.09)**
400-m walk gait speed (m/s)				
Total SB time (h/day)	−0.03 (0.04)	0.05 (0.03)	0.08	0.01 (0.02)
Breaks (breaks/day)	0.01 (0.004)*	−0.001 (0.003)	0.01	
Break Rate (breaks/h)	0.11 (0.04)*	−0.03 (0.02)	0.003	
Av Bout Length (min)	−0.03 (0.01)*	0.01 (0.01)	0.01	
20 min bouts[a] (h/day)	−0.06 (0.03)	0.04 (0.02)	0.01	
40 min bouts[a] (h/day)	−0.07 (0.03)*	0.03 (0.02)	0.004	
60 min bouts[a] (h/day)	−0.09 (0.03)*	0.02 (0.02)	0.01	

Values are regression coefficients (standard error). Models controlled for age, wear time, and MVPA. Combined analysis presented where gender by SB interaction is non-significant. Gender controlled for in combined analysis

SB sedentary behavior, *MVPA* moderate-to-vigorous physical activity

Asterisks denote significance of the regression coefficient. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

$P_{interaction}$ is the P value for the gender by sedentary variable interactions

[a] Denotes the amount of time spent in SB (h/day) in bouts lasting at least as long as the value provided

to use it to describe the amount of time spent in various domains of SB. Overall, the average daily self-reported time spent in SB over the week between study visits was 9.0 ± 3.3 h/day. Men reported being sedentary for 9.5 ± 3.0 h/day while women reported 8.8 ± 3.5 h/day. Daily SB time from the questionnaire correlated poorly with activPAL-measured SB time ($r = 0.06$, $P = 0.72$). In regard to comparative validity, the SB questionnaire also had poor agreement with activPAL-measured SB time. Lin's concordance coefficient value was 0.07 (95 % CI −0.18 to 0.30). Results of the Bland–Altman analysis (Fig. 1) revealed a small non-significant [$t(43) = 0.58$, $P = 0.56$] amount of bias between the SB questionnaire and the activPAL, such that the average h/day was not different, but individual-level error was high with a wide confidence interval (0.31 h/day, 95 % CI −6.74 to 7.37). As time spent in SB increased, so did the variability in reporting. The questionnaire had moderate test–retest reliability across study visits (ICC = 0.48, $P < 0.001$) for average daily SB. Reliability for individual scales varied from poor to excellent; the worst being driving (ICC = 0.14, $P = 0.16$) and socializing (ICC = 0.29, $P = 0.02$), and the best being TV viewing (ICC = 0.74 $P < 0.001$) and computer use (ICC = 0.93, $P < 0.001$).

Participants spent the majority of their daily SB time watching television, with an average of 2.50 ± 1.77 h/day. This was followed by computer use (1.73 ± 1.76 h/day), reading (1.44 ± 1.11 h/day), and "other" activities (0.98 ± 0.68 h/day). "Other" activities most frequently cited were sitting while eating, worship and meetings. The least likely domain to accumulate SB was at work (0.21 ± 0.33 h/day) since the majority of the sample was retired. Other domains with few hours of accumulated SB were hobbies (0.47 ± 0.63 h/day), driving (0.79 ± 0.37 h/day), and socializing (0.97 ± 0.63 h/day). The only difference between genders in this pattern was women spent more time socializing than "other" activities, while the opposite was true for the men.

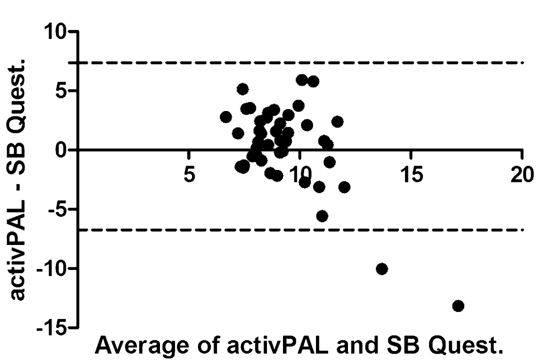

Fig. 1 Bland–Altman plot of the difference between SB questionnaire and activPAL-derived sedentary time (h/day) against the average of the two measures. *Dashed lines* represent the 95 % confidence interval around the mean difference (0.31 h/day)

Discussion

Little work has been done to examine the association between SB and health outcomes in older adults and to describe the SB of this population. We were able to gather evidence that supported our hypothesis that the manner in which SB was accumulated was important, but not our hypothesis that total SB time would be important. Strong associations were found in the males only when there was a gender by SB interaction and in both genders when such interaction was not statistically significant. These findings share a similar theme with the limited previous research that has examined this relationship; where greater self-reported sitting time was associated with self-reported reductions in physical function [4], and accelerometer-derived SB time was associated with both performance-based tests of physical function [16] and number of self-reported functional limitations [5]. However, while these studies found significant relationships with total daily SB time, we did not.

