Research Article

Longitudinal Associations of Serum 25-hydroxyvitamin D, Physical Activity, and Knee Pain and Dysfunction with Muscle Loss in Community-dwelling Older Adults

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Abstract

Aim: To describe the associations of between-person and within-person variability in serum 25-hydroxyvitamin D (25(OH)D), physical activity (PA), and knee pain and dysfunction with muscle mass, strength, and muscle quality over 10 years in community-dwelling older adults.

Method: Participants (N = 1033; 51% women; mean age 63 ± 7.4 years) were measured at baseline, 2.5, 5, and 10 years. Lower limb lean mass (LLM) was assessed using dual energy X-ray absorptiometry, lower limb muscle strength (LMS) using a dynamometer, and lower limb muscle quality (LMQ) calculated as LMS/LLM. Knee pain and dysfunction were assessed using the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index. PA was measured using pedometers. Linear-mixed effect regression models, with adjustment for confounders, were used to estimate the association of within-person and between-person variability in PA, 25(OH)D, and WOMAC scores with muscle mass, strength, and muscle quality.

Results: Both between-person and within-person increases in PA were associated with LLM, LMS, and LMQ (all P < 0.05). Within-person and between-person increases in knee pain and dysfunction were associated with LLS and LMQ, but not with LLM (all P < 0.05). Between-person effects showed that higher average 25(OH)D was associated with a higher 10-year average LLM, LMS, and LMQ (all P < 0.05), whereas within-person increases in average 25(OH)D were associated with a higher LMS and LMQ, but not with LLM.

Conclusions: Variability in 25(OH)D, pain, and dysfunction within an individual over time is related to muscle changes in that individual. Increasing one’s own PA level further increases muscle mass, strength, and quality supporting the clinical recommendation of promoting PA to reduce age-related muscle loss.

Keywords: Body composition, Pain, Physical activity, Vitamin D

Age-related loss of skeletal muscle mass, strength, and muscle quality is a major public health concern that is associated with functional disability, poor quality of life, and mortality in older people (1–4). Although loss of muscle mass and function increases with age, a significant variability exists between individuals in the rate of loss of muscle mass and function (5). Traditionally, analysis has focused on examining how risk factors for loss of muscle mass and function differ between individuals (between-person comparison). These studies showed that age-related loss of muscle mass and strength is more prevalent in older adults with lower levels of serum

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25-hydroxyvitamin D (25(OH)D) (6–8), PA (1,9,10), and higher levels of knee pain and functional limitation (1,11). As a result, public health efforts have been informed by findings from between-person comparisons but less well recognized is how variability within the same individual (within-person comparison) over time is related to muscle changes. This is important to investigate because the information we get from between-person analysis (eg, the magnitude and direction of effects) might be quite different from what a within-person analysis tells us. Besides, neither can be inferred from the other (12). Statistical methods, such as multilevel models, that properly capture the within-person processes can be used to tell us whether changes within an individual over time is related to changes in muscle in that same individual (13). Findings from within-person comparison are vital for formulating policies aimed at improving population health by promoting good health behaviours like PA at the individual level. This could include person-centered public health messages that highlight the benefits of improving one’s own level of PA.

To the best of our knowledge, there are no published studies describing associations of within-person variability in PA, 25(OH)D, and knee pain and dysfunction with loss of muscle mass, strength, and muscle quality in community-dwelling older people. Therefore, the aim of this study was to describe the associations of between-person and within-person variability in 25(OH)D, PA, and knee pain and dysfunction with age-related loss of skeletal muscle mass, strength, and muscle quality over 10 years in community-dwelling older adults. We hypothesized that older adults with a lower mean level of PA, 25(OH)D, and higher average level of knee pain and dysfunction would have a lower muscle mass, strength, and muscle quality. Furthermore, at time points when an individual had a lower level of 25(OH)D, PA, and higher knee pain and dysfunction than their own individual average, they would also have a lower muscle mass, strength, and muscle quality.

