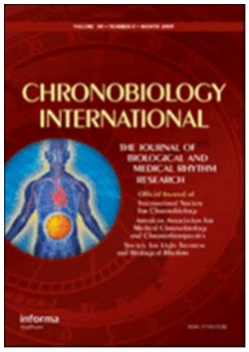

The effects of 12-week progressive strength training on strength, functional capacity, metabolic biomarkers, and serum hormone concentrations in healthy older women: morning versus evening training

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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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ABSTRACT

Previous findings suggest that performing strength training (ST) in the evening may provide greater benefit for young individuals. However, this may not be optimal for the older population. The purpose of this study was to compare the effects of a 12-week ST program performed in the morning vs. evening on strength, functional capacity, metabolic biomarker and basal hormone concentrations in older women. Thirty-one healthy older women (66±4years, 162±4cm, 75±13kg) completed the study. Participants trained in the morning (M) (07:30, n=10), in the evening (E) (18:00, n=10), or acted as a non-training control group (C) (n=11). Both intervention groups performed whole-body strength training with 3 sets of 10–12 repetitions with 2–3 minutes rest between sets. All groups were measured before and after the 12-week period with; dynamic leg press and seated-row 6-repetition maximum (6-RM) and functional capacity tests (30-second chair stands and arm curl test, Timed Up and Go), as well as whole body skeletal muscle mass (SMM) (kg) and fat mass (FM-kg, FM%) assessed by bioelectrical impedance (BIA). Basal blood samples (in the intervention groups only) taken before and after the intervention assessed low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), blood glucose (GLU), triglycerides (TG), high sensitive C-reactive protein (hsCRP) concentrations and total antioxidant status (TAS) after a 12h fast. Hormone analysis included prolactin (PRL), progesterone (P) estradiol (ESTR), testosterone (T), follicle stimulating hormone (FSH), and luteinizing hormone (LH). While C showed no changes in any variable, both M and E significantly improved leg press (+46±22% and +21±12%, respectively; $p<0.001$) and seated-row (+48±21% and +42±18%, respectively; $p < 0.001$) 6-RM, as well as all functional capacity outcomes ($p < 0.01$) due to training. M were the only group to increase muscle mass (+3±2%, $p < 0.01$). Both M and E group significantly

($p < 0.05$) decreased GLU (–4±6% and –8±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±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).

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

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

While strength and muscle mass improvements are so robust that they are observed in almost 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.

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.

Participants

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

6-RM muscular strength

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

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

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

Blood sampling and biochemical analysis

Venous blood was collected in the morning after a 12-hour fast in a standard manner from an antecubital vein. After separation of serum, samples were stored at -80°C until further analyses. Samples were assessed by immunoassay for total antioxidant status (TAS), high sensitivity C-reactive protein (hsCRP), triglycerides (TG), blood glucose (GLU) and total cholesterol (TC) concentration using a discrete photometric Clinical Chemistry Analyzer Biolis 24i Premium (Tokyo Boeki Machinery, Tokyo, Japan).

High-density lipoprotein (HDL-C), and small dense low-density lipoprotein (sdLDL-C) cholesterol were determined by detergent-based isolation and enzyme-linked colorimetric

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

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

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

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

(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.

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.

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.

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±23% and +48±22%, respectively; $p < 0.01$, Figure 2A and

2B), chair stand (+35±32%, $p < 0.01$, Figure 2C), biceps curl (+30±22%, $p < 0.01$) and TUG (–17±11%, $p < 0.01$, Figure 2D). Similarly, E significantly improved leg press and seated row 6-RM (+21±12 and +43±18%, respectively; $p < 0.01$, Figure 2A and 2B), chair stand (+34±33%, $p < 0.01$, Figure 2C), biceps curl (+36±21%, $p < 0.01$) and TUG (–20±9%, $p < 0.01$, 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×group interaction ($p < 0.01$) was found in measures of whole-body muscle mass where only M significantly (+3±2%, $p < 0.01$) increased muscle mass from pre- to post-training (Figure 2E). However, M and E both significantly decreased fat mass (–6±5% and –8±4%, respectively; $p < 0.01$, Figure 2F) and body fat % (–6±5% and –5±3%, 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±84% and 31±50%, respectively; $p < 0.05$). Both M and E groups significantly decreased the level of the GLU (–4±6% and –8±10%, 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±25%, $p < 0.01$) from pre- to post-training which differed significantly ($p < 0.01$) compared to M (+16±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±19%, $p < 0.05$) in M were observed.

