Daily patterns of physical activity by type 2 diabetes definition: Comparing diabetes, prediabetes, and participants with normal glucose levels in NHANES 2003–2006

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Abstract

Objective. Diabetes is associated with low levels of physical activity (PA), but detailed objective information about how PA patterns vary by diabetes definition is lacking.

Methods. PA was measured with ActiGraph accelerometers in older (60+) adults from the 2003–2006 National Health and Nutrition Examination Survey (n = 1,043) and analyzed in 2014. Diabetes definition (normal glucose levels, prediabetes, and diabetes) was assessed (fasting glucose, hemoglobin A1C, and self-report). Accelerometer data were used to characterize total activity counts (TAC) per day and hour-by-hour activity counts by diabetes definition. Multiple linear regression models explored the relationship between diabetes definition and TAC.

Results. Despite similar patterns of PA, diabetes participants had significantly lower TAC compared to participants with normal glucose levels and prediabetes. Diabetes participants' activity counts per hour declined more rapidly after 12 p.m., with the biggest differences between the groups occurring at 4:00 p.m. Participants with normal glucose levels and prediabetes had similar TAC and daily PA profiles.

Conclusion. Our novel methodology provides information about PA patterns by diabetes definition. Significantly lower TAC in the diabetes group, their significant drop in afternoon PA, and the similarity of PA between participants with normal glucose levels and prediabetes provide insight into potential targets for intervention.

Keywords: Diabetes; Prediabetes; PA; Cross sectional; Patterns

Introduction

Currently, type 2 diabetes (hereafter referred to as diabetes) affects 29.1 million Americans (Centers for Disease Control and Prevention, 2014b), and by 2050, its prevalence is estimated to increase by 165% (Centers for Disease Control and Prevention, 2011). While an active lifestyle can delay, prevent, and reverse diabetes (Diabetes Prevention Program Research Group, 2002; Hu et al., 2001a, 2001b; Laaksonen et al., 2005), little is known about the amount and daily patterns of physical activity (PA) in older populations with different diabetes definitions (normal glucose levels, prediabetes, and diabetes) because few studies have objectively measured PA in this population (Arnardottir et al., 2013).

Literature supporting the role of PA in diabetes prevention has mainly relied on self-reported PA (Helmrich et al., 1991; Hu et al., 2001a, 2003). However, reliance on self-report methods may fail to capture sporadic, lower intensity, and harder to recall PA (Washburn, 2000). It is also challenging to obtain accurate estimates of the absolute amount or timing of PA throughout the day with self-report methods (Sallis and Saelens, 2000).

Accelerometry provides an objective estimate of most free-living ambulatory PA (Miller et al., 2010). Researchers often apply intensity-related cut points to accelerometer data to identify time spent in sedentary, light, moderate, or vigorous PA (Evenson et al., 2012), but...
the accuracy of the cut point method, especially in older populations has been questioned (Copeland and Esliger, 2009; Lopes et al., 2009; Schrack et al., 2014; Swartz et al., 2000; Troiano et al., 2008). Total activity counts (TAC) per day, as an alternative to cut points, weights the activity of each minute according to the intensity of the movement and provides a measure of total PA volume (Schrack et al., 2014; Wolff et al., 2014).

The aims of this study are (1) to describe daily PA patterns by diabetes definition (normal glucose levels, prediabetes, and diabetes) and (2) to determine the relationship between diabetes definition and TAC in a national sample of older adults.

Methods

Data from the interview, examination, and laboratory components of the National Health and Nutrition Examination Survey (NHANES) 2003–2006 were used. The National Center of Health Statistics Research Ethics Review Board has approved all protocols and each participant gave informed consent (Centers for Disease Control and Prevention, 2003–2006). We included participants 60 years of age and older (n = 3,471), with at least 4 valid days of accelerometer wear time data (n = 2,313), who had the information required to determine diabetes definition (normal glucose levels, prediabetes, and diabetes) based on plasma glucose serum samples from the morning blood draw, hemoglobin A1C, or self-report (n = 1,120), and had information on all covariates (see below) (n = 1,043).