Data and Methods

The Tasmanian Older Adult Cohort study is a prospective, population-based study that primarily aimed at examining the causes and progression of osteoarthritis. Participants aged 50 years and older were selected using sex-stratified random sampling from the electoral roll in southern Tasmania (population 229,000). A total of 1099 adults (response rate = 57%) consented to participate. Participants were excluded if they had any implants that would prevent them from undergoing an MRI or they were living in a nursing home. Participants attended a clinic at the Menzies Institute for Medical Research, Hobart, Tasmania between March 2002 and September 2004 and follow-up clinic assessments 2.5, 5, and 10 years later. The study was approved by the Southern Tasmanian Health and Medical Research Ethics Committee, and written informed consent was obtained from all participants.

Measures

Body composition and anthropometrics

Body composition was measured using dual energy X-ray absorptiometry (DXA; Hologic Delphi, Hologic, Waltham, USA). Leg lean muscle mass (kg) was calculated as the sum of lean mass in both lower limbs. Weight (kg) was measured using electronic scales (Heine, Dover, USA). Height (cm) was measured using the Leicester stadiometer (Invicta, Leicester, UK).

Lower limb muscle strength and muscle quality

LMS in kilograms was measured simultaneously for both limbs using a dynamometer (TTM Muscular Meter, Tokyo, Japan). Participants stood on the back of a dynamometer platform with their backs against a wall and knee flexed to 115°. A bar was attached to the dynamometer, and participants lifted the bar using their lower limbs only to maximum contractile force, whilst maintaining proper head and neck posture. This test assessed the isometric strength of the whole lower limbs but predominantly of the quadriceps and hip extensors. Two trials were recorded, with the mean score taken as the criterion value for lower limbs muscle strength (9). The intra-class correlation coefficient for the first and second trial was 0.95 (95% CI: 0.94–0.96). Leg muscle quality (LMQ) was calculated as LMS divided by the sum of DXA-derived lean mass of the two lower limbs (14).

Physical activity

Habitual PA was assessed at baseline, 2.5 and 5 years. Baseline assessment was assessed over seven consecutive days using a pedometer (Omron HJ-003 & HJ-102; Omron Healthcare, Kyoto, Japan) worn on the waist band or belt above their dominant lower limb. Participants were given a diary in which they have to record the duration of pedometer use and daily step counts. At 2.5 years follow-up, Yamax pedometers (Yamax SW-200; Yamax USA, San Antonio, TX, USA) were given to the majority (98%) of the participants. A strong linear correlation (r = 0.88) was found between the estimates of the two types of pedometer, but the Omron brand recorded higher mean steps. Yamax pedometers were used by all participants at 5 years. Baseline and 2.5 years Omron estimates were multiplied by a correction factor of 0.91 to provide comparability between mean estimates for Omron and Yamax pedometers (15). Beta coefficients in the regression models were scaled to 1000 steps-per-day for ease of interpretation.

Knee pain, stiffness, and functional limitation

Knee pain, stiffness, and functional limitation were assessed by self-administered questionnaires using the WOMAC at each study visit. WOMAC is a validated and widely used measure of symptoms and...
disability in patients with osteoarthritis (16). The three WOMAC subscales such as knee pain, stiffness, and functional limitation were assessed separately with 5, 2, and 17 questions, respectively. Response to each question ranges from 0 (no symptom) to 9 (most severe knee pain, stiffness, or functional limitation). Response in each subscale was summed to create a score for knee pain (range: 0–45), stiffness (range: 0–18), and functional limitation (range: 0–153). All three subscales were also summed to create a WOMAC global score (range: 0–216).

**Serum 25-hydroxyvitamin D**

25(OH)D was assessed at baseline, 2.5 and 10 years follow-up. Serum samples of blood drawn from the participants were treated initially with acetonitrile to rapidly extract vitamin-D and other hydroxylated metabolites. Thereafter, 25(OH)D was assayed using liquid-phase radioimmunoassay (Immunodiagnostics Systems Ltd), which detects both 25(OH)D2 and 25(OH)D3. The intraassay and interassay coefficients of variation were 1.8% and 3.3%, respectively. Participants had their baseline and follow-up interview at different seasons, and as 25(OH)D is known to vary with seasons, we de-seasonalized the 25(OH)D measurement as described previously [13] to account for differences in the time of the year that blood was taken.

