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1 **Effects of different strength training frequencies on maximum strength, body composition**
2 **and functional capacity in healthy older individuals**

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25 **ABSTRACT**

26 There is controversy in the literature regarding the dose-response relationship of strength
27 training in healthy older participants. The present study determined training frequency
28 effects on maximum strength, muscle mass and functional capacity over 6 months following
29 an initial 3-month preparatory strength training period. One-hundred and six 64-75 year old
30 volunteers were randomly assigned to one of four groups; performing strength training one
31 (EX1), two (EX2), or three (EX3) times per week and a non-training control (CON) group.
32 Whole-body strength training was performed using 2–5 sets and 4–12 repetitions per
33 exercise and 7–9 exercises per session. Before and after the intervention, maximum
34 dynamic leg press (1-RM) and isometric knee extensor and plantarflexor strength, body
35 composition and quadriceps cross-sectional area, as well as functional capacity (maximum
36 7.5m forward and backward walking speed, timed-up-and-go test, loaded 10-stair climb
37 test) were measured. All experimental groups increased leg press 1-RM more than CON
38 (EX1: 3±8%, EX2: 6±6%, EX3: 10±8%, CON: -3±6%, $P<0.05$) and EX3 improved more than EX1
39 ($P=0.007$) at month 9. Compared to CON, EX3 improved in backward walk ($P=0.047$) and EX1
40 in timed-up-and-go ($P=0.029$) tests. No significant changes occurred in body composition.
41 The present study found no evidence that higher training frequency would induce greater
42 benefit to maximum walking speed (i.e. functional capacity) despite a clear dose-response in
43 dynamic 1-RM strength, at least when predominantly using machine weight-training. It
44 appears that beneficial functional capacity improvements can be achieved through low
45 frequency training (i.e. 1–2 times per week) in previously untrained healthy older
46 participants.

47

48 **Keywords:** walking; timed-up-and-go; stair climb; muscle mass; aged men and women;
49 resistance exercise; lower limbs

50

51 **1 INTRODUCTION**

52 Strength training is a widely used method to combat the deleterious effects of aging and
53 age-related reduced physical activity on maximum strength, muscle mass and functional
54 capacity. There are many combinations of acute program variables (identified by Kraemer
55 and Ratamess 2004) that can influence the overall outcome of a strength-training program.
56 These variables are; the choice of exercise(s) and exercise order, number of sets/repetitions,
57 inter-set and inter-exercise rest interval, and the intensity of each exercise. The effects of
58 several of these variables on maximum strength and muscle mass development have been
59 examined over previous decades (e.g. Campos et al. 2002; Moss et al. 1997). But one
60 variable, training frequency, has received little attention (Steib et al. 2010). It is important to
61 be clear that training frequency in the present study is limited to whole-body strength
62 training (rather than split programs; training one specific muscle group per day) and the vast
63 majority of studies using training 2-3 times per week does not allow reviews/meta-analyses
64 to accurately determine the effects of different frequencies on outcome variables.

65

66 Nevertheless, physical activity guidelines from bodies such as the World Health Organization
67 and the American College of Sports Medicine recommend whole-body strength training for
68 healthy individuals above 65 years at a frequency of *at least* two times per week (Ratamess
69 et al. 2009; World Health Organization 2010). This is despite the little experimental evidence
70 to support such a recommendation regarding development of maximum strength or muscle
71 mass, and particularly functional capacity, in previously untrained healthy older individuals.

72 This is in contrast to the quite well-established evidence base to recommend progressive
73 loading and volume to promote achieving these desirable outcomes (Ratamess et al. 2009).

74

75 A seminal paper investigating training frequency (one versus two versus three times per
76 week) on improvements in maximum strength and functional capacity observed no
77 difference in improvements between groups (Taaffe et al. 1999). Also, a recent meta-
78 analysis showed no evidence of different strength improvements comparing frequencies of
79 one, two or three times per week (Silva et al. 2014). Maintenance of muscle mass is another
80 important consideration for older adults given its role in force production and also
81 metabolic regulation. However, to our knowledge, no study has investigated the effect of
82 training frequency on muscle hypertrophy in healthy older individuals. The effect of training
83 frequency on muscle hypertrophy would be pertinent to examine since most studies use
84 either two or three times per week, which has been shown to exert little difference
85 (Wernbom et al. 2007), but recent evidence suggests these frequencies are more beneficial
86 than one time per week (Schoenfeld et al. 2016), which does support the physical activity
87 guidelines.

88

89 One important methodological consideration when evaluating these studies is the existing
90 training status of the participants. All four original articles that we identified in the literature
91 investigating training frequency in healthy older individuals used previously untrained
92 participants (DiFrancisco-Donoghue et al. 2007; Farinetti et al. 2013; Padhila et al. 2015;
93 Taaffe et al. 1999). As it is known that untrained individuals respond more robustly to a
94 variety training protocols, the use of completely untrained participants may reduce any

95 potential to identify differences in adaptive responses in response to different training
96 frequencies.

97

98 Therefore, there is a need to further study the influence of training frequency on
99 improvements in maximum strength, muscle mass and functional capacity in healthy older
100 individuals that have undergone (some) strength training prior to separation into different
101 training frequencies. Consequently, the purpose of the present study was to determine
102 whether training frequency affects improvements in maximum strength, muscle mass and
103 functional capacity over a 6-month period following an initial 3-month low-intensity
104 preparatory strength training period.

105

106 2 MATERIALS AND METHODS

107 2.1 Participant recruitment and randomization

108 This study was the second arm of a randomized controlled trial (NCT02413112). Participants
109 were 64–75-year-old men and women. Exclusion criteria were; (1) regular aerobic exercise
110 (>180 min-week⁻¹), 2) any previous strength training experience, (3) Body Mass Index >37,
111 (4) serious cardiovascular disease or lower limb injuries/disease that may lead to
112 complications during exercise or affect the ability to perform testing and training, (5) use of
113 walking aids, (6) use of medication that affect the neuromuscular or endocrine systems, (7)
114 previous testosterone-altering treatment, and (8) smoking. Therefore, participants were
115 otherwise healthy apart from conditions such as Type II diabetes, high blood pressure,
116 and/or high cholesterol in several cases, were not frail or obese, were not engaged in
117 systematic fitness training, and were able to perform strength training with no restrictions.
118 While the participants did not engage in aerobic exercise, it was clear from the pre-study
119 interviews that typical ‘Nordic’ low-intensity physical activity (e.g. berry-picking, gardening,
120 forestry etc.) was part of their lifestyle – and may, in part, explain their largely healthy
121 condition despite not meeting recommended levels of physical activity (WHO 2010).

