Research Article

Circadian Characteristics of Older Adults and Aerobic Capacity

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Abstract

Background. Alteration of circadian rhythmicity with aging might depend on physical aerobic capacity.

Methods. Three groups of participants were established based on their peak oxygen consumption (Group 1 < 20 mL/min/kg; Group 2 > 20 mL/min/kg and <30 mL/min/kg; Group 3 > 30 mL/min/kg). Each participant had an individual evaluation of their circadian rhythmicity characteristics through two well-known circadian rhythms: core temperature and rest/activity cycles. Nocturnal sleep was also recorded using actimetry and diurnal vigilance tested in a car driving simulator.

Results. The amplitude of the oral temperature fluctuations for Group 1 is significantly lower (p < .05) than that of Group 3. Group 2 (p < .01) and Group 3 (p < .05) were significantly more active during the day than Group 1. The index of inactivity during the night for Groups 2 (p < .05) and 3 (p < .01) was higher than Group 1. Results of the car driving simulation showed that for Group 1, the number of lane crossings was significantly higher than Groups 2 (p < .01) and 3 (p < .01). In addition, diurnal vigilance was lower in Group 1.

Conclusions. The biological clock seems to be enhanced in older participants with a higher level of physical capacity.

Keywords: Physical activity—Physical performance—Exercise

Biological aging is commonly defined as the accumulation of diverse deleterious changes occurring in cells and tissues with advancing age that are responsible for increased risk of disease and death (1). Among these deleterious changes, there is a deterioration of the circadian rhythms (2,3). While robust high-amplitude circadian rhythms are observed in young individuals, these often lose their strength with age. Deterioration of the circadian rhythms is probably due to alterations in the circadian timing system, such as: (i) disruptions in the central biological clock due to alterations of its cells (4,5), (ii) alterations in relays of these clocks (ie, decrease in hormonal secretions such as melatonin) (6), or (iii) a decrease in the effect of the environment (zeitgeber) on the body clock (7). As a consequence of these effects, many older adults lose the ability to adapt to the periodic environment (8). In aging, a reduction in mesor (the average value around which the variable oscillates) and amplitude, an advance and a shortening of the period, and desynchronization of individual rhythm has been observed in regulation of arousal, hormone secretion, body temperature, and other circadian rhythms (9,10).

Regardless of the cause, the deterioration of the circadian rhythmicity impacts quality of life in terms of sleep and vigilance. During the night, the sleep quantity and quality decrease (11), and during the day, alertness is affected and diurnal sleepiness increases (11). For older adults, an increase in daytime sleepiness is a known risk factor for falls (12), car crashes (13), and domestic accidents (14).

A variety of modulators of the circadian timing system, such as melatonin (15), bright light (7), and manipulations of body heat (10), have been positively tested to resynchronize circadian rhythmicity. More recently, the hypothesis that physical activity might be a potent synchronizer of the biological clock has emerged (16). In young persons, regular physical activity influences the stability of the circadian rhythms and increases the amplitude of their oscillations (17). Fluctuations in body temperature amplitudes are
higher for young physically fit subjects than in sedentary subjects (17). In addition, regular physical activity has a significant positive effect on circadian rest/activity rhythms (18–21). In contrast, a decrease in physical activity by means of forced bed rest leads to rest/activity rhythm disorders (22) and to a decrease in body temperature amplitude and hormone secretion rhythms (23). In older adults, as the daily level of physical activity progressively and continuously decreases (24), part of the alteration of the circadian rhythm must be the consequence of this physical inactivity. It is well known that the level of physical activity and physical capacity are interdependent. Aerobic capacity determines the work capacity of a person, but aerobic capacity could increase after a physical training period regardless of age (25). In the relationship between physical activity and physical capacity (24), alteration of the circadian rhythm in older adults might also be dependent on their physical aerobic capacity.

