



Prospective changes in the distribution of movement behaviors are associated with bone health in the elderly according to variations in their frailty levels.

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Contributions

IRG: investigation, data curation, formal analysis, writing original draft preparation; AM &

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statistical analyses and are guarantors.

Abstract

Frailty is associated with poor bone health and osteoporosis, and physical activity (PA) is one of the best treatments for both pathologies in older adults. Nonetheless, because daily time is limited, how the time is distributed during the waking hours is critical. The waking hours are spent according to different movement behaviors: sedentary behaviors (SB), light physical activity (LPA) and moderate-to-vigorous physical activity (MVPA). The aim of this study was to use compositional data analyses to examine the effects of the change in movement behaviors on bone health during aging in older people, related to the changes in their frailty levels. We analyzed 227 older people aged 65 to 94 [125 women and 102 men] over a 4-year period. Movement behaviors were assessed using accelerometry. Both bone mineral density (BMD) and content (BMC) were determined using bone densitometry. The Frailty Trait Scale was used to divide the sample by frailty level evolution during aging. The statistical system-R was used for the compositional data analysis and, in addition, all models were adjusted for several covariates. The changes in the distribution of all movement behaviors within a waking hours period were significantly associated with spine and femoral neck BMD changes in the subgroup with a positive change in frailty level and spine BMC in the subgroup with no change in frailty level ($p \leq 0.05$). Likewise, MVPA relative to the change in other movement behaviors

was also associated in both subgroups with higher BMD and BMC, respectively, in the same body areas ($p \leq 0.05$). No significant associations were found in the negative change in frailty level subgroup. Older people who achieved a positive change in frailty level during a 4-year period showed higher BMD changes compared to those with no changes or increases in their frailty level. Therefore, increasing MVPA relative to the change in the other movement behaviors during a 4 year-period could perhaps produce bone health improvements in elderly that do not worsen their frailty level.

Keywords: Bone mineral density – light physical activity - moderate-to-vigorous physical activity – sedentary time – elderly

Introduction

Frailty is a well-recognized geriatric syndrome (1), which is characterized by a loss of reserves (energy, physical ability, cognition, health) that gives rise to vulnerability (2). Moreover, frailty is closely associated with body composition changes, poor bone health and osteoporosis (3-5). Similarly, osteoporosis and frailty share many common risk factors, such as malnutrition, sarcopenia, physical inactivity, and low vitamin D levels (6, 7), which would decrease quality of life, and increase disability, institutionalization, and mortality, making osteoporosis an important contributor to the public health burden (8). The age-related increase in frailty prevalence, that has been clearly shown in different studies (9-11), together with the bone mass deterioration produced by the aging process, increases the relevance of this syndrome in this population. However, previous cross-sectional studies have investigated the relationship between frailty and bone mineral density (BMD) and content (BMC) with contrasting results

(3, 5, 14). The reason for these inconsistencies could be the different frailty levels. The mechanisms linking frailty and bone health are likely to be multifactorial, which makes it more difficult to ascertain the effects of different treatments, like physical activity (PA), if subjects are mixed in the same group without frailty level differentiation (15). Likewise, previous studies have demonstrated that the risk of osteoporosis and bone fractures is higher in pre-frail and frail individuals (16, 17); thus, the importance of studying each frailty level change independently. Based on previous studies, we used The Frailty Trait Scale (FTS) to stratify the sample depending on frailty level changes as the FTS is a continuous scale that indicates any change in the frailty level during aging (18).

PA and an active lifestyle have been suggested as fundamental strategies to prevent frailty onset, perpetuation, and progression, as well as other determining factors in the aging process and bone mass reduction (12, 13). Furthermore, this relationship between PA and health is also dependent on the fact that time within a 24-h period is finite, and more time spent in one behavior necessarily decreases the time spent in another behavior; factors that should guide the planning of interventions and public health policy (19). Particularly, the waking hours are made up of a sequence of periods of sedentary behavior (SB), light PA (LPA) and moderate-to-vigorous PA (MVPA), which makes it essential to use a different model such as compositional data analysis (CODA) that allows us to deal directly with the fundamental nature of movement behavior data (20, 21) and moreover, avoids perfect multi-collinearity problems and deals with the co-dependence between time spent in different movement behaviors (19, 22). CODA uses multiple linear regression modeling the influence on bone mass of the whole distribution of the

waking hours and has thus recently received attention in different studies on SB and PA (19, 22-25). Moreover, previous studies have determined that it is necessary to examine all changes in movement behaviors as a whole with a longitudinal perspective in this population to obtain accurate information about the benefits of the positive changes in MVPA, given that it has been previously acknowledged that BMD in older adults may not be easily influenced by daily PA alone (19, 20, 25, 26). Similarly, in the last Copenhagen Consensus statement 2019: physical activity and aging, it was concluded that interventions with older adults that are based on established behavior change theory produce more consistent results (27).

Therefore, the aim of this study was to identify whether changes in the distribution of movement behaviors during the waking hours have a beneficial association with bone mass related to each frailty level variation. The hypothesis was that less SB, more LPA and more MVPA during aging might have a positive association with bone health, regardless of the frailty level changes.

