Functional improvements to 6 months of physical activity are not related to changes in size or density of multiple lower-extremity muscles in mobility-limited older individuals

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ABSTRACT

Older adults are encouraged to engage in multicomponent physical activity, which includes aerobic and muscle-strengthening activities. The current work is an extension of the Vitality, Independence, and Vigor in the Elderly 2 (VIVE2) study – a 6-month multicenter, randomized, placebo-controlled trial of physical activity and nutritional supplementation in community dwelling 70-year-old seniors. Here, we examined whether the magnitude of changes in muscle size and quality differed between major lower-extremity muscle groups and related these changes to functional outcomes. We also examined whether daily vitamin-D-enriched protein supplementation could augment the response to structured physical activity. Forty-nine men and women (77 ± 5 years) performed brisk walking, muscle-strengthening exercises for the lower limbs, and balance training 3 times weekly for 6 months. Participants were randomized to daily intake of a nutritional supplement (20 g whey protein + 800 IU vitamin D), or a placebo. Muscle cross-sectional area (CSA) and radiological attenuation (RA) were assessed in 8 different muscle groups using single-slice CT scans of the hip, thigh, and calf at baseline and after the intervention. Walking speed and performance in the Short Physical Performance Battery (SPPB) were also measured. For both CSA and RA, there were muscle group × time interactions (P < 0.01). Significant increases in CSA were observed in 4 of the 8 muscles studied, namely the hip flexors (1.1 HU), hip adductors (0.9 HU), knee extensors (1.9%) and the hip adductors (2.8%). For RA, increases were observed in 4 of 8 muscle groups, namely the hip flexors (1.1 HU), hip adductors (0.9 HU), knee extensors (1.2 HU), and ankle dorsiflexors (0.8 HU). No additive effect of nutritional supplementation was observed. While walking speed (13%) and SPPB performance (38%) improved markedly, multivariate analysis showed that these changes were not associated with the changes in muscle CSA and RA after the intervention. We conclude that this type of multicomponent physical activity program results in significant improvements in physical function despite relatively small changes in muscle size and quality of some, but not all, of the measured lower extremity muscles involved in locomotion.

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1. Introduction

Age-related loss of muscle mass, physical- and muscular function (i.e., sarcopenia), is associated with hospitalization, morbidity, loss of independence and increased mortality, and carries a financial burden to society (Baumgartner et al., 1998; Janssen et al., 2004; Cruz-Jentoft et al., 2019; Bhasin et al., 2020). As resistance exercise is the best known strategy to increase or maintain muscle mass and function, current physical activity guidelines recommend that older people undertake at least two sessions per week of muscle-strengthening activities, in addition to aerobic exercise and balance training (Piercy et al., 2018; Bull et al., 2020). Against this background, we previously carried out the Vitality, Independence, and Vigor in the elderly 2 Study (VIVE2) – a 6-month multicenter, randomized, placebo-controlled trial of 70-year-old community dwelling seniors (Kirn et al., 2015). A structured multicomponent physical activity program was carried out complemented with a vitamin-D-enriched protein supplement or a placebo drink. The group-based physical activity intervention, which included walking, muscle-strengthening exercises for the major locomotor muscle groups using ankle weights, and balance activities, was designed to meet current physical activity guidelines while also being feasible for older mobility-limited individuals. After 6 months, thigh muscle cross-sectional area (CSA), walking speed, and physical function (short physical performance battery (SPPB)) had increased, with no additive effects from nutritional supplementation (Fielding et al., 2017; Englund et al., 2017). Muscle composition analysis revealed a greater reduction in muscle fat infiltration and a significant increase in normal density muscle and decrease in low density muscle in the thigh region in the group receiving the supplement (Englund et al., 2017). In the current study, we performed secondary analysis on the muscle-specific effects of this physical activity program across multiple lower-extremity muscles involved in locomotion. Given the non-standardized muscle involvement in the various exercises used in multicomponent physical activity, we hypothesized that the magnitude of muscle hypertrophy and strength gains would vary between key locomotor muscles. However, this is not necessarily a problem as improvements in physical function can occur independently of morphological adaptations. This is supported by reports showing that fat infiltration of the hip muscles, but not the thighs, is associated with variability in gait speed (Addison et al., 2014). Furthermore, intramuscular fat seems to increase more in the calves than in the thigh muscles during aging (Buford et al., 2012). This could be important for both balance and physical function in older people (Scott et al., 2015). Indeed, there is evidence that muscle mass and function follow different trajectories (Clark and Manini, 2012), and several changes within muscle fibers during aging, such as reduced calcium signaling, myosin concentration and mitochondrial function, could compromise muscle function without affecting muscle size (D’Antona et al., 2003; Tieland et al., 2018).