122

123 The recruitment process and exclusion of participants is shown in Figure 1. Prior to
124 physician assessment, advertisement letters were posted to 2000 65–75-year-old men and
125 women in the Jyväskylä region and potential participants registered to the study by
126 completing an online researcher-designed questionnaire (n=454). As part of the registration
127 questionnaire, potential participants were asked about their current and previous level of
128 physical activity, medical history including any current/ongoing/permanent conditions,
129 current and previous medications and also immediate family medical history. The

130 participants were blind to the purpose of these questions (i.e. to assess eligibility). After
131 assessing the eligibility of the registered individuals for lower limb injuries, skeletomuscular
132 diseases and physical activity levels, potential participants were invited to an information
133 session (n=148). Each participant was carefully informed of the study design and potential
134 risks before the study, after which they provided written consent and attended a physician's
135 examination (n=116). During the physician's examination, potential participants were
136 interviewed by the researchers to ensure that they were eligible to be included to the study.
137 After baseline testing, the participants (n=106) were allocated an identification number and
138 a computer-generated random number sequencer was used to allocate each participant
139 into one of four groups (Figure 1); training one (EX1), two (EX2), three (EX3) times per week
140 and non-training/wait control (CON).

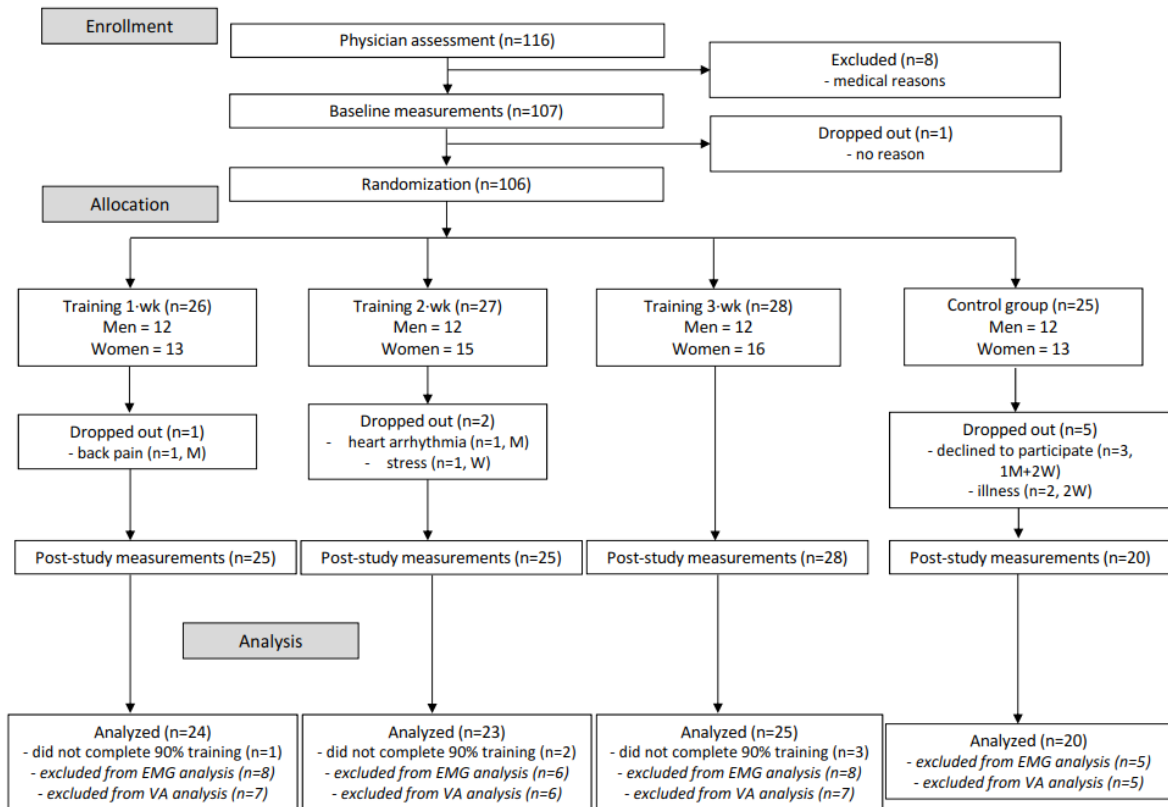
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142 During the study, one participant dropped out due to back pain induced by the strength
143 testing in month 3, one participant dropped out due to re-occurrence of heart arrhythmia
144 and one participant dropped out due to stress-related illness. Six participants failed to
145 attend at least 90% of the assigned training sessions for their group and were consequently
146 removed from the analyses (as noted in Figure 1). Furthermore, after data checking, several
147 participants' electromyography and voluntary activation level data were excluded from final
148 analysis due to technical faults. The study was conducted according to the Declaration of
149 Helsinki and was approved by the ethical committee of the University of Jyväskylä, Finland.
150 Baseline characteristics of the participants in each group are shown in Table 1, with the only
151 differences observed between men and women in height and body mass.

152

153 Some participants were taking medication during the study that was deemed not to
154 interfere with their ability to participate in training or testing. The total number users of
155 each type of medication are listed here; EX1: cholesterol medication (3 men + 3 women),
156 blood pressure medication (4 men + 3 women), blood glucose medication (1 men + 1
157 women), thyroid medication (1 men + 2 women), beta-blockers (1 woman); EX2: cholesterol
158 medication (2 men + 3 women), blood pressure medication (5 men + 6 women), blood
159 glucose medication (2 women), thyroid medication (1 man + 2 women), beta-blockers (1
160 man + 1 woman); EX3: cholesterol medication (1 man + 3 women), blood pressure
161 medication (5 men + 5 women), blood glucose medication (2 men), thyroid medication (1
162 man + 4 women), beta-blockers (1 man + 2 women); CON: cholesterol medication (2 men +
163 2 women), blood pressure medication (4 men + 3 women), beta-blockers (1 man + 1
164 woman).

165



166

167 **Fig.1.** Study flowchart from the point of physician assessment. M = men, W = women, EMG

168 = electromyography, VA = voluntary activation level.

169 Table 1. Participant characteristics and performance at baseline (mean±SD).

| | EX1 | EX2 | EX3 | Control |
|--------------------------------------|--------------|--------------|--------------|--------------|
| Sex (M/W) | 11/13 | 9/14 | 11/14 | 11/9 |
| Age (years) | 70 ± 3 | 69 ± 3 | 70 ± 3 | 69 ± 2 |
| Body mass (kg) | 76 ± 15 | 81 ± 15 | 82 ± 16 | 74 ± 11 |
| Height (m) | 1.67 ± 0.09 | 1.68 ± 0.07 | 1.67 ± 0.10 | 1.68 ± 0.09 |
| Body mass index (kg/m ²) | 27 ± 3 | 29 ± 5 | 29 ± 4 | 26 ± 3 |
| Fat mass (kg) | 25.9 ± 6.4 | 29.1 ± 9.3 | 28.3 ± 8.1 | 22.5 ± 6.5* |
| Fat-free mass (kg) | 47.0 ± 11.9 | 47.9 ± 10.6 | 48.7 ± 10.6 | 48.5 ± 10.1 |
| 1-RM load (kg) | 104.0 ± 34.7 | 115.4 ± 36.5 | 111.6 ± 37.0 | 119.5 ± 29.7 |
| KE MVC (Nm) | 153 ± 57 | 155 ± 49 | 147 ± 44 | 157 ± 45 |
| PF MVC (Nm) | 158 ± 61 | 159 ± 44 | 160 ± 40 | 160 ± 44 |
| Forward walk (s) | 3.1 ± 0.5 | 2.9 ± 0.6 | 3.0 ± 0.5 | 2.7 ± 0.4 |
| Backward walk (s) | 4.3 ± 1.2 | 4.2 ± 1.5 | 4.5 ± 1.4 | 3.7 ± 0.8 |
| TUG (s) | 9.4 ± 1.5 | 9.3 ± 1.7 | 9.4 ± 1.3 | 8.6 ± 0.8 |
| Stair climb (s) | 3.5 ± 0.9 | 3.4 ± 0.7 | 3.5 ± 0.7 | 3.1 ± 0.4 |

170 M = men, W = women, 1-RM = one-repetition maximum, KE MVC = maximum isometric knee
 171 extension torque, PF MVC = maximum isometric plantarflexion torque, TUG = timed-up-and-go. * =
 172 P=0.039 between Control and EX2.

