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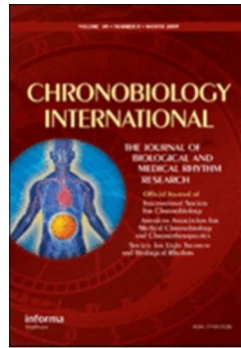
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The effects of 12-week progressive strength training on strength, functional capacity, metabolic biomarkers, and serum hormone concentrations in healthy older women: morning versus evening training

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Keywords:	Aging, Time of the day, Maximum strength, Senior fitness tests, blood lipids, resistance



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Title: The effects of 12-week progressive strength training on strength, functional capacity, metabolic biomarkers, and serum hormone concentrations in healthy older women: morning versus evening training

Running Head: Training at different times of the day in women

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($p < 0.05$) decreased GLU ($-4 \pm 6\%$ and $-8 \pm 10\%$, respectively), whereas significantly greater decrease was observed in the E compared to the M group ($p < 0.05$). Only E group significantly decreased TG ($-17 \pm 25\%$, $p < 0.01$), whereas M group increased ($+15\%$, $p < 0.01$). The difference in TG between the groups favored E compared to M group ($p < 0.01$). These results suggest that short-term “hypertrophic” ST alone mainly improves strength and functional capacity performance, but it influences metabolic and hormonal profile of healthy older women to a lesser extent. In this group of previously untrained older women, time-of-day did not have a major effect on outcome variables, but some evidence suggests that training in the morning may be more beneficial for muscle hypertrophy (i.e. only M significantly increased muscle mass and had larger effect size (M: $g = 2$ vs E: $g = 0.5$)).

Keywords: Aging, Time of the day, Maximum strength, Senior fitness tests, blood lipids, resistance

INTRODUCTION

Biological aging is associated with lower levels of physical activity that leads to the progressive loss of strength and muscle mass, and to the accumulation of body fat (Chumlea et al. 2002). As a consequence, adverse modifications to metabolism and increased prevalence of low-grade inflammation occur (Minihane et al. 2015). Hence, older individuals face challenges in maintaining functional capacity and independence, as well as to prevent non-communicable diseases, such as cardiovascular disease (CVD), which is the main cause of morbidity and mortality in the older population (Rattan 2006, Zaslavsky and Gus 2002). Conversely, older individuals that do maintain an active lifestyle demonstrate reduced mortality and morbidity from CVD, diabetes as well as physical disabilities (Gregg et al. 2003, Stessman et al. 2000).

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73 Strength training in older individuals has become one increasing research focus over the past
74 three decades in order to understand whether (and how) this form of exercise is efficacious to
75 reverse biological aging processes. In particular sarcopenia, which is defined by both loss of
76 skeletal muscle mass and muscle function (either muscle strength or functional capability), is
77 a major health issue in our aging society. Recent evidence suggests that the cause of
78 sarcopenia may be several factors including neural, hormonal and inflammatory changes,
79 along with or due to decreased physical activity connected with poor nutritional status
80 (Walston 2012). Studies in older individuals have shown increased muscular strength and
81 muscle fiber cross-sectional area (Frontera et al. 2003, Häkkinen et al. 1996, Sipilä and
82 Suominen 1995), improvements in tests of physical function (30-seconds chair stand, 30-
83 seconds arm curl) (Dias et al. 2015, Pinto et al. 2014), improved body composition including
84 lower body fat and increased upper and lower limb muscle mass (Binder et al. 2005, Galvão
85 and Taaffe 2005, Sillanpää et al. 2009).

86 More recently, improvements in metabolic biomarkers such as TG, HDL-C, LDL-C, GLU
87 (Martins et al. 2010, Tomeleri et al. 2016), as well as reduced levels of inflammatory
88 biomarker CRP (Ribeiro et al. 2015, Tomeleri et al. 2016) have also been observed. During
89 aging significant changes in endocrine function occurs and exercise appears to modify the
90 level of circulating hormones. In exercising older woman, levels of anabolic hormones
91 decrease (Ennour-Idrissi et al. 2015) and this may be connected with loss of muscle mass and
92 reduction in functional capacity. However, higher circulating estrogens have been linked with
93 risk of developing breast cancer (Key et al. 2002) and tumors (Pike et al. 1983). Hence, there
94 may be a competing need in older women to lower estrogen levels to reduce risk of disease
95 while increase levels to increase/maintain muscle mass.

96 While strength and muscle mass improvements are so robust that they are observed in almost
97 all strength training studies, the evidence for improved body composition and

metabolic/inflammatory biomarkers is weaker. For example, strength training of 8–16 weeks did not improve body composition or blood lipid profiles in several studies (Elliot et al. 2002, Hagerman et al. 2000, Joseph et al. 1999). The reason for the mixed findings is unclear, but one possibility is that there were differences in the training programs used. Given that there is no consensus on the optimal way to train older individuals (with particularly less studies performed in women), different training variables should be assessed to determine their potential role in influencing adaptations.