Based on this assumption, there is a need to evaluate the relationships between the level of physical capacity and characteristics of circadian rhythms in older persons. In the present study, participants were divided into three groups based on their peak oxygen consumption (VO2 peak). In addition, each participant had individual evaluations done of their circadian rhythmicity characteristics through two well-known circadian rhythms: core temperature and rest/activity cycles. We supposed that older participants with a high physical capacity had a higher amplitude of temperature and activity/rest circadian rhythms. Aerobic capacity could then be a marker of circadian rhythm strength. The effect of physical condition and strength of circadian rhythms on quality of life was assessed by recording nocturnal sleep and diurnal vigilance. Diurnal vigilance was assessed using an ecological driving task on a simulator known to be more effective on circadian rest/activity rhythms (26).

Methods

Participants

Twenty-nine retired persons (14 women: 60.6 ± 3.5 years and 15 men: 60.56 ± 5.7 years) volunteered to participate in this study. The ethics committee granted ethical approval (CHU Côte de nacre, Caen, France). All participants provided written informed consent after the procedures were explained in detail, then underwent a medical evaluation for their inclusion in the study.

Inclusion criteria were: no use of concomitant medication known to interfere with circadian rhythms, absence of any restricted mobility affecting pedaling motions, and absence of any cardiovascular or neurological pathology. All of the volunteers were free of any known disease and declared that they were not taking concomitant medication. An electrocardiogram at rest and a dynamic functional exploration was performed in order to assess the cardiac and vascular state of each participant before inclusion.

Participants included also were identified as having an intermediate chronotype according to the self-assessment questionnaire of Horne and Ostberg (27).

Maximal Aerobic Capacity

Participants performed a maximal incremental test between 16:00 and 18:00 hours in order to evaluate their maximal aerobic capacity after having eaten their last meal at least 3 hours before the test. The test was performed on a cycle ergometer (Ergoline ER900, Ergoline Gmbh, Germany). The work rate was increased by 20 W every 2 minutes until exhaustion. Throughout the test, the subject freely chose the pedal rate. Oxygen consumption was continuously recorded by an open-circuit sampling system (Ergocard Médisoft, Schiller, Belgium). The variable chosen to measure the aerobic capacity of the participants was the peak oxygen consumption (VO2 peak), which corresponds to the highest 1 minute mean value of oxygen uptake recorded during the test.

Group Composition

Based on the participants’ VO2 peak, three groups, based on level of aerobic capacity, were formed. Group 1 was composed of participants with a VO2 peak below 20 mL/min/kg. Group 2 was composed of participants with a VO2 peak below 30 mL/min/kg but above 20 mL/min/kg. Finally, Group 3 was composed of participants with a VO2 peak above 30 mL/min/kg. Groups were formed in order to have similar mean age. Mean age of Group 1, Group 2, and Group 3 were 60.7 ± 3.6, 60.5 ± 3.53, and 60.6 ± 3.61 years, respectively.

Characterization of Body Temperature

Circadian Rhythm

All participants took their temperature for four days and four nights with an electronic thermometer (digital Hartmann-Larochette, precision: 0.01°C) inserted sublingually. The collection was done every 2 hours in standardized conditions: the temperature recording was carried out at rest after the participant had been seated or lying down for at least 15 minutes without food or drink absorption. In their diaries, participants recorded their temperatures and the exact hour of measurement.

The evaluation of the circadian rhythm of body temperature was done using the cosinor method (28). This method is based on a mathematical computation by the least squares method of a series of data points. It gives rise to a curve exhibiting the periodicity of the component under study. The following mathematical function was used:

\[ y = M + A \cos(\omega t + \phi) \]

where, \( M = \) average daily output; \( A = \) amplitude, both in the same units as \( y; \omega = \) 2π/period; \( t = \) time, and \( \phi = \) computational acrophase. The values for \( M, A, \) and \( \phi \) were obtained by calculation. The period was fixed at 24±0 hours.

Actimetry, Circadian Rhythm of Activity, and Sleep Quality

Actimetry is a method that utilizes a miniaturized computerized wristwatch-like device to monitor and collect accelerations due to body movements over extended periods of time. Actimetry allows motor activity to be measured and is frequently used to estimate quality of sleep and the rhythm of day activity/rest (29).

For four days and four nights, all participants wore an actimeter (Gaehwiler Electronic, Switzerland) on their nondominant hand and completed a sleep/activity diary. The threshold of activity was fixed at two movements per minute (one movement corresponding to an acceleration of at least 0.1 g). This threshold is generally used when high actigraphic sensitivity is required (30).