Methodology

Study sample and design

The Toledo Study for Healthy Aging (TSHA) is a longitudinal Spanish aging study on institutionalized and community-dwelling individuals of over 65 years living in the province of Toledo, Spain. The full methodology has been described previously (11). In this specific longitudinal study, a subsample of 581 older people over 65 years of age (322 women and 259

men 77.8 ± 4.6 years) was assessed for wave 1 (i.e. baseline) (26 subjects –4%– were excluded for lack of accelerometry). Participants with dual energy X-ray absorptiometry (DXA) and accelerometry data were invited to participate in wave 2 (i.e. follow-up). A total of 227 participants (76.7 ± 4.0 years) agreed to participate in wave 2 (125 women and 102 men). No differences were found in any of the variables between this final sample and those that dropped out of the study, with the exception of the age (76.7 vs. 78.5 years, respectively). A flow diagram with information related to the sample is presented in **Figure 1**. Data were collected from July 2012 to June 2014 in wave 1 and, from May 2015 to June 2017 in wave 2; thus, the mean time between both waves was 4 years. The study was approved by the clinical research ethical committee of the Toledo Hospital Complex and all the subjects signed an informed consent to be included in the study.

Briefly, in both waves the same three stages were followed for the data collection. In the first stage, socio-demographic variables, lifestyle determinants (alcohol and tobacco), health variables and comorbidity (frailty), including blood tests, were collected by trained psychologists and nurses. Moreover, an extensive neuropsychological evaluation was performed for each subject, and all of them completed the Mini-Nutritional Assessment questionnaire to screen for malnutrition risk (28) and a brief 14-item tool to check adherence to the Mediterranean diet (29). Then, in the second stage, information was gathered regarding anthropometrics, physical performance and clinical tests. Finally, in the third stage, data related to body composition, bone health and PA and SB were obtained. All variables were normalized to the individual time gap between waves.

Frailty level

FTS was used to assess frailty (18). The FTS is one of the most useful tools for monitoring differences in frailty among individuals and also changes over time in order to better evaluate their risk for adverse outcomes, showing some advantages over other scales for measuring frailty (18). Thus, it was possible to detect changes in frailty level, which could be ignored using the classical stratification of frailty status, since a change in frailty level does not necessarily imply changes in frailty status. Furthermore, although the scale proposed by Fried et al. (10) has been the most commonly used classification, the FTS has a higher predictive value that is slightly better for mortality in individuals of over 80 years and for hospitalizations in individuals younger than 80 years (18). The FTS includes 7 different dimensions: energy balance and nutrition, PA, nervous system, vascular system, weakness, endurance, and slowness. These domains become operational through 12 items, and each item score represents a biological trait, which ranges from 0 (the best status) to 4 (the worst status) except in the “chair test”, which ranges from 0 (the worst status) to 5 (the best status). When appropriate, items are analyzed according to the item’s quintile distribution in the population. Furthermore, the variance inflation factor (VIF), tolerance and Durbin-Watson test were calculated to ensure the severity of multicollinearity and the autocorrelation in the analyses between FTS and PA. In the manner of previous studies (26, 30), only the participants who had passed at least 75% of the items in the FTS were included in this study. The total score was calculated by adding all the scores in each item divided by the total score for each individual and multiplying by 100, standardizing the measure to a range from 0 (best score) to 100 (worst score), according

to the formula: Total score $\left[\frac{\Sigma \text{ items score}}{\text{total score possible by individual}} \right] \times 100$

Changes of 5 points in the FTS have been clinically significant according to the mortality database (data not yet published). Thus, frailty level change was used to categorize patients according to whether they had a “positive change in the level”, “no change in the level”, or a “negative change in the level” during the period between the baseline and the follow-up. “Positive change in frailty level” described people who reduced their FTS score by at least 5 points (n=45); “no change in frailty level” described people with similar FTS scores (i.e. changes lower than 5 points on the FTS) (n=99) and; “negative change in frailty level” described people who increased their FTS scores by at least 5 points (n=42).

Anthropometrics

Body mass was obtained in the upright position, in underwear and barefoot using a balance-stadiometer with a precision of 100 g (Seca 711, 120 Hamburg, Germany). Height was determined using a balance-stadiometer with a precision of 1 mm (Seca 711, 120 Hamburg, Germany) in the Frankfort plane and maintaining the same condition previously used for body mass evaluation. Body mass index (BMI) was also calculated from the anthropometric data as body mass divided by height squared ($\text{kg}\cdot\text{m}^{-2}$).

Bone health, lean and fat mass

For all subjects, BMD and BMC were measured using DXA (Hologic, Serie Discovery QDR densitometer, Bedford, USA). DXA scan tests were performed with subjects in a supine position, wearing light clothing with no metal, shoes or jewelry and analyzed using Physician's Viewer, APEX System Software Version 3.1.2. (Bedford, USA). DXA equipment accuracy

was checked on a daily basis before each scanning session following the Hologic guidelines. BMC (g) and BMD ($\text{g}\cdot\text{cm}^{-2}$) were obtained for each subject from whole body scans (trunk, pelvis, arms, legs and whole body), lumbar spine (L1–L4), and proximal region of the femur (total hip, greater trochanter, inter trochanter, Ward's triangle and femoral neck). The arm region included the hand, forearm, and arm and was separated from the trunk by an inclined line crossing the scapulohumeral joint, such that the humeral head was located in the arm region. The leg region included the foot, the lower leg, and the upper leg. It was separated from the trunk by an inclined line passing just below the pelvis, which crossed the femoral neck. The trunk region included the rest of the body excluding the arms, legs, and head regions. We used the rate of longitudinal changes of these variables for the CODA analyses

(i.e. $\left[\frac{\text{follow-up femoral neck BMD} - \text{baseline femoral neck BMD}}{\text{time gap between waves}} \right]$).

Fat mass (g), lean mass (bone free) (g) and total percentage body fat mass, were also calculated from whole body scans.