One aspect of training that may influence the efficacy of the training stimulus or sport performance, which has not been extensively investigated, is the time-of-day when the training occurs (Vitale and Weydahl, 2017). It is already known that hormone concentrations exhibit circadian rhythmicity and vary throughout the day (Kraemer et al. 2001) along with body temperature (Bailey and Heitkemper, 2001) and strength performance (Sedliak et al. 2009). Since strength performance is greater in the evening compared to morning and that e.g. testosterone concentrations are higher in the morning (i.e. during the more immediate recovery period), it has been hypothesized that evening training may be more efficacious than morning training. Few studies have investigated this hypothesis during short-term training (Sedliak et al. 2009, Sedliak et al. 2017), but of those there is some evidence to suggest that muscle hypertrophy may be greater and/or systematic following evening training in young individuals (Küüsmaa et al. 2016). Furthermore, it was also recently shown that not only strength performance and hormonal concentrations vary throughout the day but also rating of perceived exertion (RPE) and mood states were affected after high intensity interval training performed at different times of the day (Vitale et al. 2017). Here, especially the evening types were more fatigued with less energy and higher RPE during morning training sessions (Vitale et al. 2017). However, considering the clear evidence that older individuals have greater vitality and/or are less fatigued in the mornings compared to the evening (Wanigatunga et al.

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2017), this hypothesis may not hold for older populations. It is important to determine possible implications of performing strength training at various times during the day, as this could enhance national and international recommendations for strength training in older individuals.

Therefore, the present study aimed to determine the effects of time-of-day on adaptations from 12-week progressive strength training program in a group of older women. This age and sex population may derive the greatest health-enhancing benefit from strength training, and it is important to optimize prescribed training methods for older women. We hypothesized that the morning training group would enhance strength and functional performance to a greater extent than the evening training group, while the differences in body composition, biochemical and hormonal outcomes between the groups will be similar.

MATERIALS AND METHODS

Study design

Thirty-one elderly women were allocated into a morning (n=10) or an evening (n=10) training group or a non-training control group (n=11). Morning (07:30) and evening (18:00) training groups performed a 12-week progressive strength-training program with a frequency of 2 days per week, while the control group continued their normal daily activities. Seven days before and seven days after the experiment, evaluations consisting of anthropometric measures, tests of 6-repetition maximum (6-RM), functional capacity tests, body composition assessment by bioelectrical impedance (BIA), and blood draws for biochemical analyses were performed. For strength and functional capacity tests, the morning training group was tested in the morning hours (from 07:30), and the evening group was tested in the evening hours (from 18:00) to match their training times. The study was conducted between February and June.

148

149 **Participants**

150 Participant recruitment was carried out through newspaper advertisements and personal
151 meetings. All potential participants completed a questionnaire focused on health history and
152 physical activity. Inclusion criteria for this study were as follows: female sex, at least 60
153 years old, physically independent, no orthopedic and cardiac problems pass a medical
154 evaluation, no medication affecting blood pressure or cholesterol, glucose or hormonal
155 concentrations. At the beginning of the experiment 40 women enrolled to the study, but 4 did
156 not pass the medical examination and were excluded. Therefore, 36 women were randomized
157 to the morning training group (M: n=12), evening training group (E: n=12), and the control
158 group (C: n=12) (Fig 1). After the initial evaluation process, and following intervention or
159 control period there were 31 women who completed the study (age: 66 ± 4 years, height: 162
160 ± 4 cm, body mass: 75 ± 13 kg). **None of the participants in this study were sarcopenic.**
161 **Sarcopenia was calculated as skeletal muscle mass index by the standard equation: muscle**
162 **mass/(height²). Cut-off point that may indicate sarcopenia in older woman according to**
163 **European working group on sarcopenia was 7.2 kg/m^2 (Bahat et al. 2016).** The reasons for
164 withdrawal from the study were different, for instance: personal reasons, lack of time, did not
165 meet the inclusion criteria (Fig. 1). All participants read and signed informed consent where
166 detailed description of study design, training program and evaluation process was listed prior
167 to the study. This study was performed according to the Declaration of Helsinki, and the local
168 University Ethic Committee approved this experiment.

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170 **6-RM muscular strength**

171 Maximal dynamic strength was assessed using the 6-RM (modified 1-RM test) (Ribeiro et al.
172 2017) test for the horizontal leg press and seated-row exercises. Testing was preceded by a

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warm-up set which consisted of 8-12 repetitions, with approximately 70% of the estimated load used in the first attempt of the 6-RM. After 2 minutes of rest the testing procedure began. The participants were instructed to perform 6 repetitions with the highest possible load within 3-4 attempts in both exercises. Two experienced coaches supervised the testing to ensure reliability and safety of the participants. The duration of the concentric and eccentric phase was 2 seconds, respectively and was controlled by the coaches. The 6-RM was recorded when the last successful repetition with a given load was lifted and the participant was not able to accomplish the next repetition. Inter-correlation coefficient (ICC) for the leg-press and seated row 6-RM was 0.95 and 0.78, respectively. Participants were verbally encouraged throughout each test. Rest intervals between attempts were 3-4 minutes and 7 minutes between exercises.