The cosinor method (see above) was used to determine participants’ circadian rhythm of activity based on the raw actimetry data.

The diary was used to take into account each event that could disturb the interpretation of the actimetric data (eg, withdrawal, watching TV) and to track the time of lights off/on when in bed.
Activity during the night (sleep period) and during the day (waking period) was determined. To know the quality of sleep during the night, we used the index of inactivity (inactivity period/total night time ratio*100). The quality of sleep was estimated based on the hypothesis that the number of periods of activity interrupting sleep (ie, the immobility of sleep) is directly proportional to poor sleep quality (31). Thus, the index of inactivity is important since inactivity is associated with better sleep quality (more restful).

Vigilance (1 Hour of Driving Simulation)
A 1-hour driving simulation (INRETS, France) test was performed to study diurnal vigilance.

Before the test session, participants were familiarized with the simulator with a 60-minute driving session. This pretest ensures that participants are accustomed to the driving conditions. The experiment was performed from 14:00 to 17:00 hours, the time of day associated with the 2nd highest peak in circadian-based sleepiness (32). The simulated environment was a two-lane road with few curves and no traffic. Each lane was 3.5 m wide and had a hard shoulder of 3 m. For realism, the system was fitted with realistic operational control instrumentation to simulate normal braking and acceleration to maintain trajectory. The system also reproduced the noise of the engine. The test was carried out in dim light in order to increase the simulated contrast of the scene and to facilitate loss of noise of the engine. The test was carried out in dim light in order to increase the simulated contrast of the scene and to facilitate loss of vigilance. No external stimulations were introduced during the test. Participants were instructed to drive on the right side of the road and to respect the speed limit set at 110 km/h during the test session. The position of the cars during the test was recorded. The variables used for analysis were the SD of the lateral position of the car relatively to the middle of the road and the number of lane crossings.

Statistical Analysis
For each variable, with exception of lane crossings, differences between physical capacities in group comparisons (Group 1, Group 2, and Group 3) were tested using an analysis of variance if Levene’s test (Brown–Forsythe version) and the Shapiro–Wilk test did not reject homoscedasticity and normality or the residuals, respectively. If any of the assumptions was not fulfilled, the Kruskal–Wallis test and Nemenyi’s test for pairwise multiple comparisons (with p < .05), a protected least significant difference Fisher test did not reject homoscedasticity and normality or the residuals, respectively, was performed as a post hoc analysis.

The lane crossing variable is different to all other measures, being a count variable. Therefore, the analysis of group differences was based on the generalized linear model framework, taking various distributional options into account (eg, Poisson, negative binomial, with and without zero inflation).

All statistical tests were processed using R software v3.1.2 (www.r-project.org).

Results
Aerobic Capacity
For each group, the value of the VO2peak was calculated. The resulting distribution indicates that the groups (Group 1: n = 6, Group 2: n = 10, Group 3: n = 12) are significantly different (F(2,26) = 57.6; p < .001; see Table 1). The mean VO2peak for all groups was 27.6 ± 7.8 mL/min/kg. The mean heart rate and blood lactate concentration recorded at the end of the VO2peak tests were 156 ± 14 beats/min and 7.52 ± 2.25 mmol/L, respectively. No age differences were observed between groups (χ2 = 1.185, p = .553).

Circadian Rhythm of Temperature
The analysis of variance applied to the cosinor parameters, mesor (F(2,26) = 2.84; p = .076) and acrophase (χ2 = 0.852, p = .653) of the circadian rhythm of oral temperature, did not differ between groups (Table 2). However, the amplitude of temperature rhythm depended on the group (F(2,26) = 4.12; p = .028). The amplitude of the oral temperature for Group 3 was significantly higher (p = .008) compared to that of Group 1 (Table 2).

Circadian Rhythms of the Rest/Sleep Cycle and Actimetry
The levels of activity, mesor (F(2,26) = 1.07), amplitude (F(2,26) = 1.18), and acrophase (F(2,26) = 1.36) are not significantly different (p > .05) between the three groups (Table 3).

The level of motor activity in the groups tested differed during the day (F(2,26) = 6.43; p = .005). Group 2 (p = .111) and Group 3 (p = .002) were significantly more active during the day than Group 1 (Table 4). Groups did not differ significantly in sleep duration (χ2 = 0.2396, p = .887).