Collectively, it seems that different muscle groups respond differently to physical inactivity and aging, and not all changes affect physical function. As most previous research has focused on changes in whole-body physical fitness components and/or thigh muscle function, the specific effects of multicomponent physical activity interventions on several major locomotor muscle groups remain unexplored. Determining whether specific training adaptations observed in key locomotor muscles are related to changes in physical function could help refine the prescription of exercise activities for older adults at risk for disability.

We performed this secondary analysis using data obtained from the VIVE2 study to examine: i) whether multicomponent physical activity prescribed for community-dwelling seniors results in increases in muscle size and reductions in fat infiltration in multiple lower-extremity muscle groups, ii) whether the magnitude of these muscular adaptations differs between muscle groups, and iii) whether they are associated with improvements in physical function. A secondary objective was to address the question of whether daily protein- and vitamin-D-enriched nutritional supplementation could enhance adaptations to the multicomponent physical activity regime and whether the response was muscle-group specific. Although physical function data have been published for the entire cohort (Fielding et al., 2017), it was necessary to re-examine these data specifically for the Swedish cohort, as the CT measurements over the hip, thigh and calves that we relate to physical function were exclusive to this sub-cohort.

2. Methods

The general design has been described in detail elsewhere (Kirn et al., 2015). The VIVE2 study was a multicenter study performed in Sweden and the United States. In brief, men and women aged >70 years old were recruited and included in the study if they had serum 25(OH)D between 9 and 24 ng/ml, a short physical performance battery (SPPB) score ≤ 9, mini-mental state examination (MMSE) score ≥ 24, could complete a 400 m walk within 15 min without support, and were not participating in any structural physical activity for more than 30 min/week. The CT images on hip and calf muscle were performed only on the Swedish arm of the VIVE2 participants. Eleven individuals from the original Swedish cohort were excluded (one died, eight did not finish the study and two did not have complete image sets). Randomization was stratified for sex (and for study site in the main study), and separate blocks of randomization were used for women and men. Each randomization block included 10 subjects (5 to the nutritional supplement group and 5 to placebo). A research assistant with no direct contact with the study staff or participants administered the group assignments. A total of 55 individuals (n = 25 in the intervention group and n = 30 in the placebo group) had complete CT images. Of these individuals, 6 were considered non-adherent to the exercise regime (2 from the intervention group and 4 from the control group). Since our primary aim in this secondary analysis was to investigate the exercise effects on the different muscle groups, these individuals were removed from further analysis (Supplemental Fig. 1). Since all individuals were supposed to perform the exercise intervention, intention to treat analysis was not performed. After the removal of the 6 non-adherent individuals, statistical testing of baseline parameters was performed (Table 1). All participants provided written informed consent prior to inclusion, and the study was approved by the Regional Ethical Review Board in Uppsala, Sweden (Dnr 2012/154), and the Tufts University Health Science Institutional Review Board, and was performed in accordance with the Declaration of Helsinki.

Table 1

Baseline characteristics for the intervention group (nutritional supplement) and control group (placebo). Statistical testing using unpaired t-test between intervention (n = 23) and control group (n = 26). Data are mean (±SD).