Functional capacity tests

Testing procedures followed the standard SFT protocol (Rikli and Jones, 2013). A battery of tests was used to examine functional capacity of the participants. A 30-second chair-stand test measured the number of times that a participant can stand upright from a chair and sit down. On a signal, participants stood up to a full standing position from a chair and then returned to the fully seated position (stand up and sit down = 1 repetition) and they continued to complete as many full stands as possible in 30 seconds. The chair (seat height = 43cm) was positioned against a wall and safely secured. Time was taken using stopwatch and the total number of complete repetitions was recorded. If the participant had completed only the stand phase when the time elapsed, this repetition was counted.

The arm curl test measured the number of arm curls with a 2.3 kg dumbbell in the dominant hand. Participants sat on the (same) chair while holding the dumbbell with palm facing towards the body with the arm beside the chair. During the curls, the upper arm and elbow

joint were positioned and maintained near to the body. If necessary, the tester assisted in maintaining the upper arm in the correct position. On a signal, participants began to flex (with gradually turning the palm – flexion with supination) and extend the elbow of the dominant hand, over the entire range of motion (the arm must be fully flexed and then fully extended at the elbow), as many times as possible in 30 seconds. Again, if the participants had only raised the arm, but not fully lowered the arm when time elapsed then that repetition was counted.

A Timed Up-and-Go (TUG) test was used to determine the amount of time required to stand up from a chair, walk 2.4 meters, turn around a cone, return and sit down on a chair. Time during the tests was taken by stopwatch similarly as in the original SFT and previous research. Participants completed 2 to 3 attempts from each test, and the best results were taken to further analysis. The ICCs for the all SFT tests were high 0.93 to 0.98 (Milanović et al. 2013).

Anthropometry

Body height was measured to the nearest 0.1 cm with a stadiometer attached to the scale. Height measurements were performed while the participants were standing barefoot. Body mass was evaluated to the nearest 0.1 kg using the InBody device which was used to determine muscle mass and percentage fat mass analysis (see below) (Biospace Co., Seoul, Korea). All measures were performed in the morning after overnight fast.

Body composition measurements

Whole-body muscle mass, fat mass, whole-body fat %, as well as leg and arm muscle mass was measured using the InBody 720 device (Biospace Co., Seoul, Korea). InBody 720 measures body composition by passing multiple frequencies at 5, 50, 250, 500, and 1000 kHz

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223 and reactance in mean frequencies (5, 50 and 250 kHz). Overall body impedance values were
224 calculated by summing the segmental impedance values that were analyzed separately with a
225 tetrapolar 8-point tactile electrode system. The measurement procedures were similar to a
226 previous study (Esco et al. 2015). Briefly, before the participants stood on the device their
227 soles and palms were wiped with an electrolyte tissue. According to the examiner's
228 instructions, the participants gripped the handles with the palm, fingers, and thumb in contact
229 with the hand electrodes. The participants' soles were also in contact with the foot electrodes.
230 According to the manufacturer's guidelines, the participants held their arms and legs in such
231 a position that they would not come into contact with any other body part during the
232 measurement. The arms were positioned at approximately 20° away from the trunk, and legs
233 were positioned 45° apart. Before the measurement began, the participants were instructed
234 not to move. The duration of the analysis was approximately 2-3 minutes per participant.
235 Test-retest reliability of this device was performed in previous study with good interclass
236 correlation coefficient (ICC) (SMM: ICC=0.99, FM%: ICC=0.99) (Esco et al. 2015).

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238 **Blood sampling and biochemical analysis**

239 Venous blood was collected in the morning after a 12-hour fast in a standard manner from an
240 antecubital vein. After separation of serum, samples were stored at -80°C until further
241 analyses. Samples were assessed by immunoassay for total antioxidant status (TAS), high
242 sensitivity C-reactive protein (hsCRP), triglycerides (TG), blood glucose (GLU) and total
243 cholesterol (TC) concentration using a discrete photometric Clinical Chemistry Analyzer
244 Biolis 24i Premium (Tokyo Boeki Machinery, Tokyo, Japan).
245 High-density lipoprotein (HDL-C), and small dense low-density lipoprotein (sdLDL-C)
246 cholesterol were determined by detergent-based isolation and enzyme-linked colorimetric

247 detection (Direct HDL cholesterol and direct sdLDL-C cholesterol; Randox Laboratories,
248 Crumlin, UK).

249 Total cholesterol, HDL-C and sdLDL-C were analyzed using commercial kits (Randox
250 Laboratories, Crumlin, UK). Total antioxidant status (TAS) was assessed by the Trolox-
251 equivalent antioxidant capacity assay performed with the kit supplied by Randox (Randox
252 Laboratories, Crumlin, UK). Briefly, the test was based on the formation of blue-green cation
253 radical of ABTS (2,2-Azino 3-ethyl benzthiazoline sulfonate) in the presence of
254 metmyoglobin and hydrogen peroxide. LDL-C concentration was estimated using the
255 Friedewald, Levy, and Fredrickson equation (Friedewald et al. 1972).