Physical activity and sedentary behaviors

Movement behaviors (PA and SB) were assessed using accelerometry (ActiTrainer and ActiGraph wGT3X-BT; ActiGraph, Pensacola, FL, USA). The customized software program (ActiLife Pro 6) was used to analyze all data. The full methodology has been previously described (24, 25). Briefly, all participants included were asked to wear an accelerometer on their left hip during waking hours for 7 consecutive days (it collected data using 1-minute epochs), except during any bathing or swimming activities. Similarly, non-wear time was defined as periods of at least 60 consecutive minutes of zero counts, with allowance for 2 min

of counts between zero and 100 (31). The explanation of accelerometer use, delivery and reception were made personally (32). Only the results from participants with at least four valid days including at least 480 min (8 h/day) of wear without excessive counts (i.e., >20,000 counts) were included in this study (30). Each valid wearing-time minute was classified into one of the classical intensity bands using count-based threshold: SB (<1.5 METs), LPA (1.5–2.99 METs) and MVPA (≥ 3 METs). Older adult-specific cut-off points for vector magnitude counts per minute were used in this analysis (33, 34).

Covariates

The following information was recorded during the interview and measurement sessions: socio-demographic variables: age, sex, education (no studies, primary school completed, secondary school completed or more), marital status (single, married/living together, widowed, divorced/separated), and income (coded into 10 categories ranging from no income to >5000€/month); anthropometric and body composition variables: BMI, fat mass, lean mass; lifestyle factors considered were alcohol intake, smoking and nutritional status; health variables: thyroid disease, arthritis and calcium. These were entered in the model as covariates and retained by backward elimination if the predictor was $p < 0.2$ as in previous studies (24, 25, 35).

Data analysis

R statistical system version 3.1.1. was used to conduct the statistical analyses. We used a multiple linear regression to evaluate differences in BMD and BMC as related to changes in

the distribution of the time spent in activities during waking hours, and a compositional method following the guide to CODA for PA, SB, and sleep research published by Chastin and colleagues (19). We used the compositional changes of the movement behaviors [$\Delta SB, \Delta LPA, \Delta MVPA$], which were determined by Aitchison's perturbation method (36, 37). Similarly, the ratios of each component in the distribution or sub-distribution of the movement behaviors within a waking hours period for follow-up changes from baseline, were calculated and then divided by the sum total of these ratios. Compositional descriptive statistics, including compositional geometric means for central tendency were used as standard descriptive statistics (mean \pm SD). Compositional geometric mean bar plots of the changes in the proportions of time were also generated to display the changes in the movement behavior profiles for frailty level changes ("positive change in frailty level", "no change in frailty level" and "negative change in frailty level"). CODA based on an isometric log-ratio (ilr) data transformation was conducted to determine the overall effect of PA distribution within average 24 hour periods (combination of the computed ilr-variables) and the relationship between the changes in movement behaviors and the rate of BMD and BMC decay, adjusting the models for the change in the time spent in the other behaviors. These ilr transformations were required to adequately adjust the models for time spent in the other behaviors. The p -values were identical across all the CODA analyses with cutoff for statistical significance set at $p \leq 0.05$; likewise, covariates were considered in the statistical analysis and the "normal" correction was also used for the complete models. All statistical analyses were conducted separately in the three subgroups. G*Power 3.1 (Franz Faul, University of Kiel, Kiel, Germany) (38) was used for power calculations. Different sample sizes were calculated using the variable of interest with more

variability (BMD) for multiple regressions ($\alpha = 0.05$, $1-\beta: 0.80$, $f^2 = 0.15$, 3 covariates). Thus, a minimum of 85 subjects per group was needed to detect significant associations.

Data availability

We are unable to provide the minimal dataset because of legal restrictions, i.e. the Spanish Data Protection Policy. However, there is an established infrastructure, including a website (<http://www.ciberfes.es/>) and a review committee, through which data requests are handled. The Hospital reviews and determines the purposes of the data requests and what data can be released. Data requests can be sent to: Research and teaching unit, Virgen del Valle Hospital Ctra. Cobisa S/N, 45071 Toledo – Spain, info@estudiotoledo.com.

Results

Descriptive

Of the 227 participants who finished all the stages in the follow-up, only 186 subjects in the whole sample (101 women and 85 men) were included in all analyses. From these participants, 41 subjects were excluded due to incomplete or invalid accelerometer data or the existence of metal prostheses in the body composition analysis (22% of the subjects excluded were due to accelerometry problems) (**Figure 1**). Nevertheless, no differences were found between the included and excluded participants in the analysis ($p > 0.05$). The characteristics of the participants in both evaluations are shown in **Table 1**. Body mass, height, whole body BMD and femoral neck BMD were significantly lower in the follow-up than at baseline in all

subgroups ($p < 0.05$). Moreover, BMI and femoral neck BMC were also lower in the follow-up in the subgroup with no changes in the frailty level and, whole body BMC in the subgroups with positive and negative changes in the frailty level ($p = 0.01-0.04$). Finally, the frailty score was also significantly different in the follow-up than compared to baseline in older people with a positive change in frailty level, which was lower in the follow-up ($p = 0.03$).

Changes in the distribution of the day by groups

The changes in the distribution of the day for each subgroup are presented as compositional mean bar plots. The changes for the whole sample grouped by frailty level change are represented in **Figure 2**. Older people with a positive change in frailty level decreased the time spent in SB relative to the other movement behaviors and increased the time in LPA and MVPA compared to the entire sample; conversely, older people with no change and a negative change in frailty level increased the time spent in SB relative to the other movement behaviors. Relative to LPA and MVPA, older people with no change in frailty level decreased the time spent in LPA relative to the other movement behaviors and increased the time in MVPA compared to the entire sample. The opposite trend was observed in the subgroup with a negative change in frailty level.