173

174 **2.2 Dynamic leg press performance**

175 Concentric bilateral leg press one-repetition maximum (1-RM) load (kg) was used to assess
 176 maximum dynamic strength (David Sports Ltd, Helsinki, Finland). Briefly, following warm-up,
 177 single repetitions with increments of 5kg were performed until the participants could no
 178 longer fully extend their hips and legs (full extension = 180°). Each trial was separated by 1.5
 179 min. All data were relayed to a pc via an AD converter (Micro 1401, Cambridge Electronic
 180 Design, UK) and recorded using Signal 4.04 software (Cambridge Electronic Design, UK).
 181 Data was sampled at 2000Hz and filtered by a 10-Hz low-pass filter (fourth-order
 182 Butterworth) and the best trial was used in further analyses.

183

184 **2.3 Isometric knee extension and plantarflexion performance**

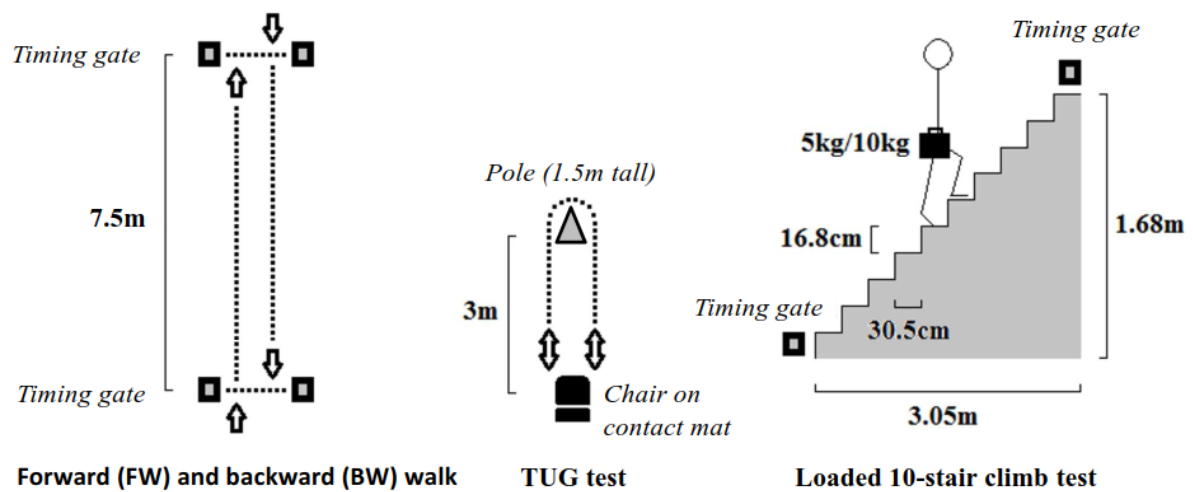
185 Maximum unilateral isometric knee extension torque of the right leg was measured using a
186 custom-built isometric force chair. Inelastic straps were used to secure the participant with
187 both hip and knee angles of 110°. Participants were instructed to kick “as fast and as hard as
188 possible” and maintain their maximum force for approximately 3s. The force signal was
189 sampled as described in the leg press trials with the highest force used in further analysis.
190 Three trials were performed with a fourth trial performed if improvements were more than
191 5%. Thereafter, two additional maximum isometric knee extension trials were performed
192 with femoral nerve stimulation delivered during the force plateau and 2s after contraction
193 cessation following similar procedures as Walker et al. (2014). Rectangular pulses (400V) of
194 200µs were delivered by a constant current stimulator (Model DS7AH, Digitimer Ltd, UK) to
195 the femoral nerve of the right leg through 5cm² self-adhesive electrodes (Polar Trode, Niva
196 Medical Ltd, Espoo, Finland) placed in the femoral triangle either side of the nerve, which
197 was identified by palpating and identifying the femoral artery. Current intensity was
198 gradually increased until no further increases were observed in peak-to-peak M-wave
199 amplitude of VL and VM. To ensure maximal activation, an additional 20% current was used
200 during subsequent stimulations. Single twitches were delivered in a resting condition to
201 determine peak-to-peak maximum M-wave amplitude. Single twitches were also delivered
202 about the maximum torque during isometric knee extension trials and 2s after contraction
203 cessation to determine voluntary activation level according to Merton’s (1954) interpolated
204 twitch technique. Maximum force was measured and then converted to torque by taking
205 into account the lever arm distance from the knee joint-center to the strain gauge.
206
207 Maximum bilateral isometric plantarflexion torque was assessed in a seated position by a
208 custom-built plantarflexion device with knees flexed to approximately 90° using similar

209 methods to Unhjem et al. (2015). The balls of the feet were positioned on a shelf connected
210 to the strain gauge (90° ankle joint-angle) and the knees were held in-place by a cushioned
211 board. Participants performed 3-4 isometric plantarflexion actions following the same
212 instructions as for the knee extension trials. Maximum force was measured and then
213 converted to torque by taking into account the lever arm distance from the ankle joint-
214 center to the strain gauge.

215

216 **2.4 Functional capacity**

217 Four maximal walking tests performed were included in the assessment of functional
218 capacity; (1) 7.5m forward walk, (2) 7.5m backward walk, (3) Timed Up-and-Go test (TUG),
219 and (4) loaded 10-stair climb test. Participants were instructed to perform the tests “as fast
220 as possible without compromising safety”. Each test was recorded by photocells except
221 TUG, which used a contact mat positioned under the chair to determine rise from and
222 return to the chair. The participants were not allowed to use their arms to assist in the chair
223 rise or return. During the 10-stair climb test, the participants carried one bag of 5kg
224 (women) or 10kg (men) and were instructed to maintain an extended elbow position and
225 prevent arm-swinging during the ascent (Figure 2). The best performance from two
226 acceptable trials was used in the analyses, and the sum result from both directions was used
227 for TUG.



228

229 **Fig.2.** Experimental set-up and procedures for the functional capacity tests. TUG = timed-up-
 230 and-go.