256 Intra-assay variability (%) was ≤ 3.0 (sdLDL-C), ≤ 1.3 (HDL-C), ≤ 2.2 (GLU), ≤ 2.5 (TG), ≤ 2.8
257 (CRP), ≤ 3.1 (TAS). Sensitivity was: 0.025 mmol/l (sd-LDL), 0.04 mmol/l (HDL-C), 0.1
258 mmol/l (GLU), 0.26 mmol/l (TG), 0.007 mmol/l (hsCRP).

259 Quantification of hormones was performed using ELISA (Enzyme-Linked Immunosorbent
260 Assay). All analyzes were performed on the DIAREADER ELX800 G (Dialab, GMBH,
261 Wiener Neudorf, Austria) with measuring range from 400 nm to 750 nm for reading 24, 48 or
262 96-well plates. ELISA assays (Dialab, Wiener Neudorf, Austria) were performed according
263 to the manufacturer's instructions. The color intensity was inversely proportional to the
264 concentration of hormones in the sample. The absorbance was determined according to the
265 manufacturer's instructions on a microplate ELISA reader - GloMax®-Multi+ Detection
266 System (Promega Corporation, Madison, USA). Seven basal hormone levels were analyzed
267 using commercially available assays: follicle stimulating hormone (FSH) (Dialab, Wiener
268 Neudorf, Austria), luteinizing hormone (LH), prolactin (PRL) (NovaTec, Immundiagnostica
269 GMBH, Dietzenbach, Germany), progesterone (P) (Dialab, Wiener Neudorf, Austria),
270 estradiol (ESTR) (NovaTec, Immundiagnostica GMBH, Dietzenbach, Germany), aldosterone
271 (ALD) (NovaTec, Immundiagnostica GMBH, Dietzenbach, Germany), and testosterone (T)

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(Dialab, Wiener Neudorf, Austria). Intra-assay variability (%) was ≤ 6.4 (FSH), ≤ 9.2 (LH), ≤ 3.5 (PRL), ≤ 4.0 (P), ≤ 9.0 (ESTR), ≤ 10.0 (ALD), ≤ 7.0 (T). Sensitivity was 1.0 mIU/ml (FSH), 0.2 mIU/ml (LH), 0.1 ng/ml (PRL), 0.1 ng/ml (P), 8.7 pg/ml (ESTR), 0.01 ng/ml (ALD), 0.1 ng/ml (T).

Strength training program

Supervised strength training was performed during the morning (07:30) and evening hours (18:00). Four weeks before the first pre-training measures and analyses, the participants completed 6 familiarization sessions with a frequency of 1-2 days per week in order to learn the correct exercise techniques. During these sessions only exercise technique (exercises used in training program) with light loads was performed. During the entire study, the participants were personally supervised by qualified instructors to ensure safety and consistency during training sessions. Participants trained two times per week, on Mondays and Thursday. Whole-body strength training program comprised of 8 exercises in the following order: dumbbell bench press, horizontal leg press, seated row, knee extension, lat pull-down, leg curl, machine chest fly, and seated calf raise. The participants performed 3 sets of 10-12 repetition maximums. The same load was kept from set 1 to set 3, and participants always finished the prescribed repetition range, which ended with concentric failure in the final set. Rest periods between sets were 2-3 minutes and 3 minutes between exercises. Participants were constantly instructed to inhale during the eccentric phase and exhale during the concentric phase. Tempo during the lifting was approximately 1 second for concentric and 2 seconds for eccentric phase. External load was gradually increased in the following manner: for upper body exercises ~3-5% and for lower body exercises ~5-8% every second week (after 4 training sessions) (Ribeiro et al. 2015). Participation in the training program was sufficient, with all participants participating in >90% of the total training sessions. In

addition, each participant received a 25g dose of whey isolate protein after each training session to ensure a similar anabolic effect during the immediate period after strength training. The Control group was asked to maintain similar physical activity as they did before the study. All groups were also assessed by International Physical Activity Questionnaire (IPAQ) to assess physical activity performed external to the intervention.

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303 **Statistical analyses**

Analysis of covariance (ANCOVA) with repeated measures was used for comparison of all three groups (3 group×2 time) and the two training groups (2 group×2 time) using baseline values as covariate. One-way ANOVA was used to assess between-group differences at baseline and post-training in all three training groups. Hedge's *g* estimates were employed, where small (<0.3), medium (0.3–0.8), and large (>0.8) effect sizes were identified to determine the magnitude of the training-induced changes between the three training groups (Hopkins 2012). Pearson product moment correlation (*r*) determined relationships between pre-training value and changes during the training. Alpha was set at 0.05. Descriptive statistics and statistical methods were calculated using statistical software IBM SPSS 22.

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314 **RESULTS**

Results of the IPAQ showed no significant differences between groups at any point (from pre- to post-training) during the study. All measured (absolute values) maximum strength, functional capacity and body composition data are shown in table 1.