Compositional data

The CODA models, which show the distribution of the change in movement behaviors within a waking hours period on each bone variable, are reported in **Table 2** for the subgroup with a positive change in frailty level, **Table 3** for the subgroup with no change in frailty level, and

Table 4 for the subgroup with a negative change in frailty level. In the subgroup with a positive change in frailty level, the change in the distribution of movement behaviors within a waking hours period as a whole was significantly associated with spine and femoral neck BMD changes ($p=0.05$ and $p=0.02$, respectively). An increase in the time spent in LPA relative to the change in other movement behaviors was associated with lower femoral neck BMD changes ($p=0.02$) and, an increase in the time spent in MVPA relative to the change in other movement behaviors was associated with higher leg and spine BMC and BMD ($p=0.02-0.03$) and femoral neck BMD ($p=0.01$). In the no change in frailty level subgroup, change in the distribution of movement behaviors as a whole within a waking hours period was significantly associated with spine BMC changes ($p=0.04$). An increase in the time spent in MVPA relative to the change in other movement behaviors was associated with higher changes in spine BMC ($p=0.05$). No significant associations were found in the negative change in frailty level subgroup.

Discussion

To our knowledge, the present study is the first to use a CODA to longitudinally examine the associations between the change in the distribution of time spent in SB, LPA and MVPA and both BMD and BMC changes in older people related to the frailty level changes that occurred during a 4-year period. In this way a more appropriate model for assessing bone health in each group of frailty level change was able to be determined. Our results showed that the change in the distribution of movement behaviors, decrease or maintenance in SB and LPA and an increase in MVPA, was significantly associated with spine BMD changes and also a decrease

or maintenance in LPA and SB and an increase in MVPA with femoral neck BMD changes in older people with a positive change in frailty level. Likewise, the change in the distribution of movement behaviors, a decrease or maintenance in SB and LPA and an increase in MVPA, was significantly associated with spine BMC changes in those older people with no change in frailty level. Additionally, a positive effect of the change in MVPA related to the change in other movement behaviors, was also present in the leg region both for BMC and BMD variables.

Normally frailty increases steadily with age (9, 10, 39), although in our study, some older people had beneficial changes in their frailty level over 4 years. This group with a positive change in frailty level showed some changes in their movement behavior profile which reflect that they had increased time spent in MVPA and decreased time spent in SB, when compared to the entire sample. Therefore, just like other previous studies (12, 13, 26, 30, 40), we have also demonstrated that increasing MVPA and decreasing SB could improve frailty level. In fact, the older people with a positive change in frailty level during these 4 years showed a significant effect on their leg and spine BMC and BMD and femoral neck BMD, by increasing time spent in MVPA related to the change in the other movement behaviors. Similarly, other meta-analyses and longitudinal studies in men and women have also reported positive associations with bone variables (41), especially in those individuals who engaged in MVPA regularly (42). Thus, due to the fact that it is the change in MVPA that is associated with the bone health benefits, our sample increased engagement concerning PA and an active lifestyle. This significant association of the MVPA changes related to the changes in the other behaviors

could be explained because of the magnitude of gravitational impact forces experienced with both muscle force generation and body weight at this intensity (43, 44). Some intervention studies demonstrated that older men and women, who practiced high-impact exercise or high-intensity resistance training, managed to gain or maintain BMD compared to a control group (45-47). Regarding the non-significant effect of the change in LPA related to the changes in the other movement behaviors, The Healthy Ageing Initiative recently showed that the effect of this movement behavior did not seem to be relevant and clear in older men and women (48). These associations could also be explained by the multifactorial relation between PA, body composition and frailty. An improvement in frailty level also generates health improvements and the reduction of the different associated pathologies (49-51). Therefore, the benefits of PA on BMD and BMC could be seen more clearly when older people manage to improve their frailty level. It would be important to highlight those significant improvements in BMD and BMC that were found in both spine and femoral neck, which are the most appropriate body sites to diagnose osteoporosis and its relationship with bone fracture incidence (52, 53). Furthermore, for years, femoral neck fractures have been considered to be the most important osteoporotic fracture from a public health viewpoint (54); increasing in this way the importance that the changes in MVPA could have on bone health in this particular population.

Minor consequences of the change in PA or SB on BMD and BMC have been found in the group with no change in frailty level. In fact, only significant results were found in relation to the spine BMC changes. Their change in the movement behavior profile showed particularly an increase of SB and decrease of LPA related to the entire sample, which did not produce

frailty level changes probably because of the little increase of time spent in MVPA. Indeed, in a recent longitudinal study, Gajic-Veljanoski et al. (55) demonstrated that for improving the frailty level it would be necessary to achieve a recommended weekly amount of PA of at least 1000 kilocalories, which is approximately equal to 30 min of walking per day (56, 57). Probably for these reasons, the general health of these subjects did not improve, which would increase the difficulty of showing the benefits of BMD and BMC of the changes in the movement behaviors. However, this small increment of MVPA related to the other behaviors could be responsible for the relationship between the complete model and spine BMC changes. The associations with the spine BMC could be due to the important influence that PA exerts on weight bearing skeletal areas (58), as is the case of the spine. Thus, the influence of the changes in the movement behaviors could have an impact on this bone area, which is of great relevance both for bone health and public health policies, as described above. For these reasons, when older people maintain their frailty level, to increase time spent in MVPA related to the changes in the other movement behaviors could still be beneficial regarding osteoporosis and the risk of bone fractures; helping to improve quality of life and avoid physical dependence.