**Potential confounders**

Age, sex, medical history, including a previous diagnosis of diabetes, rheumatoid arthritis, and cardiovascular diseases (hypertension, bronchitis/emphysema, or heart attack), employment status, and smoking history were recorded using a questionnaire.

**Data Analysis**

Measurements for PA, 25(OH)D, and WOMAC scores varied across occasions for individuals and were modelled as time-varying predictors (13,17). Time-varying predictors provide two sources of information in longitudinal analysis; consequently, we decomposed each time-varying predictor into between-person and within-person components. The between-person component of the time-varying predictor is an individual's average 25(OH)D, PA, and WOMAC scores across all measurement occasions. The within-person component is the deviation of an individual's measurement at a particular phase of the study from his or her average score across all measurement occasions. We first calculated intraclass correlation coefficients for each predictor variable to ensure that there was sufficient variance to warrant decomposing the predictors into between-person and within-person effects (13,18). All intraclass correlation coefficients of our predictor variables were > 0.3, suggesting a substantial variability in each predictor variable (18).

Linear-mixed effect regression models were used to estimate the association of between-person and within-person variability in 25(OH)D, PA, and WOMAC scores over 10 years with LLM, LMS, and LMQ. Models were adjusted for age, sex, number of chronic conditions, employment status, and smoking status. Multiplicative interaction between age, sex, and between-person and within-person variability in serum 25(OH)D, PA, and knee pain and dysfunction was assessed for each outcome variable. All models were estimated using the maximum likelihood method. The fit of models with different fixed and random effects was compared using the likelihood ratio test and Akaike Information Criterion (19). Data were analyzed using Stata version 13 (StataCorp, TX, USA).

**Results**

A total of 1033 participants with complete body composition and muscle assessments at baseline were included in the analysis. Of these, 853 (82%), 752 (73%), and 559 (54%) respectively attended the 2.5, 5, and 10 years follow-up assessments. Participants were lost to follow up because of reasons such as death, withdrawal of consent, institutionalization, moving interstate or overseas, and having a joint replacement. Compared with those who have completed the 10 year follow-up assessment, participants lost to follow up were older at baseline (64.7 ± 8.0 vs 61.4 ± 6.5 years, P < 0.001) and had a lower lean muscle mass (23.9 ± 5.3 vs 24.8 ± 5.3 kg, P = 0.005), a weaker LMS (87.7 ± 47.0 vs 96.9 ± 50.5 kg, P = 0.003), and muscle quality (5.3 ± 2.3 vs 5.7 ± 2.4, P = 0.019), but no difference in the proportions of males and females was observed (P = 0.524).

Baseline characteristics of the participants are shown in Table 1. The mean age of the participants at baseline was 63 ± 7.4 and 51% were female. The associations of between-person and within-person variability in 25(OH)D, PA, and knee pain and dysfunction with LLM, LMS, and LMQ are shown in Table 2.

**Physical Activity and LLM, LMS and LMQ**

Both between-person and within-person increases in PA were associated with muscle changes (between-person: LLM (β = 0.03; 95% CI 0.02, 0.05), LMS (β = 1.25; 95% CI 0.61, 1.88), and LMQ (β = 0.10; 95% CI 0.06, 0.13); within-person: LLM (β = 0.02; 95% CI 0.001, 0.04), LMS (β = 0.56; 95% CI 0.01, 1.10), and LMQ (β = 0.04; 95% CI 0.01, 0.07)). There was an interaction between sex and between-person variability in PA and LMQ such that the positive association was stronger in women (β = 0.14; 95% CI: 0.09, 0.19) compared with men (β = 0.06; 95% CI: 0.01, 0.11).