318 **Muscular strength and functional capacity**

Time ($p < 0.01$) and time×group ($p < 0.01$) interactions were found in leg press 6-RM, seated row 6-RM, biceps curls and chair stand functional capacity test. M significantly improved leg press and seated row 6-RM ($+46 \pm 23\%$ and $+48 \pm 22\%$, respectively; $p < 0.01$, Figure 2A and

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2B), chair stand ($+35\pm32\%$, $p < 0.01$, Figure 2C), biceps curl ($+30\pm22\%$, $p < 0.01$) and TUG ($-17\pm11\%$, $p < 0.01$, Figure 2D). Similarly, E significantly improved leg press and seated row 6-RM ($+21\pm12$ and $+43\pm18\%$, respectively; $p < 0.01$, Figure 2A and 2B), chair stand ($+34\pm33\%$, $p < 0.01$, Figure 2C), biceps curl ($+36\pm21\%$, $p < 0.01$) and TUG ($-20\pm9\%$, $p < 0.01$, Figure 2D). Improvements in both training groups were significantly larger compared to the control group ($p < 0.01$) except for TUG where no significant difference between the groups was observed (Figure 2A-D). No significant differences in 6-RM strength and functional capacity tests between M and E group were observed.

Body composition

A significant time \times group interaction ($p < 0.01$) was found in measures of whole-body muscle mass where only M significantly ($+3\pm2\%$, $p < 0.01$) increased muscle mass from pre- to post-training (Figure 2E). However, M and E both significantly decreased fat mass ($-6\pm5\%$ and $-8\pm4\%$, respectively; $p < 0.01$, Figure 2F) and body fat % ($-6\pm5\%$ and $-5\pm3\%$, respectively; $p < 0.01$) from pre- to post-training. No significant differences between all the three groups in measures of body composition were recorded.

Figure 3 shows effect sizes which favor both M and E compared to C in maximum strength, functional capacity and body composition outcomes. Of note is that M showed a large effect size ($g=2$) for muscle mass, while E showed only a medium effect size ($g=0.5$).

Biomarker and hormone concentrations

Table 2 shows results (absolute values) of the metabolic and inflammatory biomarkers and hormone level after the training. Significant difference between pre- to post-training in M and E were found in sdLDL-C where both groups increased their levels ($79\pm84\%$ and $31\pm50\%$, respectively; $p < 0.05$). Both M and E groups significantly decreased the level of the GLU ($-4\pm6\%$ and $-8\pm10\%$, respectively; $p < 0.05$), where the decrease in GLU level was significantly greater for E compared to M ($p < 0.05$). Only E significantly decreased the level

of TG ($-17 \pm 25\%$, $p < 0.01$) from pre- to post-training which differed significantly ($p < 0.01$) compared to M ($+16 \pm 27\%$, $p < 0.01$). No other significant increases or decreases in biochemical or inflammatory parameters were observed. Similarly, no significant changes in the hormone level, except for ESTR ($+16 \pm 19\%$, $p < 0.05$) in M were observed.

351

352 DISCUSSION

353 The main aim of this study was to compare effects of training performed at different times of
354 the day in a group of older women on multiple variables; including maximum strength,
355 functional capacity, and basal biomarker and hormonal concentrations. The results show that
356 morning and evening training groups significantly improved maximum strength, functional
357 capacity, body composition, as well as some biomarker concentrations. The findings partially
358 support our hypothesis that similar changes in body composition and blood markers between
359 the groups would be observed, but there was little evidence to support our hypothesis that
360 morning training would be more beneficial for strength and functional capacity improvement.

361 The observed improvements in strength and functional capacity tests in M and E are not
362 surprising since participants had no regular strength training or any other physical activity. A
363 large number of studies have demonstrated increased maximum strength in older individuals
364 after initiating progressive strength training (Frontera et al. 2003, Häkkinen et al. 1996). In
365 the present study, both M and E significantly improved leg press (46% and 21%; $p < 0.001$)
366 and seated-row (48% and 42%; $p < 0.01$) 6-RM. No significant differences were observed
367 when comparing M and E groups in both tests, however, M demonstrated larger increases
368 (twofold) compared to E in the leg-press 6-RM ($\sim 46 \pm 21\%$ vs. $\sim 21 \pm 12\%$). Despite non-
369 significance, effect size values favor M compared to E ($g = \sim 3.4$ and ~ 2.1 , respectively). It
370 could be speculated that M gained more in the leg press due to higher quality training, since it
371 has been shown that older individuals have greater vitality in the mornings (Wanigatunga et

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al. 2017). Nevertheless, these larger gains did not translate into greater improvements in functional capacity.

Functional capacity performance significantly improved in both M and E over 12 weeks of training. Comparable results in functional capacity have also been reported (Hanson et al. 2009, Pedersen et al. 2017, Turpela et al. 2017). Interestingly, we did not observe significant relationships ($r = -0.1$ – -0.4 , $p > 0.05$) between the changes in leg press or seated row 6-RM strength and changes in any functional capacity test. The importance of increasing maximum strength for improving functional capacity seems questionable since only one study has shown a statistically significant relationship (Santos et al. 2017), while most others have not (Moura et al. 2017, Turpela et al. 2017). Hence, it would seem likely that the effect of possibly more efficient strength training in the morning versus evening will have little or no effect on the overall magnitude of functional capacity improvement in older individuals. Collectively, the results of all of the aforementioned 6-RM strength tests and functional capacity tests indicate that training time is not a major factor influencing performance in favor of morning or evening training over 12 weeks in previously untrained older women.