Diabetes

Diabetes definition was based on elevated fasting glucose (≥126 mg/dl), A1C (≥6.5% [48 mmol/mol]), or self-report of: “Yes” in response to the questions “Have you ever been told by a doctor you have diabetes?”, or to the use of antidiabetic medications. Prediabetes was determined based on impaired fasting glucose (100–125.9 mg/dl) or A1C (5.7% [39 mmol/mol]–6.49% [47.9 mmol/mol]). Normal glucose levels corresponded to <100 mg/dl, and A1C <5.7% [39 mmol/mol] (American Diabetes Association, 2010).

Accelerometry

Participants wore an ActiGraph AM-7164 accelerometer (ActiGraph, Fort Walton Beach, FL) over the right hip for seven consecutive days, removing it during sleeping, bathing, or other aquatic activities. The Fort Walton Beach, FL accelerometer wear time was determined by subtracting non-wear time from 24 h. Non-wear time was defined by an interval of at least 60 consecutive minutes of zero accelerometer counts, allowing for 1–2 min of counts between 0 and 100 (Troiano et al., 2008). The average of the median activity counts per hour, for each hour across all valid days were calculated. A measure of total PA, TAC per day, was calculated across all valid days.

Statistical analysis

Data analysis was conducted with SAS software (Research Triangle Park, NC). Following the recommended guidelines from the NCHS the appropriate 4-year sampling weight (MEC4YR) (2003–2006) was used for analyses to account for the complex sampling design utilized by the NHANES (U. S. Department of Health and Human Services, 2006), and methods for subpopulation analysis within SAS survey procedures were employed. Participant characteristics by diabetes definition were assessed using X2 test (categorical variables), analysis of variance (normally distributed continuous variables), and Wilcoxon–Mann–Whitney test (non-normal continuous variables). The effects of sex on TAC and wear time were compared within diabetes definitions using linear regression. For participants with at least 1 weekday and 1 weekend day of data, paired t-tests were used to compare weekday and weekend TAC within diabetes definitions. Curves were generated to describe median activity counts per hour and cumulative TAC by diabetes definition. Wilcoxon–Mann–Whitney tests with a Bonferroni adjusted p-value (0.05/2) = 0.00069 (three groups compared across 24 h of the day) compared median activity counts per hour between groups.

The skewed distribution of TAC was log transformed to bring the distribution closer to normal, and multiple regression analysis examined the relationship between diabetes definition (normal glucose levels = reference category) and other covariates on log TAC. Variables related to both PA and diabetes based on bivariate analysis (race, waist circumference, education level, and metabolic syndrome diagnosis) or known to effect the outcome variable PA (age, sex, employment status, cigarette smoking, cardiovascular disease, waist circumference, and accelerometer wear time) were included in the final multivariable analysis. The relationship between diabetes definition and log TAC was evaluated in model 1. Additional confounding variables including: age, sex, employment status (working, not employed), education (less than high school, high school, more than high school), cigarette smoking (never, former, current), cardiovascular disease (angina, congestive heart failure, coronary heart disease, heart attack, stroke) (yes, no), waist circumference, and accelerometer wear time were included in model 2. Race and metabolic syndrome were not significant in multivariable analyses and did not remain in model 2. Log TAC was compared in prediabetes and diabetes participants by estimating the difference in regression coefficients for each group using the estimate option in SAS. Regression coefficients were exponentiated to interpret as the percent difference in TAC associated with a 1–unit change in the predictor. Sensitivity analyses were conducted with the exclusion of highly active individuals (top 1% and top 5% of TAC). Significance was set at p < 0.05.

Results

A large number of older adults (60+) were excluded from the analytic sample because of inclusion criteria. Those included in the study (n = 1,043) were not significantly different from those excluded (n = 2,428) regarding waist circumference, race, sex, or smoking status but were significantly younger, higher educated, less likely to have cardiovascular disease, and more likely to be employed than those excluded. Of the 1,043 included, 302 (29%) were defined as having normal glucose levels, 471 (45%) prediabetes, and 270 (26%) diabetes (Table 1). A greater proportion of women were defined as having normal glucose levels or diabetes. Diabetes participants had lower educational levels compared to those with normal glucose levels. Diabetes participants also had greater waist circumference, elevated fasting glucose, and A1C, were more likely to have metabolic syndrome, and cardiovascular disease than prediabetes or those with normal glucose levels. Median TAC (126,645) for diabetes participants was significantly lower than prediabetes (167,082) and those with normal glucose levels (189,498) (Table 1, and Fig. 2), but there were no significant differences in median TAC between participants with normal glucose levels and prediabetes. Average wear time (14.6 h/day), and proportion of wear time derived from weekdays (73%) were consistent across groups.