231

232 2.5 Quadriceps electromyography measurement and analysis

233 Electrode locations for electromyography (EMG) recordings were marked by indelible ink
 234 tattoo to allow accurate replacement during all test sessions. Bipolar Ag/AgCl electrodes
 235 (5mm diameter, 20mm inter-electrode distance, common mode rejection ratio >100dB,
 236 input impedance > 100MΩ, baseline noise <1μV rms) were positioned following shaving and
 237 skin abrasion on the vastus lateralis (VL) and medialis (VM) of the right leg according to
 238 SENIAM guidelines. Raw EMG signals were sampled at 2000Hz and amplified at a gain of 500
 239 (sampling bandwidth 10-500Hz). Raw signals were sent from a hip-mounted pack to a
 240 receiving box (Telemyo 2400R, Noraxon, Scottsdale, USA), then were relayed to an AD
 241 converter (Micro1401, Cambridge Electronic Design, UK) and recorded by Signal 4.04
 242 software (Cambridge Electronic Design, UK). Offline, EMG signals were band-pass filtered at
 243 20-350Hz and root mean square was obtained from approx. 65° to full leg extension (i.e.

244 180°) during 1-RM trials with values from VL and VM averaged $(VL+VM/2)$ from the best trial
245 and used in further analysis.

246

247 **2.6 Body composition**

248 Participants fasted overnight for 12 hours and were instructed to drink 0.5 liters of water 1
249 hour before measurements. After determination of height by a fixed wall-mounted scale,
250 participants underwent full body scanning by dual-energy X-ray absorptiometry (DXA) in
251 minimal clothing (LUNAR Prodigy Advance with encore software version 9.3, GE medical
252 systems, USA). The legs were separated by a polystyrene block and secured by inelastic
253 straps about the ankles. Total body fat mass and fat-free mass, as well as fat-free mass of
254 the legs was determined using software-generated analysis.

255

256 **2.7 Muscle cross-sectional area**

257 Muscle cross-sectional area (CSA) measurements of the right leg were taken 1-2 days prior
258 to dynamic leg press performance tests and 6-7 days after the final training session to
259 account for any exercise-induced swelling. CSA of the vastus lateralis, vastus intermedius,
260 gastrocnemius medialis and lateralis was assessed by B-mode axial-plane ultrasound (model
261 SSD- α 10, Aloka Co Ltd, Tokyo, Japan) using a 10 MHz linear-array probe (60 mm width)
262 coated with water-soluble transmission gel with the extended-field-of-view mode (23 Hz
263 sampling frequency). This method has been used during several of our training studies
264 (Walker et al. 2014, 2015) and has been shown to be valid (Ahtiainen et al. 2009). Indelible
265 ink tattoos on the medial and lateral sides of the target muscles ensures accurate
266 replacement of scanning track. Oriented in the axial-plane, the probe was moved manually
267 with a slow and continuous movement from medial to lateral along a marked line on the

268 skin. Great care was taken to diminish compression of the muscle tissue. Images were
269 obtained throughout the movement. As the orientation of each image relative to adjacent
270 images is known, the software builds a composite image. Four panoramic CSA images were
271 taken at; (1) 50% femur length from the lateral aspect of the distal diaphysis to the greater
272 trochanter and (2) 30% lower limb length from the lateral articular cleft between the femur
273 and tibia condyle to the lateral malleolus following the methods used by Rosenberg et al.
274 (2014). Upon visual inspection of the composite images three were selected to undergo
275 further analysis. CSA was determined by manually tracing along the border of each muscle
276 using Image-J software (version 1.37, National Institute of Health, USA). The mean of the
277 two closest values for each muscle were taken as the CSA result.

278

279 **2.8 Strength training program**

280 The experimental group performed whole-body strength training either one (EX1), two
281 (EX2) or three (EX3) times per week for 6 months on non-consecutive days and each session
282 was supervised by experienced gym instructors. The 6-month program was divided into two
283 3-month mesocycles (see supplementary material). All exercises were performed on
284 commercially available weight-stack equipment (Precor Vitality Series™, Precor Inc, UK)
285 apart from several free-weight exercises in mesocycle 2. The primary goal of mesocycle 1
286 was to increase maximum strength and muscle mass. The primary goal of mesocycle 2 was
287 to increase maximum strength and muscle activation/power. Intensity for all upper and
288 lower limb exercises was approximately 70–90% 1-RM with power training performed using
289 30-80% 1-RM loads. Multiple sets (2-5) were performed with repetition ranges of 4-12 and
290 inter-set rest of 1-3min depending on the training goal (loads used during training are
291 depicted in Figure 3). All participants were required to perform at least 1 set to concentric

292 failure (with the exception of power training). All participants were required to complete at
293 least 90% of all allocated training sessions prior to testing. Participants in the non-training
294 control group were instructed to maintain their normal physical activity throughout the
295 study period. All participants recorded their daily leisure-time physical activity levels in
296 diaries throughout the 6-month period and 3-day (including one weekend day) diet diaries
297 were collected during each mesocycle. The recording of physical activity followed
298 procedures of Waller et al. (2013).

299

300 **2.9 Statistical analyses**

301 All data are presented as means and standard deviations (\pm SD). All statistical methods were
302 performed using IBM SPSS statistics 24 software. The Kolmogorov-Smirnov test was used to
303 test normality and Levene's test was used to analyze homogeneity of variance. One-way
304 ANOVA was used to assess potential differences at baseline. Repeated measures analysis of
305 variance (ANOVA; 4 group \times 2 time) was used to determine significant time and time \times group
306 effects of the intervention. Bonferroni post hoc tests used to determine significant
307 differences within-group over time, while one-way ANOVA with Bonferroni post hoc tests
308 were used to determine whether the relative changes ($\Delta\%$) over time were different
309 between-groups. Effect sizes (Hedges' *g*) were calculated for the differences in relative
310 change between the experimental and control groups, where small (<0.3), medium (0.3 –
311 0.8), and large (>0.8) effect sizes were identified. Pearson's product moment correlation
312 coefficient was used to determine possible relationships between outcome measures.
313 Statistical significance was accepted when $P < 0.05$. Reliability for the performance measures
314 between the familiarization session and baseline measures were; 1-RM 0.97 and 5.5%, peak
315 power 0.94 and 11.2%, maximum isometric knee extension force 0.89 and 9.6%, maximum