<table>
<thead>
<tr>
<th></th>
<th>Intervention</th>
<th>Control</th>
<th>t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>77.4 (5.6)</td>
<td>77.1 (5.4)</td>
<td>0.86</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>82.0 (12.3)</td>
<td>80.1 (14.9)</td>
<td>0.62</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.4 (8.4)</td>
<td>167.0 (9.1)</td>
<td>0.09</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.9 (3.3)</td>
<td>28.7 (4.7)</td>
<td>0.48</td>
</tr>
<tr>
<td>Medical diagnoses</td>
<td>0.3 (0.7)</td>
<td>0.7 (1.4)</td>
<td>0.23</td>
</tr>
<tr>
<td>Prescriptions (no.)</td>
<td>1.0 (1.9)</td>
<td>1.9 (3.2)</td>
<td>0.25</td>
</tr>
<tr>
<td>Adherence</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>SPPB (score)</td>
<td>8.0 (1.1)</td>
<td>7.9 (1.5)</td>
<td>0.68</td>
</tr>
<tr>
<td>MMSE (score)</td>
<td>27.5 (1.9)</td>
<td>27.7 (1.7)</td>
<td>0.64</td>
</tr>
<tr>
<td>Vitamin D (ng/ml)</td>
<td>20.0 (6.4)</td>
<td>18.8 (5.6)</td>
<td>0.51</td>
</tr>
<tr>
<td>Walking speed (m/s)</td>
<td>0.95 (0.18)</td>
<td>0.94 (0.18)</td>
<td>0.94</td>
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<tr>
<td>Extensor torque (Nm)</td>
<td>120.4 (34.0)</td>
<td>139.1 (47.2)</td>
<td>0.14</td>
</tr>
<tr>
<td>Flexor torque (Nm)</td>
<td>48.3 (12.9)</td>
<td>50.4 (17.0)</td>
<td>0.69</td>
</tr>
<tr>
<td>Handgrip (kg)</td>
<td>25.5 (7.5)</td>
<td>26.5 (10.0)</td>
<td>0.72</td>
</tr>
<tr>
<td>Stair climb (s)</td>
<td>5.4 (1.2)</td>
<td>5.1 (1.3)</td>
<td>0.44</td>
</tr>
</tbody>
</table>
2.1. Multicomponent physical activity program

The physical activity intervention was group-based and included flexibility, balance, lower extremity strength and aerobic exercise (brisk walking), three times a week for 6 months. The duration was approximately 60 min, including warm-up, 30 min aerobic exercise, 20 min muscle-strengthening exercises, and then cool down. The warm-up and cool-down periods included balance and flexibility exercises. During the brisk walking, the participants were asked to walk at a rating of perceived exertion (6–20 Borg RPE scale, (Borg, 1970)) of 13 (somewhat hard), which is considered moderate intensity exercise in the current physical activity guidelines (Piercy et al., 2018; Bull et al., 2020). An exercise intensity below 11 (fairly light) and over 15 (hard) was discouraged. Muscle-strengthening exercises were focused on the lower extremities (using ankle weights), including side hip raises, chair rises, knee extensions, knee flexion and calf rises. Two sets of 10 repetitions for each exercise were conducted at an intensity of 15–16 (Borg Scale), with progressive increases in absolute loads during the intervention period. Physical activity outside of the group was encouraged, with an overall goal to meet the recommendation of 150 min of moderate intensity physical activity a week. If the participants attended at least 60% of the group sessions, they were considered adherent to the intervention.

2.2. Nutritional supplementation

All participants were randomized to daily intake of a 119 ml nutritional beverage containing 150 kcal, 800 IU of vitamin D, 20 g of whey protein, 350 mg of calcium, and other vitamins and minerals, or a placebo drink of 119 ml containing 30 kcal per serving. The beverage was taken once every day for the entire 6 months. Both drinks were provided by Nestle Health Science, Vevey, Switzerland. Each participant recorded their intake in paper diaries. At least 60% of the drinks had to be consumed for a participant to be considered adherent.