Several explanations for this discrepancy could be proposed. Our sample of older adults was comparatively small and had an overall decidedly high level of physical function. With a small sample size, we could have lacked the statistical power to reach statistical significance. Also, our sample's lack of a sufficiently wide range of physical function may have decreased our ability to establish a relationship with SB. Median SF-36 physical function subscale scores for our sample were 90 for the males and 82.5 for the females, which are well above the 75th percentile for both genders. Normative scores for adults aged 65–74 on this subscale are 45.74 for males and 41.85 for females [17]. A second explanation concerns the accuracy of the tools to measure SB between studies. As previously mentioned, the ability of the activPAL to detect changes in posture reduces the amount of measurement error compared to the previous study's methods of self-report [4] and ActiGraph-derived SB time [5, 16]. This could increase the likelihood of spurious statistically significant associations.

What the current study adds to this body of literature is a confirmation of this relationship with the use a device specifically designed to measure time spent in SB and a series of performance-based tests of physical function. In addition, a strength of this study was the consideration of effect modification by gender and MVPA, which has not been explored before. Though no modification by MVPA was detected, we found striking results when stratifying by gender. A lack of significant associations between SB and physical function in the females is in contrast to the findings of Seguin et al. [4], who found such a relationship in a sample of 62,000 women from the Women's Health Initiative. It is unknown why differences between genders were found, especially for the 400-m walk. One explanation could be the difference between males and females in the achievement of $\geq$150 min/week of MVPA. Half of the males and only a third of the females achieved this amount of weekly MVPA. Though we did not find effect modification by this level of MVPA in the whole sample, we did not explore this modification by level of gender (a gender by MVPA by SB interaction). Unfortunately, due to our sample size, we lacked the ability to explore multiple interactions.

In regard to the secondary purpose of this study, we found moderate test–retest reliability of the SB questionnaire and poor comparative validity between it and the activPAL. Despite there being no difference in their estimation of average daily time spent in SB, performance of the SB questionnaire worsened as time spent in SB increased and there was almost no correlation between the two measures. These findings, in addition to those for the individual scales, are similar to those from the questionnaire's original validation paper [13], where it was compared to ActiGraph-derived SB time, and suggest that this instrument should not be used to quantify SB time in individuals. In terms of self-report, Matthews et al. [18] have found that a 24-h recall has substantially better measurement properties for assessing SB than what we have found here. For descriptive purposes, we found our older adults to spend the majority of their SB time watching television, using the computer, and reading. This was slightly different than the top three from the Australian sample in Gardiner et al., where socializing was more frequent than computer use in Australian older adults. Television watching, reading, and socializing were also the most frequent sedentary activities of older adults from the 2003–04 Bureau of Labor Statistics' American Time Use Survey [19]; however, computer use was not assessed.

In addition to the strengths of this study mentioned above, this study had several limitations. First, the cross-sectional design of this study limits our ability to make causal inferences. We are unable to say definitively that increasing SB causes reduced physical function, because it is also a possibility that reduced physical function causes increased SB. However, the successes of physical activity interventions to improve physical function [20–22] provide evidence to suggest some directionality to the relationship; making it probable that SB may similarly influence function. Second, a number of our performance-based tests of physical function lacked a sufficient range of scores. For instance, the median score for both genders on the SPPB subscores of balance and gait speed was 4.0 out of 4.0. Also, our sample had a postural stability score of 0.40 in the males and 0.35 in the females. To give those scores some perspective, the overall postural stability scores measured by the Biodex Balance System, in a study of 40 nursing home residents with a normal average SF-36 physical function, where smaller values indicate better function, were 5.88 for males and 5.09 for females [10]. The lack of a wide range of functional ability in our sample not only limited our ability to more completely investigate its relationship with SB, but also the generalizability of our findings.

Conclusions

In summary, we found evidence to suggest that an activity pattern characterized by longer bouts and fewer breaks in SB is negatively associated with physical function in older adults. This relationship seemed to be modified by gender in some cases, but not by participation in MVPA adequate to meet physical activity guidelines. Future studies should address this relationship with another series of performance-based tests of physical function that is less susceptible to ceiling and floor effects to gain a more comprehensive understanding of this relationship. Lastly, our sample of older adults spent the majority of their SB time watching television, using the computer and reading. This information can be used in the design of future intervention to reduce sedentary time in older adults.

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Human and Animal Rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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