The index of inactivity during the night differed between the groups. Statistical analysis indicates a significant effect (F(2,26) = 4.13; p = .028). The post hoc analysis showed that Group 2 (p = .044) and Group 3 (p = .009) are more inactive during the night than participants from Group 1 (Table 4).

Table 1. Mean ± SD, Minimum and Maximum Values of VO2peak by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>VO2peak (mL/min/kg)</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 6)</td>
<td>17.6 ± 2.2†</td>
<td>14.8</td>
<td>19.5</td>
</tr>
<tr>
<td>Group 2 (n = 10)</td>
<td>25.3 ± 4†</td>
<td>20.7</td>
<td>29</td>
</tr>
<tr>
<td>Group 3 (n = 12)</td>
<td>35.4 ± 3.9</td>
<td>30</td>
<td>43.6</td>
</tr>
</tbody>
</table>

Notes: †Significantly different from Group 2 (p < .05).
†Significantly different from Group 3 (p < .05).

Table 2. Mean ± SD of Mesor, Amplitude, and Phase of Body Temperature Rhythm by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mesor (°C)</th>
<th>Amplitude (°C)</th>
<th>Phase (h:min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 6)</td>
<td>36.75 ± 0.63</td>
<td>0.43 ± 0.09†</td>
<td>15:15 ± 05:40</td>
</tr>
<tr>
<td>Group 2 (n = 10)</td>
<td>36.43 ± 0.34</td>
<td>0.69 ± 0.34</td>
<td>15:17 ± 03:44</td>
</tr>
<tr>
<td>Group 3 (n = 12)</td>
<td>36.90 ± 0.47</td>
<td>0.83 ± 0.32</td>
<td>16:10 ± 03:58</td>
</tr>
</tbody>
</table>

Notes: †Significantly different from Group 3 (p < .05).

Table 3. Mean ± SD of Mesor, Amplitude, and Phase of Rhythms of Cycle Rest/Sleep by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mesor (mL/min)</th>
<th>Amplitude (mL/min)</th>
<th>Phase (h:min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 6)</td>
<td>13.3 ± 6.4</td>
<td>24.9 ± 14.7</td>
<td>12:51 ± 01:11</td>
</tr>
<tr>
<td>Group 2 (n = 10)</td>
<td>19.5 ± 8.3</td>
<td>39.9 ± 18.5</td>
<td>13:34 ± 01:40</td>
</tr>
<tr>
<td>Group 3 (n = 12)</td>
<td>17.1 ± 10.1</td>
<td>31.5 ± 23.8</td>
<td>12:37 ± 01:06</td>
</tr>
</tbody>
</table>

Notes: mL/min = movements.
Driving Simulator

The number of lane crossings and the SD of lane position and speed are shown in Table 5.

The number of lane crossings is a count variable and subject to strong overdispersion (mean = 5.46, variance = 129.62). In addition, both homoscedasticity (p = .03) and normality of the residuals (p < .001) are rejected. Consequently, the common analysis of variance setting does not constitute an appropriate modeling approach. Among various natural alternatives (Poisson/negative binomial regression via log-link, with and without zero inflation modeled either canonically or by a hurdle-type approach), the simple negative binomial regression setting was strongly supported by the Akaike information criterion. Moreover, Vuong’s non-nested test (33) did not support the zero-inflated count models. Visual inspection of the data suggested that Group 2 and 3 may not differ significantly from each other. Therefore, we compared three different models: first, a null model without any group effect (m0); second, a model with group effect but assuming no difference between Group 2 and 3 (mL); third, a full model with three levels of the group factor. We compared the models by means of a likelihood ratio test. Supplementary Appendix Table A shows the results indicating a significant difference between Group 1 and Groups 2 and 3 combined (likelihood ratio = 7.72, p = .005). Moreover, differences between participants in Groups 2 and 3 are not supported (likelihood ratio = 0.09, p = .76).

Investigation of the model coefficients (see Supplementary Appendix Table B) shows an estimate of 15.33 lane crossings in Group 1, which is significantly different from zero (p < .001). Moreover, the number of lane crossings in Groups 2 and 3 combined is significantly lower (p = .005), resulting in an estimation of 2.50 lane crossings.