**Knee pain and dysfunction and LLM, LMS, and LMQ**

Between-person increase in WOMAC global score was associated with a significantly lower LMS (β = −0.21; 95% CI −0.28, −0.13), LMQ (β = −0.02; 95% CI −0.02, −0.01), but not with LLM (β = 0.001; 95% CI−0.002, 0.003). Similar associations were observed for the WOMAC subscales. Sex modifies the relationship between LMQ and between-person variability in WOMAC knee pain subscale (men: β = −0.06, 95% CI: −0.09, −0.03; women: β = −0.10, 95% CI: −0.12, −0.07) and WOMAC knee dysfunction subscale (men: β = −0.02, 95% CI: −0.03, −0.01; women: β = −0.03, 95% CI: −0.04, −0.02) such that the negative associations were stronger for women compared with men.

Within-person increase in WOMAC global score was associated with a lower LMS (β = −0.09; 95% CI −0.15, −0.03) and LMQ (β = −0.006; 95% CI −0.01, −0.002), but not with LLM (β = 0.002 95% CI −0.0003, 0.004). Furthermore, at time points when WOMAC knee dysfunction subscale scores were higher than average LMS (β = −0.14; 95% CI −0.22, −0.06) and LMQ (β = −0.01; 95% CI −0.01, −0.003), but not with LLM (β = 0.002 95% CI −0.001, 0.005) were significantly lower. No significant within-person association was found between WOMAC knee pain and knee stiffness subscales and LLM, LMS, and LMQ. The association between within-person variability in WOMAC global score and LMQ was modified by age such that the association was significant in participants aged 50–69 years (β = −0.01; 95% CI: −0.01, −0.004) but not significant in participants aged 70 years and older (β = 0.005; 95% CI: −0.001, 0.01). A similar relationship was observed between within-person variability in knee dysfunction subscale and LMS.
**Table 2. Association of Between-person† and Within-person Variability‡ in Serum 25-hydroxyvitamin D, PA, Knee Pain and Dysfunction with LLM, LMS, and Muscle Quality (N = 1033)**

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>β (95% CI)</th>
<th>Lower limb muscle strength, kg</th>
<th>Between-person effect</th>
<th>Within-person effect</th>
<th>Lower limb muscle quality, kg/kg</th>
<th>Between-person effect</th>
<th>Within-person effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA (per 1000 steps/day)</td>
<td>0.03 (0.02, 0.05)</td>
<td>0.10 (0.06, 0.13)</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.04 (0.01, 0.07)</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.04 (0.01, 0.07)</td>
<td></td>
</tr>
<tr>
<td>WOMAC global score</td>
<td>0.004 (0.001, 0.007)</td>
<td>0.10 (0.06, 0.13)</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.04 (0.01, 0.07)</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.04 (0.01, 0.07)</td>
<td></td>
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<tr>
<td>Knee pain</td>
<td>0.004 (0.001, 0.007)</td>
<td>0.10 (0.06, 0.13)</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.04 (0.01, 0.07)</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.04 (0.01, 0.07)</td>
<td></td>
</tr>
<tr>
<td>Knee stiffness</td>
<td>0.002 (0.001, 0.004)</td>
<td>0.10 (0.06, 0.13)</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.04 (0.01, 0.07)</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.04 (0.01, 0.07)</td>
<td></td>
</tr>
<tr>
<td>Serum 25(OH)D(nmol/L)</td>
<td>0.008 (0.005, 0.010)</td>
<td>0.10 (0.06, 0.13)</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.04 (0.01, 0.07)</td>
<td>0.06 (0.03, 0.09)</td>
<td>0.04 (0.01, 0.07)</td>
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</tr>
</tbody>
</table>

Data in bold indicate statistical significance at \( P < 0.05 \). Models are adjusted for age, sex, number of chronic conditions (diabetes, rheumatoid arthritis, cardiovascular disease: hypertension, bronchitis/emphysema, or heart attack), employment status (currently employed vs. not currently employed), smoking status, linear time, and quadratic time.