2.3. CT scans

The procedure to obtain images has been described in detail earlier (Rasch et al., 2007). In brief, axial bilateral images with 5 mm thickness were obtained to assess muscle CSA (mm²) and RA (Hounsfield units, HU) at three different levels: hips, thighs, and calves (Fig. 1). The images were obtained using a spiral CT scanner (General Electric Medical Systems, London, UK). To avoid influence from fluid shifts on muscle size, all scans were performed after 30–60 min of supine rest (Berg et al., 1993). Straps around the feet were applied to standardize limb position. The hip image was obtained at the upper part of foramen ischiadicum (the first image where the opening between sacrum and os. ischium is seen). Principal hip extensors (Gluteus maximus), principal hip abductors (Gluteus medius + minimus) and hip flexors (iliopsoas) were assessed. The thigh section was located 20 cm proximal to the knee joint. Here, three different muscle groups were assessed: knee extensors (vastus lateralis, vastus medialis, vastus intermedius and rectus femoris), knee flexors (biceps femoris caput longum, semimembranosus and semitendinosus), and hip adductors (gracilis, adductor magnus and adductor longus). The calf section was localized 13 cm distal of the knee joint. Here, ankle plantar flexors (gastrocnemius, soleus, tibialis posterior, flexor digitorium longus and flexor hallucis longus), and ankle dorsiflexors (tibialis anterior, extensor hallucis longus, extensor digitorium longus and peroneus) were measured. To divide the posterior from the anterior muscle group, a line was drawn from the anterior edge of fibula to tibia. Pre and post scans were analyzed (Image J, NIH, Bethesda, US) side by side in a blinded manner. All muscle groups were manually circled around the muscle border as previously described (Rasch et al., 2007; Aubrey et al., 2014) and as shown in Fig. 1. The mean CSA and RA of the right and left sides were used for the statistical analysis. The inter-rater reliability of our method was recently found to be high, and also to correlate well with semi-automated thresholded method using pre-set HU to define muscle (Berg et al., 2020).

2.4. Functional measurements

Gait function was assessed by walking speed, measuring the average walking speed (m/s) during a 400 m walk (for reference values see (Vestergaard et al., 2009)), stair climb (10 sets of stairs as fast as possible). The Short Physical Performance Battery (SPPB) including balance testing, gait speed and chair stand (Guralnik et al., 1994) were assessed to characterize physical function as described earlier (Kirn et al., 2015; Fielding et al., 2017). One individual had missing scores for walking speed.

2.5. Muscle strength

Knee extensor and knee flexor maximal isometric torque were measured at baseline and after 6 months in both legs using a Biodex System 3 Isokinetic Dynamometer (Biodex Medical Systems, Shirley, NY). Maximal force was applied for 5 s with 30 s rest in between, alternating between extension and flexion. The highest maximal isometric peak torque generated over three attempts was recorded for each leg. Peak torque was expressed as the average of the right and the left leg. For the torque measurement, two participants from the control group had missing scores. Grip strength was measured as reported previously (Kirn et al., 2015, Fielding et al., 2017). Two individuals had missing values for grip strength.

2.6. Statistics

The statistical analysis was performed in R studio version 1.1.442.

Fig. 1. Example images of the axial sections analyzed. To the left, a section from the hip at the upper part of foramen ischiadicum (the first image where the opening between sacrum and os. ischium is seen). Here, three different muscle groups were assessed: knee extensors (vastus lateralis, vastus medialis, vastus intermedius and rectus femoris), knee flexors (biceps femoris caput longum, semimembranosus and semitendinosus), and hip adductors (gracilis, adductor magnus and adductor longus). The calf section was localized 13 cm distal of the knee joint. Here, ankle plantar flexors (gastrocnemius, soleus, tibialis posterior, flexor digitorium longus and flexor hallucis longus), and ankle dorsiflexors (tibialis anterior, extensor hallucis longus, extensor digitorium longus and peroneus) were measured. To divide the posterior from the anterior muscle group, a line was drawn from the anterior edge of fibula to tibia. Pre and post scans were analyzed (Image J, NIH, Bethesda, US) side by side in a blinded manner. All muscle groups were manually circled around the muscle border as previously described (Rasch et al., 2007; Aubrey et al., 2014) and as shown in Fig. 1. The mean CSA and RA of the right and left sides were used for the statistical analysis. The inter-rater reliability of our method was recently found to be high, and also to correlate well with semi-automated thresholded method using pre-set HU to define muscle (Berg et al., 2020).
Unpaired t-test was performed to compare baseline characteristics between the two groups. Three-way ANOVA with CSA and RA as dependent variables, time and muscle group as independent within variables, and group (nutrition or placebo) as the independent between variable, was performed. To follow up on significant interactions, a paired two-tailed t-test was used. The false discovery rate procedure was used to compensate for multiple comparisons of the different muscle groups. Paired two tailed t-tests were used to compare muscle torque and physical function from pre to post intervention. An alpha level of 5% ($P < 0.05$) was considered significant. Since the effect of the nutritional supplement already had been investigated in the whole study population in relation to isometric muscle torque and physical function, we did not re-analyze the effect of the nutritional supplement on these variables in the current study.