Many previous strength training studies have shown positive effects on body composition over 8 to 18 weeks in older women (Bouchard et al. 2009, Santos et al. 2017, Sipilä and Suominen 1995, Tomeleri et al. 2016). In the present study, both M and E significantly decreased whole-body fat mass (kg) ($-6 \pm 5\%$ and $-8 \pm 4\%$, respectively; $p < 0.01$) and body fat percentage ($-6 \pm 5\%$ and $-5 \pm 3\%$, respectively; $p < 0.01$). However, only M significantly increased whole body muscle mass in the present study (kg) (M: $+3.4\%$, $p < 0.01$; E: $+0.7\%$). This increase in muscle mass may help to explain the larger gains in leg press 6-RM, but correlation analyses suggest that this influence was small and not statistically significant ($r = 0.373$, $p > 0.05$, $n = 20$). The reason why only the M group significantly increased muscle mass is not clear. But this finding is in contrast to the trends found in young individuals by

397 Kūusmaa et al. (2016), while several papers by Sedliak and colleagues (2009, 2017) have
398 shown equal hypertrophy in young men. This is a potentially important finding for practical
399 applications when aiming to counteract age-related loss of muscle mass and should be
400 explored further.

401 The results in metabolic and inflammatory biomarkers are somewhat contradictory in our
402 study compared to others' findings. Both M and E significantly improved GLU from pre- to
403 post-training (M: $-4 \pm 6\%$, E: $-8 \pm 10\%$; $p < 0.05$), and the improvement in E group was
404 significantly higher compared to M ($p < 0.05$). Tomeleri et al. (2016, 2017) found reductions
405 in glucose level after 8- and 12-weeks of resistance training from 6% to 20 %, respectively.
406 Improved basal glucose concentrations may have been due to improvements in insulin
407 sensitivity brought about by loss of fat (Boden 2002). Studies have observed significant
408 relationships between changes in body fat and changes in glucose concentration (Tomeleri et
409 al. 2016), however, in our study we did not observe such a relationship. Further, it is difficult
410 to attribute that fat loss would be a major factor in reduced glucose concentration since both
411 M and E lost fat mass to a similar extent. It neither seems likely that muscle hypertrophy
412 would play such an important role considering that M increased muscle mass more than E,
413 but E reduced glucose concentration more than M. Regardless of the possible mechanisms, an
414 important finding from a general health perspective is that a significant relationship between
415 baseline glucose level and changes during the training was observed ($r = -0.491$, $p < 0.05$,
416 $n=20$). Thus, those individuals with higher basal glucose concentration gain the most benefit
417 from strength training, regardless of whether training is performed in the morning or evening.

418 Only E significantly decreased TG (-17% , $p < 0.01$) while M actually showed an increase in
419 TG ($+16\%$, $p < 0.01$). Strength training may decrease lipid concentrations by the ability of
420 skeletal muscle to use fat stores during physical activity (Mann et al. 2014). However, the
421 results in M are hard to explain, particularly given the muscle mass results, and we can only

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speculate what mechanism(s) may be responsible for this result (e.g. dietary intake, intra-individual differences, and daily/seasonal variation in TG, synthesis of tissue/hormones from cholesterol). Once again correlation analyses between baseline values and changes during training revealed a negative relationship ($r = -0.677$, $p < 0.01$, $n=20$). Therefore, individuals with higher initial levels benefit most from beginning strength training.

Small dense low-density lipoprotein is a new emerging risk factor associated with cardiovascular diseases because it is more atherogenic than LDL-C. sdLDL-C can be used as a predictor of future CVD and other conditions associated with dislipidemia (Ivanova et al. 2017). Our study is the first to examine the effects of strength training on sdLDL-C concentration, and it is difficult to explain why both groups increased the level of sdLDL-C and whether strength training is the cause of such change.

Except for estradiol in M ($+16 \pm 19\%$, $p < 0.05$) no significant changes in basal hormone concentrations were observed from pre- to post-training. These results match findings in the younger as well as in the older population (Häkkinen et al. 2000, Sallinen et al. 2006). However, this result should be interpreted with caution because it is unknown whether this change is due to greater production or lower uptake of ESTR in M, and therefore, it is unclear whether this is a positive effect related to strength training.

This study has some limitations that should be mentioned; 1) It was not possible to objectively control physical activity during daily living despite participants being instructed to avoid any exhaustive activities or beginning new exercises that could potentially affect results of the study. Instructions were also provided regarding nutritional intake. 2) Sample size in the present study may not have been sufficient to determine statistical significance in some biomarkers, since the pattern of change suggested improvements in both M and E for HDL-C (M: $+3 \pm 12\%$, E: $+10 \pm 12\%$) and TAS (M: $+10 \pm 12\%$, E: $+14 \pm 11\%$), and E only for hsCRP (M: $+0.02 \pm 47\%$, E: $-4 \pm 41\%$). 3) Our strength training program was focused on

“hypertrophic” type of training. Therefore, it is not known whether manipulation of other program variables (i.e. shorter rest-interval, higher number of repetitions) may have led to greater benefit on metabolic and inflammatory biomarker concentrations. Nevertheless, one strength of the study was that both training groups received a 25 g-dose of whey isolate protein after each training session. Hence, both groups had similar conditions for immediately post-training anabolic effects.