No effect was found on the SD of the lateral position of the car (γ² = 0.2383, p = .879) or on the SD of speed of the car (F_{2,34} = 2.6; p = .08).

Discussion

In our study, three groups were formed based on the level of VO₂peak of participants. The mean values of VO₂peak recorded for the second group (25.3 mL/min/kg) was consistent with data previously observed on sedentary older persons (34). Participants in the first group had a VO₂max of 17.6 mL/min/kg, approaching the threshold for loss of independence (34). Participants in Group 3 had a VO₂peak (35.4 mL/min/kg) close to values reported for older persons having had regular physical training for several years (34).

Aerobic capacity is both innate (genetic) and acquired (through physical activity). However, with age, the proportion of the acquired components should increase. In healthy sedentary adults of both genders, the VO₂max declines by approximately 10% per decade after age 25–30 while the rate of decline in VO₂peak with advancing age has been as much as 50% lower in endurance exercise-trained athletes (35). Thus, the aerobic capacity of older adults depends on their level of physical activity (34). Conversely, aerobic capacity determines physical capacity of subjects and thus could restrict it (36).

Actigraphy is an indirect method of appraising the level of overall physical activity. Over four days, our recordings show that Group 2 and Group 3 were more active than Group 1 during waking periods. The physical capacity of the first group of participants seems to restrict their motor activity. These results show that the amount of motor activity throughout waking periods develops proportionately with the aerobic capacities of the participants. These results are in line with previous studies which reported a concomitant increase in diurnal activity and physical capacity after a physical training period (19,20).

An important result of our study is that the amplitude of temperature rhythm depends on the level of aerobic capacity in older participants. Amplitude is believed to indicate the “strength” of the circadian system (37). By strength, the ability to resist change (e.g., phase shifts) is meant. A strong correlation has been shown between the amount of day-to-day variability in a rhythm and the rhythm’s amplitude (38). Classically, a decrease in amplitudes of different circadian rhythms, such as that of core temperature, is observed with aging.

Our results show that the oral temperature amplitude for Group 3 is significantly higher than the oral temperature amplitude recorded for Group 1. Participants with greater aerobic capacity seem to be those who are also more active during periods of wakefulness and should have larger amplitudes of core temperature rhythms.

Regular physical training increases the strength of biological rhythm. Atkinson and Davenne (39) reported that physically active young adults have higher biological rhythm amplitudes than inactive young adults. In older adults, an increase in the amplitude of core temperature rhythm has been observed after a fitness-training program (18–20).

In our study, the greater amplitude of the core temperature rhythm observed in Group 3 may have been due to motor activity during the day which was also greater, resulting in what is called a “masking effect” (40). However, several arguments provide a basis for rejection of this hypothesis. First, Group 2 and Group 3 have a different mean motor activity during the day, while the amplitude of core temperature rhythm is not significantly different between these two groups. Second, regardless of the group, the rhythms of activity/rest and core temperature are not in phase. Time to peak for core temperature rhythm is longer than the activity/rest rhythm (for Group 3: 16:10 vs 12:37 hours). Lastly, in previous studies, based on a fitness-training program in the older subject (19,20), a clear dissociation between diurnal motor activity and the amplitude of core temperature rhythm was reported.

The effect of physical activity on circadian rhythmicity might be mediated by the circadian clocks in the central nervous system. The greater recorded physical capacity in Group 3 compared to

Number of Lane Crossings and SD of the Lateral Position and Speed of the Car by Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Lane Crossings</th>
<th>SD of Lateral Position</th>
<th>SD of Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>15.3 ± 21.4*†</td>
<td>0.49 ± 0.16</td>
<td>7.6 ± 3.9</td>
</tr>
<tr>
<td>Group 2</td>
<td>2.7 ± 2.9</td>
<td>0.44 ± 0.05</td>
<td>15.4 ± 8.1</td>
</tr>
<tr>
<td>Group 3</td>
<td>2.3 ± 3.5</td>
<td>0.43 ± 0.07</td>
<td>15.5 ± 7.6</td>
</tr>
</tbody>
</table>

Notes: *Significantly different from Group 2 (p < .05).
†Significantly different from Group 3 (p < .05).
Group 1 results from higher diurnal physical activity. In Group 1, the low VO$_2_{peak}$ restricts diurnal physical activity. The hypothesis is that physical activity directly or indirectly impacts the circadian clock, which is the major vector of biological rhythm, and thus fights against circadian clock aging.