Finally, in the older people group with a negative change in frailty level, no effects of the distribution of movement behaviors within a waking hours period or significant associations were found. The reason could be the general deterioration in health and the increase of comorbidities that are linked with the increase in frailty. Furthermore, with frailty, PA impact might not be considered clinically meaningful, given that it is much smaller than the effect of fractures and obesity (55). The negative consequences of obesity on frailty have been

previously suggested, just like the multifactorial relationship between bone health and frailty, making it more difficult to differentiate the PA effect (15). Thus, it could be necessary to consider other factors when bone health is studied (15, 55). In the same way, a controlled exercise intervention trial found no effect on BMD over 4 years (59). A cross-sectional study about the same associations demonstrated that PA was not related to bone health in frail older people when the whole sample was analyzed, although MVPA could be a good strategy to improve bone health before reaching frailty level (35). Similarly, previous studies also found a small beneficial relationship between walking and decreased BMD loss (60, 61).

Despite the fact that changes in the distribution of movement behaviors within a waking hours period showed a beneficial change in BMC and BMD during a period of 4 years, related to the frailty changes, the most relevant aspect from a public health perspective would be that significant improvements in both bone variables were found in both spine and femoral neck. Therefore, increasing MVPA and maintaining or reducing SB and LPA may be a good approach for reducing osteoporosis and the risk of bone fractures, which have a strong association with the increase of morbidity and mortality. Additionally, given that the prevalence of older people is continuously increasing, improving the quality of life of this population acquires a higher relevance (25). Thus, this study also confirms previous statements as being physically active is a key factor in maintaining health and in the normal functioning of physiological systems across the life-course (27). Furthermore, the movement behavior profile of the older people with a positive change in frailty level after 4 years of follow-up

showed that to decrease time in SB and increase time in LPA and MVPA would be a distinctive characteristic of this specific population.

Our study is not without limitations. SB could have been overestimated because the accelerometers may not detect differences between sitting and standing positions (62). However, this instrument has been widely validated and moreover, specific cut-off points for older adults were used in order to determine their movement behaviors (33, 34). Similarly, sleep was not recorded in our study, although we had objectively measured all the waking hours. Related to the data, the probability that some results might be attributed to chance due to multiple comparisons cannot be excluded; however, the complete models were corrected and compositional data analyses deals with the co-dependence between time spent in different movement behaviors (22). Finally, in spite of the high validity of our measurements, with the use of the gold standard DXA method for assessing body composition and accelerometry to measure movement behaviors objectively, the sample size was limited, especially in the subgroups with positive and negative changes in frailty level. Therefore, caution should be applied when interpreting the results. Future research should increase the sample and explore whether the benefits of PA changes in older people are the most suitable method to enhance and maintain bone health depending on frailty level changes. On the other hand, the main strength of our study was related to the use of a longitudinal perspective to assess BMD and BMC decay according to the changes in frailty level. Thus, it is the first longitudinal research to include this novel analytical approach to deal with all waking hour data with objectively assessed PA, SB, body composition and bone health according to the frailty changes. Furthermore, longitudinal data provide better estimates of the rate of bone loss compared to

the rate of change estimated from cross-sectional data that could be overestimated (63). Additionally, some potential confounding factors were introduced in all models as covariates in order to eliminate the possible influence on bone.

Conclusion

The present longitudinal study showed that the changes in the distribution of movement behaviors within a waking hours period seem to be beneficial for bone health according to the frailty changes. We observed in our study that reducing or maintaining time spent in sedentary behaviors and light physical activity and increasing moderate-to-vigorous physical activity produce improvements in bone mineral density and bone mineral content when older people improve their frailty level; however, these associations are reduced if older people just maintain their frailty level and even disappear when their frailty level deteriorates. Therefore, and particularly, the benefits of the increase of moderate-to-vigorous physical activity relative to the changes in the other movement behaviors on bone mineral density could be highlighted if older people manage to improve their frailty level during aging. Furthermore, due to the fact that these benefits favor the spine and femoral neck bone mineral density and bone mineral content, it is important to emphasize the possible effect of this strategy from the public health perspective through osteoporosis and bone fracture risk reduction. Future research might explore which changes come first and what is happening between frailty and the distribution of the movements in this population.

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References

1. Ahmed N, Mandel R, Fain MJ. Frailty: an emerging geriatric syndrome. *Am J Med* 2007;120(9):748-53.

2. Rockwood K, Song X, MacKnight C, Bergman H, Hogan DB, McDowell I, et al. A global clinical measure of fitness and frailty in elderly people. *Can Med Assoc J*. 2005;173(5):489-95.
3. Cheng MH, Chang SF. Frailty as a Risk Factor for Falls Among Community Dwelling People: Evidence From a Meta-Analysis. *J Nurs Scholarsh*. 2017;49(5):529-36.
4. Blain H, Rolland Y, Beauchet O, Annweiler C, Benhamou C-L, Benetos A, et al. Usefulness of bone density measurement in fallers. *Joint Bone Spine*. 2014;81(5):403-8.
5. Yoneki K, Kitagawa J, Hoshi K, Harada M, Watanabe T, Shimoda T, et al. Association between frailty and bone loss in patients undergoing maintenance hemodialysis. *J Bone Miner Res*. 2018:1-9.
6. Rolland Y, Van Kan GA, Benetos A, Blain H, Bonnefoy M, Chassagne P, et al. Frailty, osteoporosis and hip fracture: causes, consequences and therapeutic perspectives. *J Nutr Health Aging*. 2008;12(5):a319-a30.
7. Chang C-I, Chan D-CD, Kuo K-N, Hsiung CA, Chen C-Y. Vitamin D insufficiency and frailty syndrome in older adults living in a Northern Taiwan community. *Arch Gerontol Geriatr*. 2010;50:S17-S21.
8. Cauley JA. Public health impact of osteoporosis. *J Gerontol A Biol Sci Med Sci*. 2013;68(10):1243-51.
9. Liu L-K, Lee W-J, Chen L-Y, Hwang A-C, Lin M-H, Peng L-N, et al. Association between frailty, osteoporosis, falls and hip fractures among community-dwelling people aged 50 years and older in Taiwan: results from I-Lan Longitudinal Aging Study. *PLoS one*. 2015;10(9):e0136968.