Within all diabetes definitions, men had significantly greater TAC than women, controlling for confounders. Men with normal glucose levels and prediabetes had higher accelerometer wear time compared to women with the same diabetes definition (Table 2). Participants had greater TAC on weekdays than on weekend days regardless of diabetes definition. Those with diabetes had significantly less TAC than the other groups on weekdays and weekend days (Table 2).
context. After controlling for confounding, the model 2 parameter between diabetes de-

tac. Excluding highly active individuals did not change the relationship

tences between those with normal glucose levels and prediabetes
tences between the groups occurring at 4:00 p.m. There were no differ-
tances (model 2). Controlling for age, sex, employment classi-

gave 14.2% lower TAC than those with normal glucose levels (β = −0.001). There was no difference in TAC

groups all people ≥ 85 years of age and older as 85-years-old. Prediabetes patients signi-
cantly different from both diabetes patients (p < 0.001). Patients with normal glucose levels patients significantly different from diabetes patients (p < 0.05). Participants with normal glucose levels patients significantly different from prediabetes patients (p < 0.05). Average age not representative of study population; NHANES groups all people ≥ 85 years of age and older as 85-years-old. Prediabetes patients significantly different from diabetes patients (p < 0.05).

Evaluation of 24-h patterns revealed that PA began between 6:00 a.m. and 8:00 a.m. in all groups, and activity counts per hour increased rapidly in the morning. Median activity counts per hour increased 366% from 7:00 to 8:00 a.m., 63% from 8:00 to 9:00 a.m., 34% from 9:00 to 10:00 a.m., and 9% from 10:00 to 11:00 a.m. (Fig. 1A). Diabetes participants were significantly less active than those with normal glucose levels from 10:00 a.m. to 8:00 p.m. and significantly less active than the prediabetes participants from 10:00 a.m. to 6:00 p.m. (Wilcoxon–Mann–Whitney test for non-normal continuous variables, and chi-square test for proportions. Pairwise comparisons: 1Participants with normal glucose levels patients significantly different from diabetes patients (p < 0.05). 2Participants with normal glucose levels patients significantly different from prediabetes patients (p < 0.05). 3Average age not representative of study population; NHANES groups all people ≥ 85 years of age and older as 85-years-old. Prediabetes patients significantly different from diabetes patients (p < 0.05).