In order to explore the effect of the exercise-intervention on muscle CSA, density and functional variables, we used Orthogonal Partial Least Squares (OPLS), which is similar to PCA although handles classification instead of correlation. OPLS regression is applicable when the X-independent variables are highly correlated and the matrix of predictors has more variables than observations. An OPLS Discriminant Analysis (OPLS-DA) classification model was constructed using the ropls-library in R (Thevenot et al., 2015). The contribution of each variable is represented by a loading value compared with a prediction, i.e., baseline or post intervention in the current study. An OPLS model locates the multidimensional direction in the X space that explains the maximal variance in the Y space. All data was mean centered and scaled to unit variance before analysis. To assess the validity of the model (Q2 value), bootstrapped cross-validation, yielding 95% confidence intervals for the contribution of each of the variables in the group classification was used. In the model, X represents the regressor variables (area, RA and functional outcome variables) and Y represents the response variable (Pre-and Post-intervention). Variable Important for Projection (VIP) was used to quantify the importance of the variables. VIP was normalized with the average squared VIP value of 1. VIP > 1 indicates that the variable is of importance for the projection, and values < 0.5 indicates that the variable is not important for the projection (Trygg, 2002). These analyses were performed in R version 3.5.3.

### 3. Results

#### 3.1. Baseline characteristics

There were no differences in baseline data in the adherent individuals, (including physical function and vitamin-D levels; Table 1). During the intervention, both groups had a small weight loss of 0.2 kg in the intervention group and 0.6 kg in the control group.

#### 3.2. Muscle size and radiological attenuation

For both CSA and RA, there were muscle group x time interactions ($p < 0.01$, after Greenhouse-Geisser correction $p < 0.05$), suggesting that the response to the intervention differed between muscle groups. Significant increases in CSA were seen in knee extensors (1.9%, $p < 0.01$) and hip adductors (2.8%, $p < 0.05$). For RA, significant increases were seen in hip flexors (1.1 HU; $p < 0.01$), hip adductors (0.9 HU; $p < 0.05$), knee extensors (1.2 HU; $p < 0.001$) and ankle dorsiflexors (0.8 HU; $p < 0.05$) (Fig. 2 and Table 2). There were no additive effects of nutritional supplementation on CSA or RA (all group x time interaction effects: $p > 0.05$).

#### 3.3. Functional measurements

Functional measurements have previously been published for the whole cohort (Fielding et al., 2017). In the current subpopulation of Swedish participants, and merged across the two groups, there were significant improvements in walking speed ($p < 0.001$, 13%), stair climbing time ($p < 0.01$, 6%) and in SPPB ($p < 0.001$, 38%).

#### 3.4. Muscle strength

There was an increased knee flexor (22%; $p < 0.001$) but not knee extensor strength (merged across the two groups). This is in line with the results for the entire VIVE2 cohort (Englund et al., 2017). Grip strength did not change over the intervention ($p = 0.9$).

Fig. 2. Mean changes (with 95% CI) for different muscle groups after 6 months of physical activity merged across the intervention group (nutritional supplement) and the control group (placebo).
daily intake of a daily protein- and vitamin D-enriched supplement. The VIVE2 cohort (United States and Sweden) (Englund et al., 2017) and this substudy extends the findings of our previous report on the entire tors, knee extensors, and ankle dorsiflexors, with no additive effects of individuals with low vitamin D levels. The physical activity program muscles of the hip, thigh, and calf in community-dwelling older in

dividuals with low vitamin D levels. The physical activity program

musc

Table 2
Cross-sectional area (mm$^2$) and radiological attenuation (HU) of the different muscle groups involved in locomotion (i.e., hip, thigh, and calf muscles), how individuals (Tiggemann et al., 2010). As these participants were also