- Accepted Article
10. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*. 2001;56(3):M146-M57.
 11. Garcia-Garcia FJ, Avila GG, Alfaro-Acha A, Andres MA, Aparicio ME, Aparicio SH, et al. The prevalence of frailty syndrome in an older population from Spain. The Toledo Study for Healthy Aging. *J Nutr Health Aging*. 2011;15(10):852-6.
 12. Lim S, Joung H, Shin CS, Lee HK, Kim KS, Shin EK, et al. Body composition changes with age have gender-specific impacts on bone mineral density. *Bone*. 2004;35(3):792-8.
 13. Liu CK, Fielding RA. Exercise as an intervention for frailty. *Clin Geriatr Med*. 2011;27(1):101-10.
 14. Cook MJ, Oldroyd A, Pye SR, Ward KA, Gielen E, Ravindrarajah R, et al. Frailty and bone health in European men. *Age Ageing*. 2016;46(4):635-41.
 15. Dawson A, Dennison E. Measuring the musculoskeletal aging phenotype. *Maturitas*. 2016;93:13-7.
 16. Kojima G. Frailty as a predictor of disabilities among community-dwelling older people: a systematic review and meta-analysis. *Disability and rehabilitation*. 2017;39(19):1897-908.
 17. Álvarez-Sánchez N, Álvarez-Ríos AI, Guerrero JM, García-García FJ, Rodríguez-Mañas L, Cruz-Chamorro I, et al. Homocysteine levels are associated with bone resorption in pre-frail and frail Spanish women: The Toledo Study for Healthy Aging. *Exp Gerontol*. 2018;108:201-8.

18. García-García FJ, Carcaillon L, Fernandez-Tresguerres J, Alfaro A, Larrion JL, Castillo C, et al. A new operational definition of frailty: the Frailty Trait Scale. *J Am Med Dir Assoc* 2014;15(5):371. e7-. e13.
19. Chastin SF, Palarea-Albaladejo J, Dontje ML, Skelton DA. Combined effects of time spent in physical activity, sedentary behaviors and sleep on obesity and cardio-metabolic health markers: a novel compositional data analysis approach. *PloS one*. 2015;10(10):e0139984.
20. Dumuid D, Stanford TE, Martin-Fernández J-A, Pedišić Ž, Maher CA, Lewis LK, et al. Compositional data analysis for physical activity, sedentary time and sleep research. *Stat Methods Med Res*. 2018;27(12):3726-38.
21. Gupta N, Mathiassen SE, Mateu-Figueras G, Heiden M, Hallman DM, Jørgensen MB, et al. A comparison of standard and compositional data analysis in studies addressing group differences in sedentary behavior and physical activity. *Int J Behav Nutr Phys Act*. 2018;15(1):53.
22. Carson V, Tremblay MS, Chaput J-P, Chastin SF. Associations between sleep duration, sedentary time, physical activity, and health indicators among Canadian children and youth using compositional analyses 1. *Appl Physiol Nutr Metab*. 2016;41(6):S294-S302.
23. Carson V, Tremblay MS, Chastin SF. Cross-sectional associations between sleep duration, sedentary time, physical activity, and adiposity indicators among Canadian preschool-aged children using compositional analyses. *BMC Public Health*. 2017;17(5):848.
24. Rodríguez-Gómez I, Mañas A, Losa-Reyna J, Rodríguez-Mañas L, Chastin S, Alegre L, et al. Associations between sedentary time, physical activity and bone health among older people using compositional data analysis. *PloS one*. 2018;13(10):e0206013-e.

25. Rodríguez-Gómez I, Mañas A, Losa-Reyna J, Rodríguez-Mañas L, Chastin SF, Alegre LM, et al. Compositional Influence of Movement Behaviours on Bone Health during Ageing. *Med Sci Sports Exerc* 2019.
26. del Pozo-Cruz B, Mañas A, Martín-García M, Marín-Puyalto J, García-García FJ, Rodríguez-Mañas L, et al. Frailty is associated with objectively assessed sedentary behaviour patterns in older adults: Evidence from the Toledo Study for Healthy Aging (TSHA). *PloS one*. 2017;12(9):e0183911.
27. Bangsbo J, Blackwell J, Boraxbekk C-J, Caserotti P, Dela F, Evans AB, et al. Copenhagen Consensus statement 2019: physical activity and ageing. *Br J Sports Med*. 2019:bjsports-2018-100451.
28. Garry PJ, Vellas BJ. Practical and validated use of the Mini Nutritional Assessment in geriatric evaluation. *Nutrition in clinical Care*. 1999;2(3):146-54.
29. Martínez-González MA, García-Arellano A, Toledo E, Salas-Salvado J, Buil-Cosiales P, Corella D, et al. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. *PLoS One*. 2012;7(8):e43134.
30. Mañas A, del Pozo-Cruz B, Guadalupe-Grau A, Marín-Puyalto J, Alfaro-Acha A, Rodríguez-Mañas L, et al. Reallocating Accelerometer-Assessed Sedentary Time to Light or Moderate-to Vigorous-Intensity Physical Activity Reduces Frailty Levels in Older Adults: An Isotemporal Substitution Approach in the TSHA Study. *J Am Med Dir Assoc* 2017.
31. Colley R, Connor Gorber S, Tremblay MS. Quality control and data reduction procedures for accelerometry-derived measures of physical activity. *Health Rep*. 2010;21(1):63-9.