| Characteristics | Diabetes definition (n = 1043) |
|-----------------|--------------------------------|
| normal glucose levels (n = 302) | Prediabetes (n = 471) | Diabetes (n = 270) | χ², p<sup>b</sup> |
| Sex, % (n) | | | |
| Men | 35.1 (135) | 50.3 (256) | 44.3 (139) | 0.002<sup>c</sup> |
| Women | 64.9 (167) | 49.7 (215) | 55.7 (131) | 0.120<sup>d</sup> |
| Age (years)<sup>c</sup>, M (SE) | 69.8 (0.6) | 70.9 (0.4) | 70.6 (0.5) | 0.239 |
| Race, % (n) | | | |
| Non-Hispanic white | 84.9 (200) | 84.5 (300) | 76.4 (135) | 0.015<sup>f</sup> |
| Non-Hispanic black | 6.9 (39) | 6.7 (39) | 11.9 (56) | 0.38 |
| Hispanic | 2.9 (50) | 3.2 (84) | 4.7 (67) | 0.205 |
| Other | 5.3 (13) | 5.5 (23) | 6.9 (12) | 0.205 |
| Education, % (n) | | | |
| <High school | 19.2 (89) | 24.1 (160) | 28.2 (109) | 0.38 |
| High school | 27.8 (76) | 28.3 (126) | 33.2 (73) | 0.001<sup>h</sup> |
| Work status, % (n) | 52.9 (137) | 47.4 (185) | 38.6 (88) | 0.001<sup>h</sup> |
| Smoking, % (n) | | | |
| Never | 43.2 (133) | 37.8 (187) | 48.5 (126) | 0.051<sup>j</sup> |
| Former | 42.3 (126) | 49.7 (230) | 40.8 (112) | 0.205 |
| Current | 14.5 (43) | 12.5 (54) | 10.7 (32) | 0.001<sup>h</sup> |
| Waist circumference (cm), M (SE) | 95.1 (1.0) | 101.7 (0.8) | 106.6 (1.1) | 0.0001<sup>l</sup> |
| Fasting glucose (mg/dL), median (IQR) | 92.9 (91.9–94.0) | 105.4 (104.3–106.5) | 133.7 (128.3–139.2) | 0.0001<sup>l</sup> |
| A1C [% (mmol/mol)], median (IQR) | 5.3 [34] (5.2 [33]–5.3 [34]) | 5.6 [38] (5.6 [38]–5.7 [39]) | 6.4 [46] (6.1 [43]–6.7 [50]) | 0.0001<sup>l</sup> |
| Metabolic syndrome, % (n) | 9.4 (21) | 30.5 (161) | 47.3 (136) | 0.0001<sup>l</sup> |
| Cardiovascular disease, % (n) | 19.2 (61) | 25.4 (111) | 37.2 (91) | 0.001<sup>h</sup> |
| Wear time (h/day), M (SE) | 14.5 (0.1) | 14.6 (0.1) | 14.6 (0.2) | 0.748 |
| Proportion wear from weekdays, M (SE), % | 75.8 (0.2) | 75.7 (0.4) | 72.9 (0.6) | 0.336 |
| TAC, median (IQR) | 189498 (173036–205960) | 167082 (156600–177722) | 126645 (106883–146047) | 0.001<sup>h</sup> |
| Log TAC, median (IQR) | 12152 (10624–12240) | 12027 (11964–12090) | 11749 (11591–11908) | 0.001<sup>h</sup> |

Cardiovascular disease (angina, congestive heart failure, coronary heart disease, heart attack, stroke); n = number, M = mean, SE = standard error, IQR = interquartile range. □Four-year sampling weights (MEC4YR) were applied. □-values for overall group comparisons, analysis of variance for normally distributed continuous variables, Wilcoxon–Mann–Whitney test for non-normal continuous variables, and chi-square test for proportions. Pairwise comparisons: 1Participants with normal glucose levels patients significantly different from diabetes patients (p < 0.05). 2Participants with normal glucose levels patients significantly different from prediabetes patients (p < 0.05). 3Average age not representative of study population; NHANES groups all people ≥ 85 years of age and older as 85-years-old. Prediabetes patients significantly different from diabetes patients (p < 0.05).

Table 2

Aerobic TAC and wear time comparison between men and women by diabetes definition (n = 1043), and within groups on weekdays and weekend days (n = 1007); 2003–2006 NHANES.

| Characteristics | n | Total activity counts M (SE) | Wear time (h/day), M (SE) |
|-----------------|---|-----------------------------|--------------------------|
| Normal glucose levels men | 135 | 205671 (9688) | 14.8 (0.2) |
| Normal glucose levels women | 167 | 194521 (8613) | 14.4 (0.1) |
| Prediabetes men | 256 | 197806 (5801) | 14.8 (0.1) |
| Prediabetes women | 215 | 172456 (8779) | 14.3 (0.1) |
| Diabetes men | 139 | 167920 (11918) | 14.7 (0.3) |
| Diabetes women | 131 | 139142 (9031) | 14.4 (0.3) |
| Normal glucose levels | 0.008 | 0.47 |
| Normal glucose levels weekdays | 291 | 202011 (7317) | 14.5 (0.1) |
| Normal glucose levels weekend | 187861 (7213) | 0.0001<sup>n</sup> |
| Prediabetes weekdays | 455 | 189505 (6572) | 14.6 (0.1) |
| Prediabetes weekend days | 170890 (5720) | 0.0001<sup>n</sup> |
| Diabetes weekdays<sup>+</sup> | 261 | 155520 (9497) | 14.6 (0.2) |
| Diabetes weekend days<sup>+</sup> | 140302 (8242) | 0.0004<sup>n</sup> |

M = mean, SE = standard error. □Four-year sampling weights (MEC4YR) were applied, and adjusted for age, employment status, education, smoking category, cardiovascular disease, waist circumference, and wear time. □M does not equal total N because mean differences were calculated within individuals with at least 1 valid week and weekend day and did not take into account covariates. □Diabetes significantly different from both other groups on weekdays and on weekend days. Significant differences (p-value < 0.05) are bolded.
estimates indicated that estimated TAC for participants with normal glucose levels, prediabetes, and diabetes—assuming mean values for each continuous covariate and reference group for each categorical covariate—were 162755, 162267, and 139665, respectively.