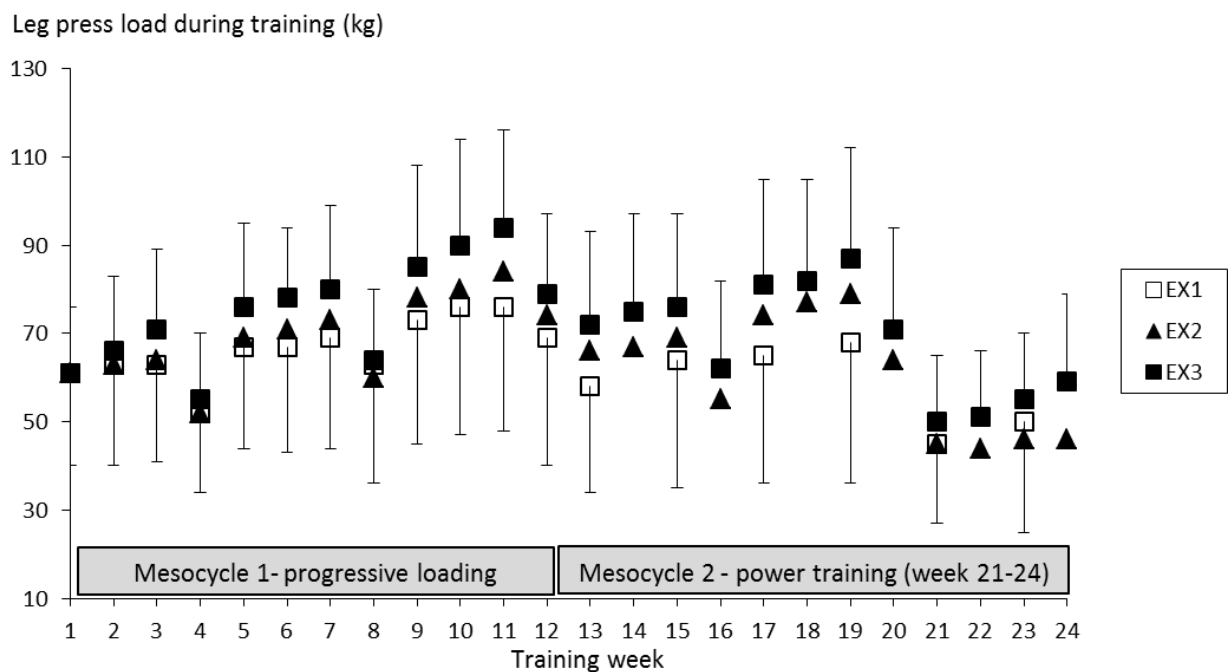
316 isometric plantarflexion force 0.87 and 9.7%, forward walk 0.82 and 6.3%, backward walk
317 0.81 and 8.3%, TUG 0.89 and 3.2%, 10-stair climb 0.96 and 3.2%, and CSA 0.94 and 4.2% for
318 Intra-class correlation coefficient (r) and coefficient of variation (%), respectively.

319

320 3 RESULTS

321 3.1 Loads used during the strength training intervention

322 All training loads used throughout the study are presented in the supplementary material
323 for the leg press, knee extension and chest/bench press exercises. Figure 3 shows the
324 highest loads used during each week for the leg press exercise in all training groups.



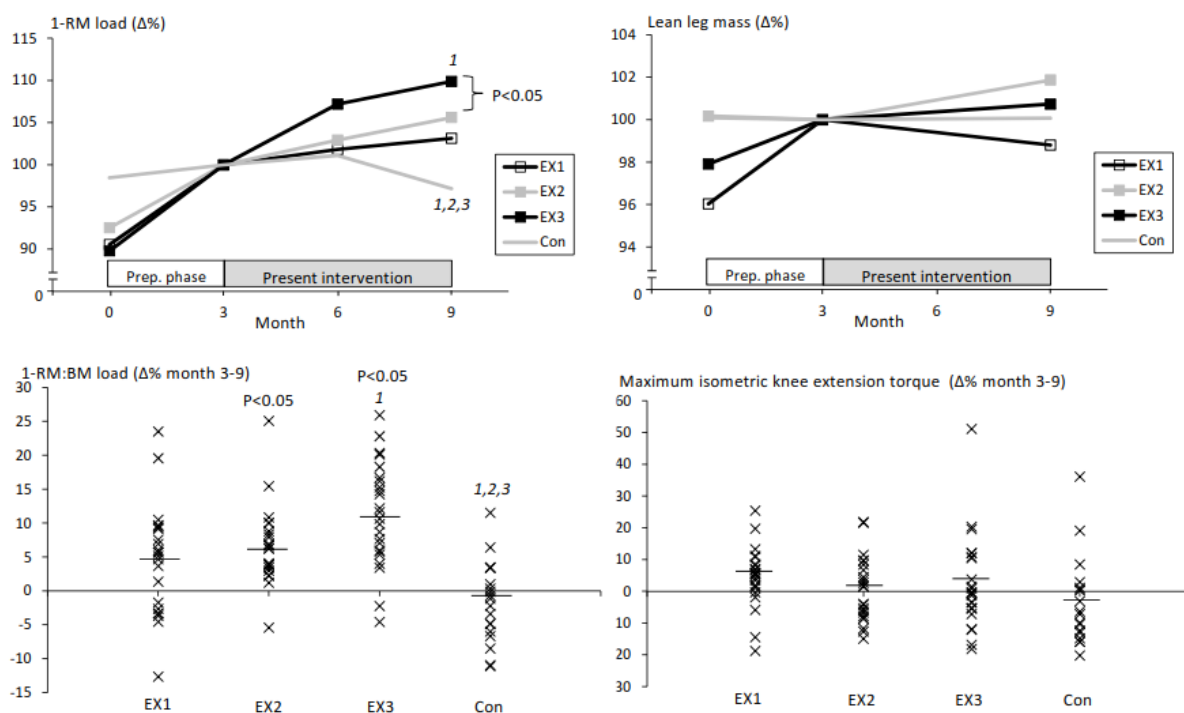
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326 **Fig.3.** Highest (weekly) load used during the leg press exercise (mean±SD). Every fourth
327 week was used as a 'tapering week' with lighter loads used. Progressive resistance training
328 was used during mesocycle 1, which began again at the beginning of mesocycle 2. The last
329 four weeks training was power training using loads of approx. 30–60% 1-RM.

330

331 **3.2 Neuromuscular performance and muscle activation**

332 Statistically significant main effects for time ($F=25.8$, $P<0.001$) and time \times group interaction
 333 ($F=12.7$, $P<0.001$) were observed in leg press 1-RM, where post hoc tests revealed that EX2
 334 ($6\pm 6\%$, $P<0.001$) and EX3 ($10\pm 8\%$, $P<0.001$) increased strength significantly over the
 335 intervention period. At month 9, between-group differences were observed for all
 336 experimental groups versus control and between EX3 and EX1 ($P=0.007$, 95% confidence
 337 interval=1.3 to 12.4%, $g=0.89$). The same results were observed when 1-RM was expressed
 338 relative to BM (Figure 4). There were no significant (time \times group) main effects for maximum
 339 isometric knee extension (Figure 4) or plantarflexion.



340

341 **Fig.4.** Changes in maximum strength and muscle mass for each group during the study. 1-
 342 RM load (top left), lean leg mass (top right), 1-RM normalized to body mass (bottom left),
 343 maximum isometric knee extension torque (bottom right). In the bottom panels, each
 344 participant's data is marked by an x and the line indicates the group mean. Significant
 345 within-group differences are marked with $p<0.05$, significant between-group differences are

346 marked above the data points with the number corresponding to the group to which it
347 differs.