32. Matthews CE, Hagströmer M, Pober DM, Bowles HR. Best practices for using physical activity monitors in population-based research. *Med Sci Sports Exerc* 2012;44(1 Suppl 1):S68.
33. Aguilar-Farías N, Brown WJ, Peeters GG. ActiGraph GT3X+ cut-points for identifying sedentary behaviour in older adults in free-living environments. *J Sci Med Sport* 2014;17(3):293-9.
34. Santos-Lozano A, Santin-Medeiros F, Cardon G, Torres-Luque G, Bailon R, Bergmeir C, et al. Actigraph GT3X: validation and determination of physical activity intensity cut points. *Int J Sports Med*. 2013;34(11):975-82.
35. Rodríguez-Gómez I, Mañas A, Losa-Reyna J, Rodríguez-Mañas L, Chastin SF, Alegre LM, et al. The Impact of Movement Behaviors on Bone Health in Elderly with Adequate Nutritional Status: Compositional Data Analysis Depending on the Frailty Status. *Nutrients*. 2019;11(3):582.
36. Van den Boogaart KG, Tolosana-Delgado R. *Analyzing compositional data with R*: Springer; 2013.
37. Aitchison J, Ng K. The role of perturbation in compositional data analysis. *Statistical Modelling*. 2005;5(2):173-85.
38. Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G* Power 3.1: Tests for correlation and regression analyses. *Behavior research methods*. 2009;41(4):1149-60.
39. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. *The Lancet*. 2013;381(9868):752-62.

- Accepted Article
40. Mañas A, del Pozo-Cruz B, García-García FJ, Guadalupe-Grau A, Ara I. Role of objectively measured sedentary behaviour in physical performance, frailty and mortality among older adults: A short systematic review. *Eur J Sport Sci.* 2017;1-14.
 41. Shea B, Bonaiuti D, Iovine R, Negrini S, Robinson V, Kemper H, et al. Cochrane Review on exercise for preventing and treating osteoporosis in postmenopausal women. *Eura Medicophys.* 2004;40:199-210.
 42. Shephard RJ, Park H, Park S, Aoyagi Y. Objective Longitudinal Measures of Physical Activity and Bone Health in Older Japanese: the Nakanajo Study. *J Am Geriatr Soc.* 2017;65(4):800-7.
 43. Kohrt WM, Barry DW, Schwartz RS. Muscle forces or gravity: what predominates mechanical loading on bone? *Med Sci Sports Exerc* 2009;41(11):2050.
 44. Popp KL, McDermott W, Hughes JM, Baxter SA, Stovitz SD, Petit MA. Bone strength estimates relative to vertical ground reaction force discriminates women runners with stress fracture history. *Bone.* 2017;94:22-8.
 45. Allison SJ, Folland JP, Rennie WJ, Summers GD, Brooke-Wavell K. High impact exercise increased femoral neck bone mineral density in older men: a randomised unilateral intervention. *Bone.* 2013;53(2):321-8.
 46. Kohrt WM, Bloomfield SA, Little KD, Nelson ME, Yingling VR. Physical activity and bone health. *Med Sci Sports Exerc* 2004;36(11):1985-96.
 47. Zhao R, Zhao M, Xu Z. The effects of differing resistance training modes on the preservation of bone mineral density in postmenopausal women: a meta-analysis. *Osteoporosis international.* 2015;26(5):1605-18.

48. Johansson J, Nordström A, Nordström P. Objectively measured physical activity is associated with parameters of bone in 70-year-old men and women. *Bone*. 2015;81:72-9.
49. Marzetti E, Calvani R, Tosato M, Cesari M, Di Bari M, Cherubini A, et al. Physical activity and exercise as countermeasures to physical frailty and sarcopenia. *Aging clinical and experimental research*. 2017;29(1):35-42.
50. Grassi C, Landi F, Delogu G. Lifestyles and ageing: targeting key mechanisms to shift the balance from unhealthy to healthy ageing. *Stud Health Technol Inform*. 2014;203:99-111.
51. McPhee JS, French DP, Jackson D, Nazroo J, Pendleton N, Degens H. Physical activity in older age: perspectives for healthy ageing and frailty. *Biogerontology*. 2016;17(3):567-80.
52. Adachi JD, Adami S, Gehlbach S, Anderson FA, Boonen S, Chapurlat RD, et al., editors. Impact of prevalent fractures on quality of life: baseline results from the global longitudinal study of osteoporosis in women. *Mayo Clinic Proceedings*; 2010: Elsevier.
53. Jones G, Nguyen T, Sambrook P, Kelly P, Gilbert C, Eisman J. Symptomatic fracture incidence in elderly men and women: the Dubbo Osteoporosis Epidemiology Study (DOES). *Osteoporos Int*. 1994;4(5):277-82.
54. Aloia JF, Vaswani A, Ross P, Cohn SH. Aging bone loss from the femur, spine, radius, and total skeleton. *Metab Clin Exp*. 1990;39(11):1144-50.
55. Gajic-Veljanoski O, Papaioannou A, Kennedy C, Ioannidis G, Berger C, Wong AKO, et al. Osteoporotic fractures and obesity affect frailty progression: a longitudinal analysis of the Canadian multicentre osteoporosis study. *BMC Geriatr*. 2018;18(1):4.