†Beta coefficients expressed as a change in 10-year average of the outcome variable per 1 unit increase in the 10-year average predictor variable (25(OH)D, WOMAC, and PA). ‡Beta coefficients expressed as a change in individual's usual level of PA, further increases in PA have additional beneficial effects on muscle mass, strength, and muscle quality. For instance, at time points when an individual (either with higher or lower 10-year average PA) increased their steps-per-day by 1000, that individual had a 0.56 kg increase in LMS. The effect of PA on muscle mass was stronger in the between-person analysis (eg, a 1000 steps/day increase resulted in a 1.25 kg increase in 10-year average LMS).

Discussion

To our knowledge, this is the first prospective study to determine the associations between both within-person and between-person variability in serum 25(OH)D, PA, and knee pain and dysfunction and LLM, LMS, and LMQ in community-dwelling older adults. Consistent with between-person findings, fluctuations in 25(OH)D, knee pain and dysfunction, and PA within an individual were related to variations in muscles in that individual. Importantly, this work builds on our knowledge from between-person analysis, as it demonstrates that within-person variability in these factors has independent effects on muscles. Specifically, having higher 25(OH)D and lower knee pain and dysfunction compared with an individual’s average was associated with a greater muscle strength and quality but not muscle mass. Furthermore, when participants engaged in more PA than their average level, they had a higher LMM, LMS, and LMQ.

Both between-person and within-person increases in PA were associated with a higher muscle mass, strength, and muscle quality. The within-person findings suggest that, irrespective of an individual’s usual level of PA, further increases in PA have additional beneficial effects on muscle mass, strength, and muscle quality. For instance, at time points when an individual (either with higher or lower 10-year average PA) increased their steps-per-day by 1000, that individual had a 0.56 kg increase in LMS. The effect of PA on muscles was stronger in the between-person analysis (eg, a 1000 steps/day increase resulted in a 1.25 kg increase in 10-year average LMS).

The discrepancy between the within- and between-person estimates may be because error variance associated with important individual differences is reduced in the within-person estimates compared with the between-person comparisons. For instance, differences such as genetic makeup, pain perception, and motivation to engage in PA, which are not captured in the between-person comparison, are held constant in the within-person comparison. Nonetheless, our previous study (9) and others (1,10) report that the pedometer-determined PA is associated with sarcopenia. Hence, PA has been recognized as one of the most feasible and inexpensive strategies to delay age-related loss of mass and function with clinical guidelines emphasizing PA to promote health including preventing incidence of sarcopenia in older people (20,21). We found that the magnitude of the associations of between-person increases in PA with LMQ was higher in women compared with men. This observation has been seen in some studies (9,22), but not in all previous studies (23). Although the underlying mechanism of this sex difference is unclear, one potential explanation may be that because women have smaller muscles, ambulatory activity stimulates a greater improvement in muscle quality in
women than men who may require a greater stimulus. This present study builds on previous knowledge by showing that increasing one's own PA above their "usual" level results in further increases in LLM, LMS, and LMQ.

Within-person increase in WOMAC global score and functional limitation subscale was associated with a lower muscle strength and quality, whereas between-person increase in WOMAC global score and knee pain, stiffness, and functional limitation subscales was associated with a lower muscle strength and quality. Muscle mass was not associated with WOMAC global score or the subscales in either between or within person analysis. Knee pain and dysfunction may lead to a lower muscle strength and muscle quality through activity limitation due to pain-related fear (24,25) or via reflex muscle inhibition (26,27). The significant within-person association suggests that at time points when the participants reported higher knee dysfunction, they may also have experienced greater activity limitations; thus, they had a weaker LMS and LMQ. It could also imply that the participants were less able to exert a maximal contraction due to actual or anticipated pain, which may explain why the decrease in muscle strength does not translate to a loss of muscle mass. Pain represents a major clinical symptom in older people and it is a predictor of transition towards sarcopenia (1,28). Yet, it is under-assessed and under-treated potentially due to the perception that pain is an unavoidable consequence of ageing (29). The within-person analysis emphasizes the importance of adequate pain management in older people, particularly on occasions when their pain is higher than their "usual" pain level, in order to prevent further loss of muscle strength and quality. Interestingly, the between-person association between knee pain and dysfunction and muscle parameters was stronger in women compared with men. This finding is consistent with our previous study (11) and may be due to the fact that women are less capable of generating force in the presence of pain during strength testing compared with men (30). Furthermore, the within-person association between pain and muscle measures was modified by age such that an increase in knee pain was associated with a higher loss of muscle strength and quality in the younger (50–69 years) compared to the older age category (70 years and above). The reason for this is unclear. Notably, the association of between-person and within-person variability in knee pain and dysfunction with muscle strength and quality is mostly independent of PA (Supplementary Table). This demonstrates that there are multiple pathways including direct inhibition of muscle function through which knee pain and dysfunction contribute to loss of muscle function (26,27).