dividuals (Tiggemann et al., 2010). As these participants were also

inactivity-induced changes in the most affected muscle compartments

in the elderly. There is limited information on the specific training responses of different muscle groups in the elderly. Knee extensors have been reported to atrophy more than posterior thigh muscles with age, which may suggest that the hypertrophic response in knee extensors, but not in flexors, reflects a more rapid recovery from inactivity-induced changes in the most affected muscle compartments (Brown et al., 1990; Sipila and Suominen, 1995; Frontera et al., 2008; Taaffe et al., 2009). The multicomponent physical activity program involved most major muscle groups at the hip, thigh, and calves. However, the observed changes were rather small also in the muscle groups that responded to training. This is in line with several other studies reporting low to moderate muscle hypertrophy of the thigh muscles following moderate-intensity physical activity in the elderly (Goodpaster et al., 2008; Gylling et al., 2020). Although the capacity to induce muscle hypertrophy appears to be impaired in sarcopenic individuals (Bao et al., 2020), there is still evidence at the meta-analysis level that very old individuals generally respond to resistance-type training (Beckwée et al., 2019). Importantly, the multicomponent physical activity program used in the present study was not primarily designed to maximize muscle hypertrophy. Rather, the goal was to test a feasible physical activity intervention that meets current physical activity guidelines (Piercy et al., 2018; Bull et al., 2020) and can be adhered to by community-dwelling seniors over time. Physical activity levels were increased in a structured manner as part of their normal life, and included both moderate intensity aerobic exercise and muscle-strengthening activities. The strategy used to guide participants was based on exercise intensity, with lower extremity strengthening exercises performed at an RPE of 15 to 16 according to the RPE Borg scale. It has been reported that sedentary individuals report a higher RPE for the same relative intensity (% of 1 RM) compared to more trained individuals (Tiggemann et al., 2010). As these participants were also other studies that typically report on the quadriceps muscle group. The response to the intervention varied between muscle groups, with only knee extensors and hip adductors showing a significant increase in CSA. While hypertrophy of individual muscles is typically detected as increased CSA, quantitative changes in contractile muscle mass can also be demonstrated by increased RA of CT images, indicating reduced fat infiltration and thus a greater relative proportion of contractile elements (Goodpaster et al., 2000; Goodpaster et al., 2001). Coherent with the changes in CSA, several, albeit not all, muscle groups of the hip, thigh, and calves responded to this physical activity program in terms of increased RA.

In contrast to our previous report (Englund et al., 2017) on the entire thigh muscle (i.e., including both knee extensors, flexors, and hip adductors), we found no increase in RA in the group that consumed a daily protein- and vitamin D-enriched supplement. There are several factors that may explain these slightly contradictory results, apart from the fact that the current sub-study of the Swedish arm of participants included less than half of the total cohort, which reduces the statistical power to detect small changes. First, the response to training could have differed because of differences in baseline levels of physical activity across the Swedish population. Second, in our previous report, muscles were divided into low- and high-density muscle, which is a somewhat different approach than the one used here where the attenuation was treated as a continuous variable. Third, in the main report, only muscles between 0 and 100 HU were included and intermuscular fat was measured separately, whereas in this study there were no HU limits. Fourth, in the current study, we measured the three main muscle compartments of the thigh separately to obtain a higher resolution of muscle-specific effects. Regardless of these differences in results and methodological approaches, it appears that any additive effect of protein/vitamin D supplementation on muscle-specific adaptations to physical activity in this well-nourished, community-dwelling population is very small.

The observation that not all muscle groups responded could reflect either differences in remodeling capacity or differences in muscle involvement in the training program. There is limited information on the specific training responses of different muscle groups in the elderly. Knee extensors have been reported to atrophy more than posterior thigh muscles with age, which may suggest that the hypertrophic response in knee extensors, but not in flexors, reflects a more rapid recovery from inactivity-induced changes in the most affected muscle compartments (Brown et al., 1990; Sipila and Suominen, 1995; Frontera et al., 2008; Taaffe et al., 2009). The multicomponent physical activity program involved most major muscle groups at the hip, thigh, and calves. However, the observed changes were rather small also in the muscle groups that responded to training. This is in line with several other studies reporting low to moderate muscle hypertrophy of the thigh muscles following moderate-intensity physical activity in the elderly (Goodpaster et al., 2008; Gylling et al., 2020). Although the capacity to induce muscle hypertrophy appears to be impaired in sarcopenic individuals (Bao et al., 2020), there is still evidence at the meta-analysis level that very old individuals generally respond to resistance-type training (Beckwée et al., 2019). Importantly, the multicomponent physical activity program used in the present study was not primarily designed to maximize muscle hypertrophy. Rather, the goal was to test a feasible physical activity intervention that meets current physical activity guidelines (Piercy et al., 2018; Bull et al., 2020) and can be adhered to by community-dwelling seniors over time. Physical activity levels were increased in a structured manner as part of their normal life, and included both moderate intensity aerobic exercise and muscle-strengthening activities. The strategy used to guide participants was based on exercise intensity, with lower extremity strengthening exercises performed at an RPE of 15 to 16 according to the RPE Borg scale. It has been reported that sedentary individuals report a higher RPE for the same relative intensity (% of 1 RM) compared to more trained individuals (Tiggemann et al., 2010). As these participants were also