56. Haskell WL, Lee I-M, Pate RR, Powell KE, Blair SN, Franklin BA, et al. Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Circulation*. 2007;116(9):1081.
57. Kokkinos P, Myers J. Exercise and physical activity: clinical outcomes and applications. *Circulation*. 2010;122(16):1637-48.
58. Nordström P, Pettersson U, Lorentzon R. Type of physical activity, muscle strength, and pubertal stage as determinants of bone mineral density and bone area in adolescent boys. *J Bone Miner Metab*. 1998;13(7):1141-8.
59. Huuskonen J, Väisänen S, Kröger H, Jurvelin J, Alhava E, Rauramaa R. Regular physical exercise and bone mineral density: a four-year controlled randomized trial in middle-aged men. The DNASCO study. *Osteoporos Int*. 2001;12(5):349-55.
60. Palombaro KM. Effects of Walking-only Interventions on Bone Mineral Density at Various Skeletal Sites: A Meta-analysis. *J Geriatr Phys Ther*. 2005;28(3):102-7.
61. Bleicher K, Cumming R, Naganathan V, Seibel M, Blyth F, Le Couteur D, et al. Predictors of the rate of BMD loss in older men: findings from the CHAMP study. *Osteoporos Int*. 2013;24(7):1951-63.
62. Chayes F. A priori and experimental approximation of simple ratio correlations. *Concepts in geostatistics*: Springer; 1975. p. 106-37.
63. Melton Iii L, Khosla S, Atkinson E, O'Connor M, O'fallon W, Riggs B. Cross-sectional versus longitudinal evaluation of bone loss in men and women. *Osteoporos Int*. 2000;11(7):592-9.

Figure legends

Figure 1. Flow diagram of the Toledo Study of Healthy Ageing (TSHA).

Figure 2. Compositional geometric mean bar plots for the whole sample comparing the changes in the proportions of time of the entire sample (value 0 in the plot), with the changes of the subgroups with a positive change in frailty level, no change in frailty level and a negative change in frailty level for sedentary behavior (SB), light physical activity (LPA) and moderate-to-vigorous physical activity (MVPA).

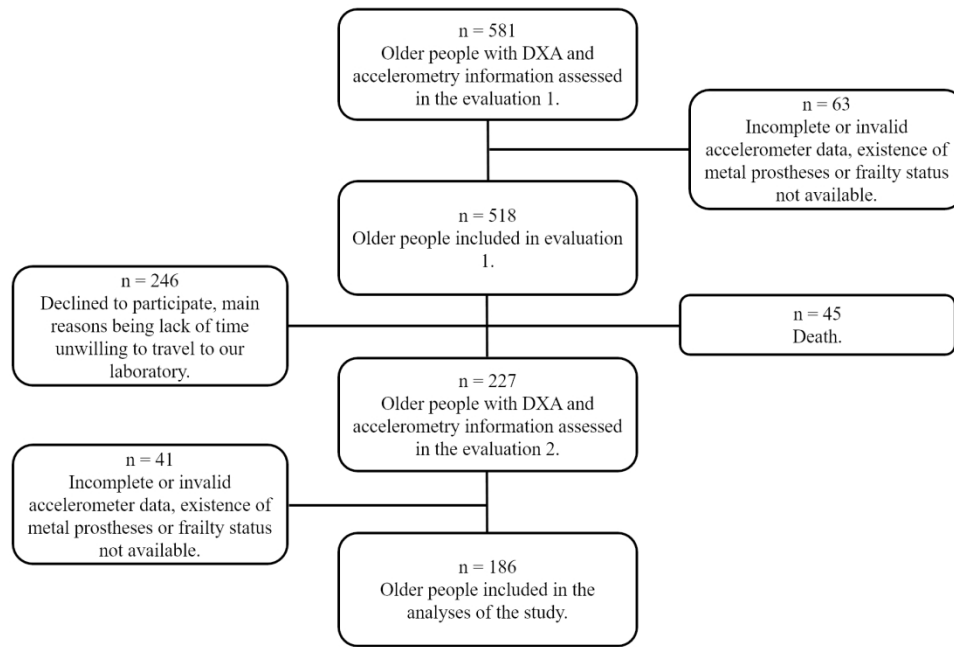


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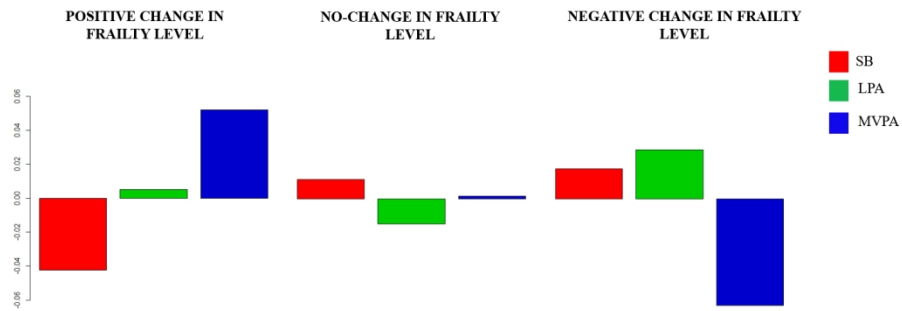


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143x65mm (300 x 300 DPI)