453

454 CONCLUSIONS

455 This study showed that improvements in maximum strength, functional capacity and some
456 metabolic biomarkers in previously untrained older woman occur regardless of whether
457 training was performed in the morning or evening. However, morning trainers gained more in
458 leg press 6-RM and whole-body muscle mass compared to evening trainers, which appears in
459 contrast to some (limited) findings in young individuals. An important finding, which
460 requires further detailed study, was that these greater gains in maximum strength and muscle
461 mass did not influence the changes in functional capacity performance, fat loss, or blood
462 profile of these women.

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468 DECLARATION OF INTEREST

469 The authors of this study declare that there is no conflict of interest.

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FIGURES

Figure 1 Flow diagram showing subject enrollment, allocation and follow-up.

Figure 2 Changes in leg press (A) and seated row (B) 6RM strength, chair stand (C) and Timed Up and Go (D) performance, and whole-body muscle mass (E) and fat mass (F) over the study duration. Stars within the bars = within-group differences, Stars above bars = between-group differences, The horizontal (black) line denotes the Covariate baseline value.

Figure 3 Effect size plot (Hedge's g) showing training-induced changes in maximum strength, functional capacity tests and body composition adjusted for control group changes. Closed squares=morning group; Open squares=evening group

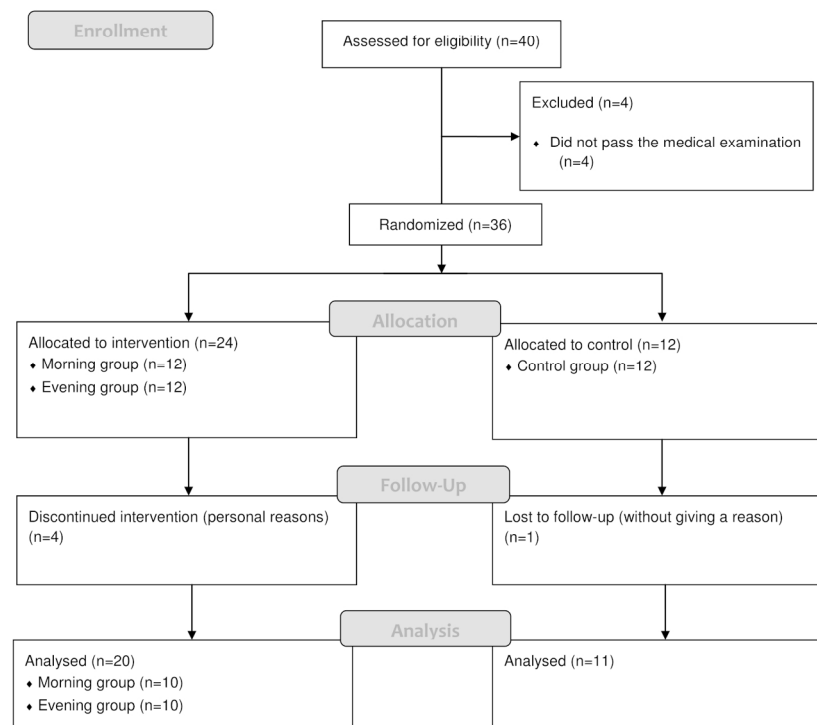


Figure 1 Consort flow diagram showing subjects enrollment in this experiment

599x776mm (72 x 72 DPI)

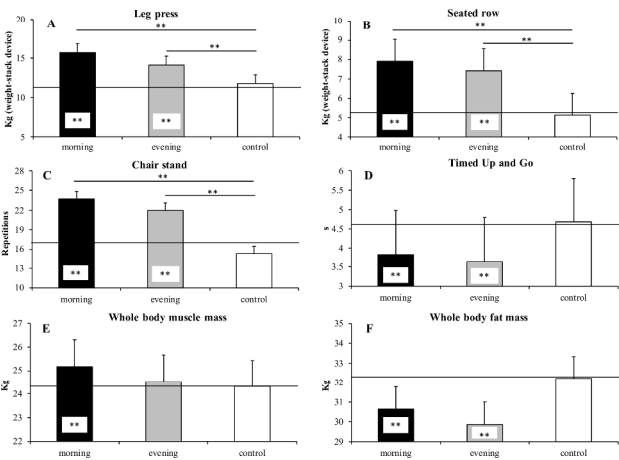


Figure 2 Changes in leg press (A) and seated row (B) 6RM strength, chair stand (C) and Timed Up and Go (D) performance, and whole-body muscle mass (E) and fat mass (F) over the study duration. Stars within the bars = within-group differences, Stars above bars = between-group differences, The horizontal (black) line denotes the Covariate baseline value.