<table>
<thead>
<tr>
<th>Muscle Group</th>
<th>Pre</th>
<th>Post</th>
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<tbody>
<tr>
<td>Area (mm$^2$)</td>
<td>Delta %</td>
<td>Radiological attenuation (HU)</td>
</tr>
<tr>
<td>Hip extensors Pre</td>
<td>3474.7</td>
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<tr>
<td>Post</td>
<td>3466.5</td>
<td>16.1 (14.7)</td>
</tr>
<tr>
<td>Hip abductors Pre</td>
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<td>-0.2</td>
</tr>
<tr>
<td>Post</td>
<td>4521.2</td>
<td>22.5 (14.5)</td>
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<tr>
<td>Hip flexors Pre</td>
<td>1365.6</td>
<td>1.6</td>
</tr>
<tr>
<td>Post</td>
<td>(368.2)</td>
<td>49.9 (5.9)</td>
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<tr>
<td>Knee extensors Pre</td>
<td>4631.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Post</td>
<td>(977.5)</td>
<td></td>
</tr>
<tr>
<td>Knee abductors Pre</td>
<td>4712.4</td>
<td>45.0 (6.8)</td>
</tr>
<tr>
<td>Post</td>
<td>(969.8)</td>
<td></td>
</tr>
<tr>
<td>Knee flexors Pre</td>
<td>2631.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Post</td>
<td>(581.3)</td>
<td></td>
</tr>
<tr>
<td>Hip adductors Post</td>
<td>1831.7</td>
<td>2.8</td>
</tr>
<tr>
<td>Post</td>
<td>(574.0)</td>
<td></td>
</tr>
<tr>
<td>Hip adductors Pre</td>
<td>1875.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Post</td>
<td>(578.9)</td>
<td></td>
</tr>
<tr>
<td>Ankle plantarflexors Pre</td>
<td>4260.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Post</td>
<td>(992.2)</td>
<td></td>
</tr>
<tr>
<td>Ankle plantarflexors Post</td>
<td>4273.2</td>
<td>40.2 (14.0)</td>
</tr>
<tr>
<td>Post</td>
<td>(988.5)</td>
<td></td>
</tr>
<tr>
<td>Ankle dorsiflexors Pre</td>
<td>1763.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Post</td>
<td>(316.6)</td>
<td></td>
</tr>
<tr>
<td>Ankle dorsiflexors Post</td>
<td>1770.3</td>
<td></td>
</tr>
<tr>
<td>Post</td>
<td>(324.7)</td>
<td></td>
</tr>
</tbody>
</table>

3.5. CSA, RA and functional relationships

The OPLS model differentiating post from pre-exercise samples correctly classified 61% (R2Y = 0.61, p = 0.01) of the observations in the dataset, with a predictive (Q2 value) of 0.43 (p = 0.01) after 1000-fold cross-validation (Fig. 3). The model identified three variables that contributed significantly to the model (SPPB, walking speed and knee flexor torque) (Fig. 4). The results are summarized in Suppl. Table 1, along with the corresponding parametrically tested changes. The relationship between muscle CSA, RA, physical function and strength measures is shown in a correlation plot (Fig. 5).

4. Discussion

The current study examined the effects of a structured multicomponent physical activity program combined with nutritional supplementation on muscle size and density of several major locomotor muscles of the hip, thigh, and calf in community-dwelling older individuals with low vitamin D levels. The physical activity program resulted in small but significant increases in CSA of the knee extensors and thigh adductors and increases in RA of the hip flexors, hip adductors, knee extensors, and ankle dorsiflexors, with no additive effects of daily intake of a daily protein- and vitamin D-enriched supplement. The strongest contributors to the intervention effect were improvements in SPPB, 400 m walking speed, and knee flexion torque, and these changes were largely unrelated to muscle-specific changes in CSA and RA.