348

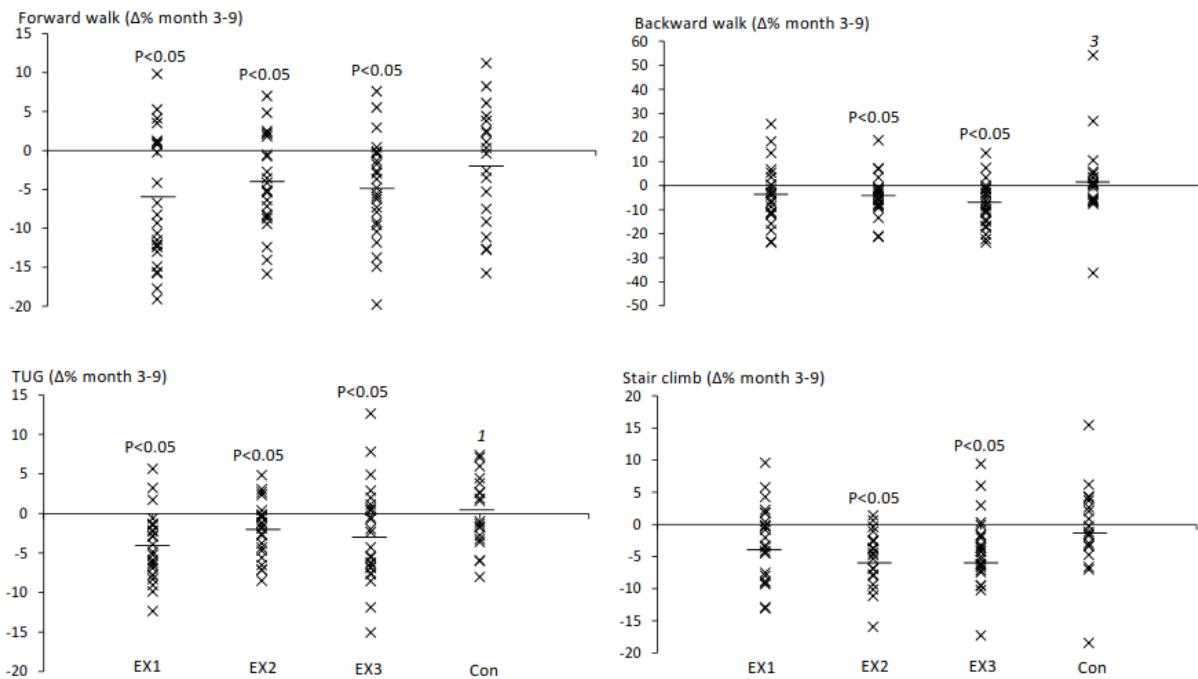
349 Statistically significant main effects for time ($F=40.8$, $P<0.001$) and time \times group interaction
350 ($F=4.4$, $P=0.007$) were observed in muscle activity of the quadriceps (VL+VM/2) during leg
351 press 1-RM, where post hoc tests revealed that all experimental groups increased
352 significantly over the intervention period (EX1: $25\pm 29\%$, $P=0.003$; EX2: $31\pm 37\%$, $P=0.002$;
353 EX3: $43\pm 31\%$, $P<0.001$). Between-group differences were observed for EX3 and control
354 ($P=0.005$, 95% confidence interval= 7.9 to 63.2% , $g=1.18$). However, once normalised to
355 maximum M-wave amplitude there were no significant main effects, and within-group
356 changes were no longer statistically significant. Furthermore, there were no significant main
357 effects for voluntary activation level assessed by the twitch interpolation technique during
358 isometric knee extension trials.

359

360 **3.3 Functional capacity**

361 Statistically significant main effects for time and trends for time \times group interaction were
362 observed for backward walk ($F=9.9$, $P=0.002$, and $F=2.7$, $P=0.053$, respectively), TUG ($F=19.9$,
363 $P<0.001$, and $F=2.6$, $P=0.056$, respectively) and loaded 10-stair climb ($F=26.8$, $P<0.001$, and
364 $F=2.7$, $P=0.051$, respectively). A significant main effect for time ($F=35.9$, $P<0.001$) was
365 observed for forward walk. From month 3 to 9, EX1 improved forward walk ($-7\pm 8\%$,
366 $P=0.002$) and TUG ($-4\pm 4\%$, $P<0.001$) performance only. EX2 and EX3 improved performance
367 in all functional capacity tests from month 3 to 9; forward walk (EX2: $-4\pm 6\%$, $P<0.001$; EX3: -
368 $5\pm 6\%$, $P=0.001$), backward walk (EX2: $-4\pm 8\%$, $P=0.02$; EX3: $-8\pm 9\%$, $P=0.001$), TUG (EX2: -
369 $2\pm 3\%$, $P=0.011$; EX3: $-3\pm 6\%$, $P=0.033$) and loaded 10-stair climb (EX2: $-5\pm 4\%$, $P<0.001$; EX3: -

370 4±5%, P=0.001). At month 9, between-group differences were observed for EX3 and control
 371 in backward walk (P=0.047, 95% confidence interval=-19.7 to -0.1%, g=0.72, Figure 5) and
 372 EX1 versus control in TUG (P=0.029, 95% confidence interval=-8.2 to -0.3%, g=0.95, Figure
 373 5).



374
 375 **Fig.5.** Changes in time to complete all functional capacity tests from month 3 to 9. 7.5m
 376 forward walk (top left), 7.5m backward walk (top right), timed-up-and-go test (bottom left),
 377 loaded 10-stair climb (bottom right). Each participant's data is marked by an x and the line
 378 indicates the group mean. Significant within-group differences are marked with p<0.05,
 379 significant between-group differences are marked above the control group's data points
 380 with the number corresponding to the experimental group to which it differs.

381

382 3.4 Body composition and muscle mass

383 There were no significant main effects for total fat mass, total lean mass or lean leg mass
 384 (Figure 4), or CSA for any muscle assessed.

385

386 **3.5 Habitual physical activity and nutritional intake**

387 Habitual physical activity external to the prescribed intervention was similar between all
388 groups (average minutes per week = EX1: 136±125, EX2: 121±110, EX3: 108±109, control:
389 172±69). Walking was the most common mode of physical activity, and this did not differ
390 between groups either in total time or in proportion of total physical activity (EX1: 107±122,
391 EX2: 80±70, EX3: 65±77, control: 122±65 min·week⁻¹; EX1: 69±33, EX2: 70±32, EX3: 60±36,
392 control: 70±26 % of total physical activity).