**Discussion**

Using objectively measured PA in a large sample of U.S. older adults (60+), this study demonstrated that despite similar overall patterning

Table 3

| Dependent variable | Model 1 | Model 2 |
|--------------------|---------|---------|
| Log daily cumulative activity counts | $R^2 = 0.04$ | $R^2 = 0.37$ |
| Intercept | 12.062 | 12.000 |
| Diabetes patients | $-0.299$ | $-0.153$ |
| Prediabetes patients | $-0.081$ | 0.93 |
| Prediabetes patients vs. diabetes patients | $-0.218$ | $-0.15$ |
| Age | $-0.034$ | $-0.003$ |
| Female | $-0.146$ | $-0.001$ |
| Not employed | 0.17 | $-0.001$ |
| <High school | 0.129 | 0.021 |
| High school | $-0.001$ | 0.987 |
| Current smoker | $-0.216$ | $-0.001$ |
| Former smoker | 0.015 | 0.683 |
| Cardiovascular disease | $-0.146$ | $-0.001$ |
| Waist circumference | $-0.01$ | $-0.001$ |
| Wear time | 0.001 | 0.003 |

Model 1 shows the beta-coefficient and $p$-value from a simple linear regression model assessing the relationship between diabetes status (participants with normal glucose levels patients = reference category) and the log of cumulative daily activity counts. Model 2 expands upon model 1 by adding age and sex (male = reference category), employment status (employed = reference category), education (> High school = reference category), smoking (never smoker = reference category), cardiovascular disease (no = reference category), waist circumference, and wear time to the model. Variables for race, and metabolic syndrome diagnosis were not significant in multivariable analysis and not included in model 2. "Four-year sampling weights (MEC4YR) were applied. "The coefficient for prediabetes patients vs. diabetes patients was determined using the estimate statement. All confounding variables were centered to ease interpretation of the intercept (the average log daily activity counts for the average person in the data set accounting for the proportions of the categorical variables). Significant differences ($p$-value $< 0.05$) are bolded.
of PA between all three groups, adults living with diabetes accumulated less PA compared to those with normal glucose levels and prediabetes participants. Prediabetes participants and those with normal glucose levels had similar PA. The quantification of the magnitude and timing of the differences in PA between groups are novel contributions which can inform the development of primary care and community-based interventions to increase PA.

While it is well-established that physical inactivity is a risk factor for incident diabetes (Folsom et al., 2000; Jeon et al., 2007), little is known about the actual PA patterns of this high-risk population (Tudor-Locke et al., 2009). Our hour-by-hour analysis showed that overall daily PA patterns were similar between groups; however, the diabetes participants diverged from the other groups early in the day, and that the biggest differences in PA occurred between 1:00 and 6:00 p.m. This is significant because older adults tend to get most of their PA between 12:00 a.m. and 4:00 p.m. (Arnardottir et al., 2013). The P-value curve provides a visual of the statistical differences in activity counts per hour between the groups and describes how groups diverge and converge over the day. The cumulative activity counts plot demonstrates how activity accumulates over the day, showing the gap between the diabetes participants and others expanding to a maximum value at the final data point for each group, the median TAC (Bai et al., 2014). Those with diabetes were less active than the other groups on weekdays and weekend days. In line with previous findings, we observed a weekend-related drop in activity (Van Domen, et al., 2011) and a sex-related impact on activity with women having lower TAC than men in all groups (Hawkins et al., 2009).