209x297mm (300 x 300 DPI)

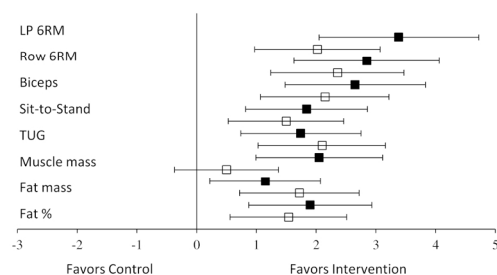


Figure 3 Effect size plot (Hedge's g) showing training-induced changes in maximum strength, functional capacity tests and body composition adjusted for control group changes. Closed squares=morning group; Open squares=evening group

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Table 1 Pre- and Post-training values (Mean±SD) in strength, functional capacity, muscle mass, fat mass and body fat % in the morning, evening and control groups

	Pre-training									
	LP (kg/weight-stack device)	SR (kg/weight-stack device)	Chair stand (reps)	Bicep curls (reps)	TUG (s)	Muscle mass (kg)	Fat mass (kg)	Body fat (%)	Leg muscle mass (kg)	Arm muscle mass (kg)
Morning	10.6±2.9	5.6±1.3	19±4	23±3	4.6±0.7	24.0±1.0	32.2±6.7	41.8±4.9	12.7±1.5	4.6±0.7
Evening	12.5±3.2	5.1±1.0	17±3	19±3	5.1±1.3	25.1±4.5	36.0±14.5	42.2±6.8	13.6±1.8	5.2±1.4
Control	11.4±2.0	5.2±1.1	15±3	21±4	4.2±0.7	23.9±2.6	29.3±8.4	38.5±5.6	12.5±2	4.4±0.9
	Post-training									
	LP (kg/weight-stack device)	SR (kg/weight-stack device)	Chair stand (reps)	Bicep curls (reps)	TUG (s)	Muscle mass (kg)	Fat mass (kg)	Body fat (%)	Leg muscle mass (kg)	Arm muscle mass (kg)
Morning	15.0±2.5** ^{SS}	8.1±1.3** ^{SS}	24±5** ^{SS}	29±2.4** ^{SS}	3.8±0.9**	24.9±0.9**	30.5±7.3**	39.5±5.7**	13.0±1.1	4.9±0.6
Evening	14.9±3.0** ^{SS}	7.3±1.4** ^{SS}	22±5** ^{SS}	26±4** ^{SS}	4.0±1.1**	25.3±4.6	33.3±13.5**	40.4±7.0**	13.4±1.7	5.1±1.2
Control	11.7±1.7	5.1±0.9	14±3	20±3	4.4±0.7	23.9±2.7	29.2±8.4	38.5±5.6	12.4±1.8	4.4±1.2

*0.05, **0.01 = within-group changes compared to pre-training; ^S0.05, ^{SS}0.01 = difference compared to control

Table 2 Pre- and Post-training values (Mean±SD) in metabolic and inflammation markers, and basal hormone levels in the morning and evening groups

Pre-training														
	LDL-C (mmol/ l)	sdLDL- C (mmol/ l)	HDL-C (mmol/ l)	GLU (mmol/ l)	TG (mmol/ l)	hsCRP (mmol/ l)	TAS (mmol/ l)	FSH (mIU/ml)	LH (mIU/ml)	PRL (ng/ml)	P (ng/ml)	ESTR (pg/ml)	T (ng/ml)	ALD (ng/ml)
Morning	2.6±0.8	0.4±0.2	1.5±0.5	5.8±0.4 _#	1.4±0.4 _#	2.0±1.2 _{##}	1.2±0.8	36.6±3.4	16.4±6.8	15.0±11.4 _#	7.4±3.3 _#	11.9±2.4	0.07±0.01	5.1±2.9
Evening	3.4±1.3	0.7±0.6	1.5±0.4	5.6±0.9 _#	1.7±1.0 _#	2.5±1.8 _{##}	1.3±0.1	36.1±5.3	15.6±8.3	11.4±7.4 _{##}	10.1±9.0 _#	12.3±3.4	0.08±0.03	5.5±3.0
Post-training														
Morning	3.0±0.9	0.8±0.4 _*	1.5±0.4	5.6±0.6 _*	1.6±0.4 _{**}	1.8±1.1	1.4±0.1	35.5±3.4	18.0±9.5	10.8±8.7	8.4±3.4	13.4±1.7 _*	0.07±0.02	5.0±2.3
Evening	3.6±1.4	0.8±0.5 _*	1.7±0.4	5.1±0.5 _{**}	1.3±0.7 _{***}	2.0±1.4	1.5±0.2	34.9±6.7	14.4±6.3	10.7±7.6	8.6±6.3	12.8±3.7	0.08±0.03	5.4±2.4

*0.05, **0.01 = within-group changes compared to pre-training; _#0.05, _{##}0.01 pre-training difference between the M and E group; _†0.05, _{††}0.01 = difference compared to the M group