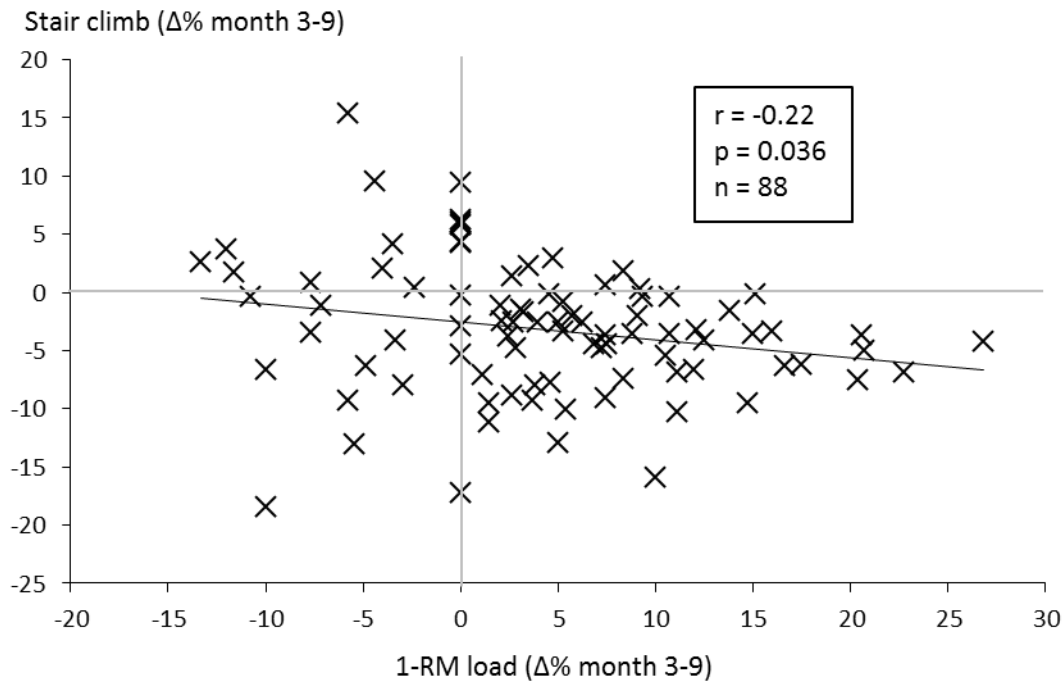
393

394 Results from the 3d diet diaries showed that there were no statistically significant
395 differences between any group for total carbohydrate (EX1: 218±60, EX2: 220±81, EX3:
396 205±74, control: 213±61 g), protein (EX1: 88±23, EX2: 89±25, EX3: 82±21, control: 86±35 g)
397 or fat (EX1: 75±24, EX2: 77±27, EX3: 84±20, control: 84±43 g) intake or when these totals
398 were normalized to body mass (e.g. protein g·kg⁻¹ = EX1: 1.2±0.3, EX2 1.2±0.3, EX3: 1.1±0.2,
399 control: 1.2±0.4).

400

401 **3.6 Correlation analyses**

402 All neuromuscular performance and functional capacity outcome measure change scores
403 were assessed to determine bivariate correlation. The only variable-pair to demonstrate
404 statistical significance was the change in leg press 1-RM and the change in loaded 10-stair
405 climb from month 3 to 9 ($r=-0.22$, $p=0.036$, $n=88$, Figure 6). There were no relationships
406 between habitual physical activity level and **change in** neuromuscular performance or
407 functional capacity.



408

409 **Fig.6.** Relationship between the change in 1-RM load (i.e. maximum dynamic strength) and
 410 the change in loaded 10-stair climb time from month 3 to 9. Each participant's data is
 411 marked by an x.

412

413 4 DISCUSSION

414 The main findings of the present study were that; 1) training frequency influenced
 415 maximum 1-RM gain in a dose-dependent manner but did not result in improved strength as
 416 measured by maximum isometric unilateral knee extension or isometric seated
 417 plantarflexion torque, 2) all experimental groups improved in some or all tests of functional
 418 capacity, but higher training frequency did not provide additional benefit, and 3) the present
 419 study did not observe any changes in body composition or muscle mass nor observe
 420 differences in muscle activity that might explain possible differences in 1-RM performance.

421

422 Taaffe and colleagues (1999) were the first to study the effects of training frequency in older
423 individuals (one versus two versus three times per week). In all 8 exercises included in their
424 study, equivalent improvements were made in 1-RM using a program of 3 sets at 80% of 1-
425 RM for 24 weeks. Furthermore, equivalent improvements were made in a chair sit-to-stand
426 test, while improvements in a 6m backward walking test (that approached statistical
427 significance) also displayed no between-group differences. The results of the present study
428 are in contrast to those of Taaffe et al. (1999) for 1-RM development but are similar
429 regarding functional capacity tests. One factor for the conflicting 1-RM data may have been
430 that the participants in the present study had undergone a 3-month preparatory strength
431 training period where 1-RM had already increased approximately 12% (unpublished data),
432 and so this would reduce the potential for further improvement in EX1 and EX2.
433 Interestingly, reducing training frequency from two to one time per week did not adversely
434 affect the gain in 1-RM strength in older women (Walker et al. 2017). When investigating
435 maximum strength only, DiFrancisco-Donoghue et al. (2007) did not observe different 1-RM
436 gain when training with a frequency of one or two times per week and, with the exception
437 of the chest press exercise, Padhila et al. (2015) did not observe differences in 1-RM gain
438 with two or three times per week. Therefore, it seems that there is potential little difference
439 between training frequencies in the magnitude of maximum strength gain over the first few
440 months of initiating strength training in older people.

441

442 The only study that we are aware of to show clear differences between training frequencies
443 was a study by Farinetti et al. (2013). Using a training-specific (but not maximum strength
444 test *per se*) a dose-response was observed for 10-RM improvement in the knee extension
445 and bicep curl exercises (3x > 2x > 1x) but not for the bench press or calf raise. While TUG

446 performance was not influenced by training frequency, chair sit-to-stand and 2m walking
447 speed improved more after two and three times compared to one time per week (Farinetti
448 et al. 2013). In comparison with the present study's results, the (shorter distance) 2m
449 walking speed test was in contrast but the TUG data were similar.

450

451 Overall, studies that have investigated the effect of training frequency on strength
452 improvement have produced conflicting results and we are unable to determine a definitive
453 conclusion. The majority of findings suggest that simply the initiation of strength training
454 itself is the main factor for improved functional capacity, rather than any single program
455 design variable. Also, it is interesting that the magnitude of strength improvement seems
456 not to be a major factor and this should be investigated in more detail in future.

457 Consequently, the underlying adaptations leading to improved functional capacity (i.e.
458 walking ability and chair rise), as well as main training program variable(s) to achieve these
459 adaptations are yet to be elicited.

460

461 One methodological consideration that may influence findings of intervention studies is the
462 choice of strength performance test. As Buckner and colleagues (2016) noted, strength
463 training for maximum lifts (i.e. 1-RM) comprises an element of (task-specific) skill. This may,
464 in some cases, falsely represent functionally relevant increases in strength. As observed in
465 the present study, there was a linear dose-response relationship between training
466 frequency and improved 1-RM performance but this difference was diminished when
467 testing isometrically. It should be noted that, at least in the present study, training was
468 conducted bilaterally using (predominantly) weight-stack devices whereas the isometric
469 knee extension action was tested unilaterally. This non training-specific test in itself would

470 reduce the likelihood of observing training-induced improvements (Abernethy & Jurimäe
471 1996; Baker et al. 1994). Similar observations have been made when training dynamically
472 and testing isometrically when investigating the effect of training frequency on back
473 extension in older adults (Graves et al. 1990) and even when comparing low- to high-
474 intensity strength training (Mitchell et al. 2012; Van Roie et al. 2013). Nevertheless, overall it
475 could be proposed that the small-to-moderate differences in improved 1-RM between
476 training frequencies observed in various studies may be a consequence of greater practice
477 rather than 'true' strength gain.