No significant difference between groups was reported for the activity/rest rhythm recorded during the four days of our study. These results are in accordance with Gruau and coworkers (19) who reported no physical activity level difference in activity/rest rhythms in heterogeneous groups. However, our result is contradictory to our observation performed on diurnal motor activity (see above). This discrepancy is likely due to a large interindividual variability in motor activity allocation throughout the day.

Another assumption is that the deterioration of circadian rhythmicity impacts the quality of life in terms of sleep and vigilance. In healthy persons, actigraphy has often been used to evaluate both the quantity and the quality of sleep (29). In quantity of sleep analysis, no difference in sleep duration was observed between the three groups in this study. However, significant differences were found in quality of sleep measured by activity during the night.

Group 2 and Group 3 have higher indexes of inactivity during the night compared to Group 1. This means that participants with greater aerobic capacities sleep better than those with poor aerobic capacity. These results confirm the positive impact of physical activity on sleep quality (18). Thus, a higher level of physical activity during the day for Groups 2 and 3 enabled them to have better sleep.

Concomitant with an increase in sleep quality and a reinforcement of circadian rhythms, an increase in diurnal vigilance is also observed in the groups with higher levels of physical activity. Vigilance was assessed using a driving simulator (41) in monotonous traffic conditions and during the post-lunch dip of vigilance. Our results show that the SD of the lateral position of the car is similar regardless of the group. However, the participants in Group 2 and Group 3 had fewer lane crossings than those in Group 1. These results indicate that participants with a higher level of physical activity have better vigilance in the early afternoon compared to those who are not active. This is important because vigilance impairment in older persons throughout the day is a cause of accidents such as falls or car crashes (12,13).

However, in our study, the effect of physical activity on the circadian clock could not be separated from the effect of physical activity on sleep quality (39) and consequently on diurnal vigilance.

Taken together, it seems that physically active participants who have a VO$_2_{peak}$ higher than 20 mL/min/kg, have a better level of vigilance during day and quality of sleep during the night. These effects might be due to an improvement of circadian rhythmicity.

Finally, in our study, it is difficult to separate the effect of physical activity from other external synchronizers such as light, which is known to impact the main body clock (42). Active participants might have spent more time outside in the sunlight, which could then explain our result. Physical activity and light could also be two disconnected synchronizer and thus have a cumulative impact on the biological circadian clock in order to combat the effect of aging.

Drawing a distinction between light and the physical activity effect on the circadian clock requires further research. Additionally, other physical status indicators such as body mass index, declared activity, muscle strength, and mobility must be correlated to physical capacity, then to circadian rhythm strength. Our participants were “young elderly” adults and our result should not be generalized to the whole elderly population. In addition, small groups are evaluated in this study and this lack of size reduces the statistical power of our analysis. Further investigation using a multivariate approach in a larger and older population is thus needed to confirm our result.

**Conclusion**

Our study highlights the fact that circadian rhythm amplitudes of core temperature, diurnal activity, vigilance while driving, and sleep quality depend on aerobic capacity in older adults. It has already been reported that physical capacity acts as a protection against cognitive decline (43,44). Our study added that a high level of aerobic capacity might also improve biological rhythmicity and quality of life. An improvement in aerobic capacity, easily obtained by regular practice of physical activity, seems necessary to combat age-related decline in biological rhythms. Based on this study and current knowledge, we recommend that older people should perform at least 30 minutes per day of continuous physical exercise involving whole body musculature (e.g., gardening, house cleaning, Nordic walking) in order to develop their aerobic capacity. Further research should be conducted in order to clearly distinguish physical activity from other circadian clock synchronizers.

**Supplementary Material**

Please visit the article online at http://gerontologist.oxfordjournals.org/ to view supplementary material.

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**Conflict of Interest**

The authors declare that there are no conflicts of interest.

**References**