The between-person findings between 25(OH)D and muscle mass, strength, and quality suggest that long-term maintenance of 25(OH)D is beneficial for muscles. These findings are supported by our previous work (7). Notably, the magnitude of the association of between-person difference in 25(OH)D and LLM was higher in men compared with women, which is potentially due to a higher PA in men. Furthermore, there was evidence for a dynamic within-person relationship between 25(OH)D and muscle strength and quality but not muscle mass. This suggests that, irrespective of their "usual" level of 25(OH)D, variability in 25(OH)D around an individual's mean value results in further improvements in muscle strength and quality but not muscle mass. Indeed, improvements in muscle strength do not necessarily overlap with an increase in mass as the two processes may be the results of different pathophysiological mechanisms (31). The within-person increase in muscle strength and quality but not muscle mass may be related to the role of vitamin D in muscle fiber neuromuscular junction (32). These findings highlight that vitamin D plays a role in age-related skeletal muscle changes and that further increases in 25(OH)D above an individual's average have additional benefit to muscle strength and muscle quality.

This study has a number of strengths including the use of a person-mean centering analysis approach, allowing us to disentangle the within-person and between-person effects of the predictor variables. This is particularly useful as we were able to show both between-person and within-person variability in 25(OH)D, PA, and knee pain and dysfunction were independently contributing to muscle mass, strength, and muscle quality. Another strength of this study is the use of objective measures of PA which likely increases the accuracy of our estimates. However, this study also has a number of limitations. Firstly, 47% of participants recruited at baseline were lost to follow up over 10 years. Such missing data are not unexpected in a long-term prospective study involving older people. The missing data were accommodated by using maximum likelihood estimation which uses available data for model estimation, rather than case-wise deletion. Secondly, although we hypothesized that higher knee pain and dysfunction would result in lower muscle mass, strength, and muscle quality, reverse causality is also possible where muscle weakness could lead to an increase in knee pain. Our study focused on the association of between-person and within-person variability in serum 25(OH)D and PA with LLM. However, it is possible that serum 25(OH)D and PA have a systemic effect on muscle mass and function. Unlike LLM, we found no evidence for an association of between-person (β = –0.04; 95% CI: 0.10, 0.02) and within-person (β = 0.01; 95% CI: –0.02, 0.04) increases in PA and ALM, potentially due to the large role the lower limbs play in mobility. Furthermore, we found no association between within-person (β = 0.0002; 95% CI: –0.004, 0.004) and between-person increases in 25(OH)D (β = –0.004; 95% CI: –0.01, 0.01) and ALM. The reason for this observation is unclear, but it is consistent with studies showing evidence for an association between vitamin D and specific muscle groups (33).

In conclusion, our findings demonstrate that both between-person and within-person fluctuations in 25(OH)D, pain and dysfunction, and PA were associated with muscle changes. The finding that within-person variation in PA levels was associated with within-person variation in muscle mass, strength, and quality is reassuring, and it adds a new perspective to public health efforts aimed at promoting PA in older people. It shows that increasing one's own ambulatory PA further increases muscle mass, strength, and quality.

**Supplementary Material**

Supplementary data is available at The Journals of Gerontology Series A: Biological Sciences and Medical Sciences online.

**Conflict of Interest**

None reported.

**References**

3. Balogun S, Winzenberg T, Wills K, et al. Prospective associations of low muscle mass and function with 10-year falls risk, incident fracture and...