By examining the response of 8 different muscle groups covering the major joints involved in locomotion (i.e., hip, thigh, and calf muscles), this study extends the findings of our previous report on the entire VIVE2 cohort (United States and Sweden) (Englund et al., 2017) and
It is possible that the intensity and effort level achieved was too low to elicit an optimal hypertrophy stimulus.

Another possible explanation for the observed small effects on muscle growth could be age-related attenuation of muscle plasticity (Petrella et al., 2006; Slivka et al., 2008; Rau et al., 2009; Rivas et al., 2014; Lee et al., 2019; Karlsen et al., 2019; Skoglund et al., 2020). Although it is known that muscle adaptations to exercise can occur in advanced age, and even if they are smaller compared to young healthy individuals, they still seem to be specific to the exercise load, type of exercise and physical function at baseline (Frontera et al., 1988; Sipila and Suominen, 1995; Harridge et al., 1999; Ferri et al., 2003; Wernbom et al., 2007; Rau et al., 2009; Aas et al., 2020). Karlsen et al. recently reported that 12 weeks of heavy resistance training in very old men and women (age 83–94) had no effect on type II fiber area, satellite cell content, or myonuclear density, supporting the observation of reduced plasticity in advanced age (Karlsen et al., 2019; Lundberg and Gustafsson, 2019). Physical function, as determined by 400-m walking speed and the SPPB test (Fielding et al., 2017), improved markedly in response to the intervention. Furthermore, in line with our previous publication (Englund et al., 2017), a significant increase peak torque of the knee flexors, but not the knee extensors, was observed in the Swedish subgroup of VIVE2 participants. Yet, there was no significant effect on flexor CSA or RA after correction for multiple analysis, even though the strength increased. There is ample evidence that changes in muscle mass and strength are not always correlated and may even follow different trajectories as we age (Clark and Manini, 2012; Loenneke et al., 2019; Aas et al., 2019). This is also consistent with the rather small changes in CSA observed in in the current cohort in combination with increased functional capacity. This clearly supports the idea that other mechanisms such as neural adaptations, improved metabolic functions and changes in the contractile machinery are important for improving physical function in the elderly (Hakkinen et al., 2000; Biolo et al., 2014). Indeed, task-specific exercise can be used as an adjunct to traditionally recommended exercise programs to improve physical function and activities of daily living (Buford et al., 2014).

In further support of the notion that physical function is improved by multicomponent physical activity independent of changes in muscle size and density of the major lower extremity locomotor muscles, multivariate analysis revealed that improvements in SPPB, walking speed, and knee flexor torque were the most important factors that differentiated the pre- and the post-study outcomes, whereas muscle-specific changes in RA and CSA had minimal impact on the model. This observation may have implications for the design of feasible physical activity programs for the elderly population, as it highlights the importance of targeting physical function and specific tasks related to daily activities, rather than specific isolated muscle groups. However, it should be acknowledged that muscle-specific adaptations such as increased muscle size and density may also have other beneficial effects, such as improved insulin sensitivity and lipoprotein metabolism, as well as improved immune function and reduced low-grade inflammation (Hughes et al., 1993; Gordon et al., 2014; McGee and Hargreaves, 2020; Bautmans et al., 2021). None of these factors were measured in the current study.

In summary, our results show that a 6-month, multicomponent physical activity program deemed feasible for community-dwelling, mobility-limited, it is possible that the intensity and effort level achieved was too low to elicit an optimal hypertrophy stimulus.
Fig. 4. Variable Important for Projection (VIP). VIP > 1 indicates that the variable is important for the projection.
mobility-limited individuals results in significant functional improvements but moderate increases in muscle size and reductions in fat infiltration in several, but not all, lower-extremity muscles involved in locomotion. Of note, the large functional improvements were not related to the changes in muscle size and density and were not augmented by protein- and vitamin D-enriched nutritional supplementation. It therefore seems plausible that processes other than skeletal muscle remodeling, such as task-specific neural, contractile, and metabolic adaptations, contributed to the functional improvement observed in this physical activity program. This may have implications for the design of physical activity programs for the elderly population.

CRediT authorship contribution statement


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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2021.111631.

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