478

479 The exact cause of disparity in 1-RM improvement between groups is difficult to discern. All
480 experimental groups demonstrated an increase in muscle activity as assessed by surface
481 EMG amplitude. This measure may or may not represent neural adaptation to strength
482 training (for review see Farina et al. 2014), but it was a systematic change in all groups,
483 whereas increased 1-RM performance was not. Correlation analysis revealed that there was
484 a weak but statistically significant relationship between the change in 1-RM load and the
485 change in EMG amplitude ($r=0.23$, $p=0.041$, $n=78$), which explained only ~5% of the
486 variance. Perhaps improvements in inter-muscle coordination during specific parts of the
487 lift, which would be very challenging to determine experimentally, may have led to
488 improved 1-RM performance and could be considered part of the improved task-specific
489 'skill' of 1-RM performance.

490

491 To the authors' knowledge, this is the first study to investigate the influence of training
492 frequency on muscle hypertrophy in healthy older individuals. Whereas the present study's
493 3 months of preparatory training induced gains in muscle mass (approx. 2% increase in lean

494 leg mass, $P < 0.05$, unpublished data), neither reduced or maintained or increased training
495 frequency led to further increases in muscle mass during the subsequent 6-month
496 intervention period of the present study (Figure 4). In young subjects, higher training
497 volume using high- to moderate-loads has been shown to be a key element of training for
498 the development of muscle hypertrophy (Campos et al. 2002; Ratamess et al. 2009), but
499 training frequency has been rarely examined directly, and there appears to be no
500 differences between a training frequency of 2 and 3 times per week in novice trainers
501 (Wernbom et al. 2007). Given that the training program in the first 3-month preparation
502 period was not optimized to increase maximum strength or muscle mass it was expected
503 that further increases would have been made during the subsequent 6-month intervention.
504 Certainly there is a large body of evidence showing that initial gains in muscle mass continue
505 over the first year of training (e.g. Häkkinen et al. 2002; Pyka et al. 1994; Taaffe et al. 1996).
506 However, there are also rare observations where older individuals do not gain muscle mass
507 at all, such as the ~70yr old men in Häkkinen et al. (1998).

508

509 One possible explanation for the lack of prolonged development in muscle mass in the
510 present study may be that older individuals are thought to be “anabolically resistant” to the
511 effect of strength training, may not demonstrate hypertrophy to the extent of younger
512 individuals (Kraemer et al. 1999) and may require greater protein intake to maximize
513 hypertrophic potential (Moore et al. 2015). Unfortunately for the development of muscle
514 mass, Mero et al. (2013) showed that older individuals habitually consumed less protein
515 (and calories in general) than young individuals, and this was also observed in the present
516 study. The self-reported protein intake of the participants in the present study (range 0.6–
517 1.8 g·kg⁻¹ body mass) perhaps indicates a sub-optimal environment for muscle hypertrophy

518 despite the more optimized training program for strength and hypertrophy. Consequently,
519 with no control of diet and/or supplementation during training to optimize protein intake,
520 the present study does not fully allow the effect of training frequency on muscle
521 hypertrophy in healthy older individuals to be determined.

522

523 Since there was no improvement in voluntary activation level during unilateral isometric
524 knee extension and no increase in quadriceps muscle mass, it is not surprising that
525 maximum knee extension torque did not increase during the present study. A lack of
526 improved voluntary activation level could be a result of using a non-specific test, as
527 discussed above, or it may indicate that no neural adaptation occurred during the present
528 study. This would seem to be supported by the finding that surface EMG amplitude was no
529 longer significantly changed due to training once normalized to the maximum M-wave
530 amplitude. Alternatively, it may be that the use of single-pulse twitches was not sensitive
531 enough to determine training-induced changes (Herbert & Gandevia 1999). It would be
532 recommended that more detailed measurements assessing muscle activation would be
533 included in future studies.

534

535 Interestingly, relationships between increased strength, muscle activity/activation or muscle
536 mass and improved functional capacity were not observed. In fact, the only statistically
537 significant (negative) relationship was observed between change in leg press 1-RM and
538 change in loaded 10-stair climb, and this would be classed as a weak relationship with only
539 5% of the variance explained. In this regard, it appears that performing strength training
540 (regardless of training frequency) is important to improve functional capacity even in

541 healthy older individuals, but the actual magnitude of strength gain has little or no influence
542 on improved maximum walking speed.

543

544 The decision to recommend strength training two-three times per week may have
545 originated from the meta-analysis of Rhea et al. (2003) who were the first to investigate the
546 effect of training frequency on maximum strength development. However, due to the
547 integration of studies utilizing different participant groups, the authors cautioned that
548 *“additional reviews are needed to verify the application of the dose-response trends to those*
549 *populations”* (p. 458). Subsequently, a meta-analysis in healthy adults over 55 years did not
550 observe the same dose-response relationship for maximum strength (Silva et al. 2014).
551 Regarding muscle hypertrophy, a recent meta-analysis did observe a benefit of higher
552 training frequency but this included studies with age-ranges throughout the lifespan
553 (Schoenfeld et al. 2016). It may well be that the present number of intervention studies
554 examining training frequency does not allow meta-analysis techniques to accurately
555 evaluate its influence on strength, muscle mass, and functional capacity. For example, 17
556 out of 21 treatments included in study by Silva and colleagues (2014) comprised training of
557 three times per week with majority of studies using 2-3 times per week. This clearly does
558 not allow valid evaluation of a training frequency of one time per week, specifically over the
559 influence of other program variables, such as intensity, volume, number of exercises per
560 muscle group etc., on outcome measures. Therefore, scientists do not currently have
561 sufficient evidence to inform policy makers as to recommendable training frequencies for
562 young or older individuals.

563

564 Finally, although habitual physical activity was tracked (daily) throughout the present study,
565 it was done so subjectively using diaries. This method of course is non-blinded, i.e. the
566 participants can see their daily activity level and this may influence the results, and also is
567 open to typical errors of subjective reporting. Future studies may wish to implement
568 objective measures of tracking physical activity in order to verify this finding. Nevertheless,
569 since there were no differences between the groups and that there were no significant
570 relationships between habitual physical activity and any performance outcome measure, it
571 appears that this potential confounding factor did not likely influence the data. In other
572 words, we can be confident that functional capacity did not improve due to physical activity
573 performed externally to the prescribed intervention. Therefore, in general it seems that the
574 act of performing strength training, and not necessarily the increase in strength or muscle
575 mass, is important to improve functional capacity in older individuals, and in order to
576 prescribe individualized training programs for older adults the precise underlying factor
577 should be identified.

578

579 **5 CONCLUSIONS**

580 The present study found no evidence that higher training frequency would induce greater
581 benefit to maximum walking speed, functional capacity or muscle mass. There is a clear
582 dose-response in dynamic bilateral 1-RM strength but this was not observed during
583 unilateral isometric tests of maximum strength. It appears that in healthy older individuals
584 with some (albeit limited) experience in strength training beneficial functional capacity
585 improvements can be achieved through low frequency training (i.e. 1–2 times per week).
586 This study suggests that a sufficient frequency for whole-body strength training to improve

587 strength, muscle mass and functional capacity are not one-in-the-same and perhaps should
588 be noted in physical activity guidelines for healthy older individuals.

589

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596

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