There is a scarcity of objectively measured PA in diabetes participants and those at high risk for developing diabetes (Morrato et al., 2003; Tudor-Locke et al., 2009). Interestingly, we found that prediabetes participants and those with normal glucose levels have similar amounts of PA, while diabetes participants had much less. For prediabetes participants, this is a particularly optimistic observation because increased PA is important for delaying or preventing the development of diabetes (Aponte, 2013; Diabetes Prevention Program Research Group, 2002; Laaksonen et al., 2005). It is estimated that 15%–30% of individuals with prediabetes will develop diabetes within 5 years (Centers for Disease Control and Prevention, 2014c); however, lifestyle modifications, such as increasing PA, can prevent the progression to diabetes by reversing prediabetes (Centers for Disease Control and Prevention, 2014a; Diabetes Prevention Program Research Group, 2002). From a public health standpoint, assessing PA of prediabetes participants may be important to determine appropriate treatment and management strategies to reduce their risk of developing diabetes. Changing in the scheduling of activities or appointments may be one method to increase PA during the afternoon. Our findings need further investigation into what factors (e.g., physician advice to exercise, resources, supportive environment, etc.) are attributable to the higher levels of activity in the prediabetes participants so these factors can be targeted for intervention.

Differences in physical activity by diabetes status were substantial in this study. Exponentiation of the estimated regression coefficient for diabetes in Model 2 of Table 3 indicates that adults with diabetes average 14.2% lower TAC than adults with normal glucose levels. For perspective, the same model indicates that an equivalent reduction in TAC is attributed to a 4.5-year increase in age.

We cannot establish the directionality of the relationship between diabetes and TAC in our cross-sectional analysis. It is unclear whether older adults with diabetes were less active than their peers without the disease due to choice or ability. Those with diabetes often have decreased capacity for exercise (LeBrasseur and Ruderman, 2005; Regensteiner et al., 1998; Schneider et al., 1984), increased physical disabilities (Gregg et al., 2000; Wolff et al., 2002), and discomfort when exercising (Thomas et al., 2004) that may contribute to lower PA levels. Fritschi and Quinn, 2010; Morrato et al., 2003; Schrack et al., 2014). In our sample, diabetes participants were more likely to have increased waist circumference, CVD, and metabolic syndrome which may influence engagement in PA (Lee, 2010; Tucker et al., 2013).

While cross-sectional data are convenient for hypothesis generation, it limits the scope of the results and precludes the ability to make causal inference. Other limitations must be acknowledged. We adjusted for potential confounders related to diabetes and PA. However, we did not have information on the duration of prediabetes and diabetes, which may impact PA. Also, because the diagnosis of diabetes was based on fasting glucose and A1C, but not OGTT, some diabetic and prediabetic patients may have been missed. OGTT was only available for NHANES 2005–2006. Using it to determine diabetes definition would have further limited our sample size. The sample was restricted to older adults due to their higher prevalence of diabetes (Centers for Disease Control and Prevention, 2003–2006); therefore, the results may not be generalizable to younger populations. Because of numerous missing data (accelerometer data, biological measures, and other covariates), a large proportion of the older population were excluded from the analysis. The missing data might not be at random, and selection bias may exist such that the study population may not be entirely representative of the U.S. older population. The use of accelerometers to objectively measure physical PA is a major strength of this study. However, accelerometers do not capture all types of PA, nor do they provide information on the type of PA performed, and their ability to accurately identify time spent in specific activity intensity categories is not above reproach (Crouter et al., 2013; Schrack et al., 2014; Strath et al., 2003). Using TAC, which incorporates all intensity categories and provides a measure of total PA volume, is one way to reduce the impact of some of these limitations (Schrack et al., 2014; Wolff et al., 2014). We also must acknowledge that it is possible that individuals wore monitors for different amounts of time on different days, which may be of greater concern when analyzing data hourly than when using daily summaries.

Conclusion

In conclusion, we found that compared to those with normal glucose levels and prediabetes, diabetes participants had less PA, and that prediabetes participants had PA levels comparable to those with normal glucose levels. Regardless of the causal direction of the relationship between PA and diabetes definition, the quantification of the timing and magnitude of differences in PA between these groups has valuable intervention implications. Findings from this study suggest that diabetes participants could benefit from increasing PA throughout the afternoon, and that all participants should be encouraged to increase weekend activity.

Author contributions

J.A.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analyses. J.A.S., R.A.M., C.M.C., V.Z., D.R.V., and T.B.H. contributed to conception and design of the study. D.R.V. prepared the data sets for analysis. J.A.S., C.M.C., and V.Z. performed statistical analyses. J.A.S. wrote the initial draft of the paper, and all authors contributed to interpretation of the data and the writing of the paper. All authors read and approved the final paper.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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