DISCUSSION

The main aim of this study was to compare effects of training performed at different times of the day in a group of older women on multiple variables; including maximum strength, functional capacity, and basal biomarker and hormonal concentrations. The results show that morning and evening training groups significantly improved maximum strength, functional capacity, body composition, as well as some biomarker concentrations. The findings partially support our hypothesis that similar changes in body composition and blood markers between the groups would be observed, but there was little evidence to support our hypothesis that morning training would be more beneficial for strength and functional capacity improvement. The observed improvements in strength and functional capacity tests in M and E are not surprising since participants had no regular strength training or any other physical activity. A large number of studies have demonstrated increased maximum strength in older individuals after initiating progressive strength training (Frontera et al. 2003, Häkkinen et al. 1996). In the present study, both M and E significantly improved leg press (46% and 21%; $p < 0.001$) and seated-row (48% and 42%; $p < 0.01$) 6-RM. No significant differences were observed when comparing M and E groups in both tests, however, M demonstrated larger increases (twofold) compared to E in the leg-press 6-RM (~46±21% vs. ~21±12%). Despite non-significance, effect size values favor M compared to E (g = ~3.4 and ~2.1, respectively). It could be speculated that M gained more in the leg press due to higher quality training, since it has been shown that older individuals have greater vitality in the mornings (Wanigatunga et

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±5% and –8±4%, respectively; $p < 0.01$) and body fat percentage (–6±5% and –5±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

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

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

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

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±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 (Häkinnen 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±12%, E: +10±12%) and TAS (M: +10±12%, E: +14±11%), and E only for hsCRP (M: +0.02±47%, E: −4±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.

CONCLUSIONS

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

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DECLARATION OF INTEREST

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

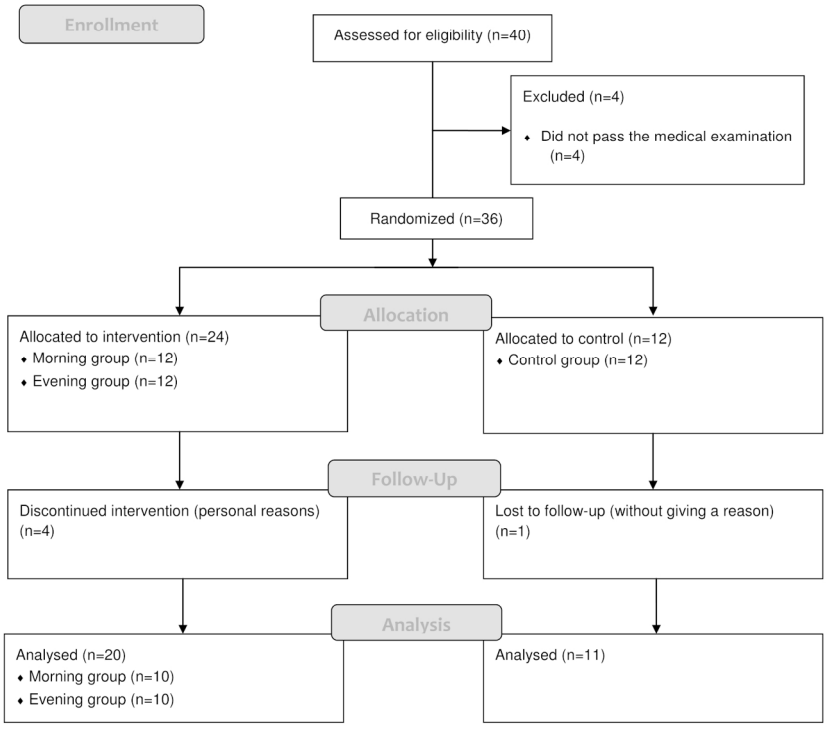

Figure 1 Consort flow diagram showing subjects enrollment in this experiment

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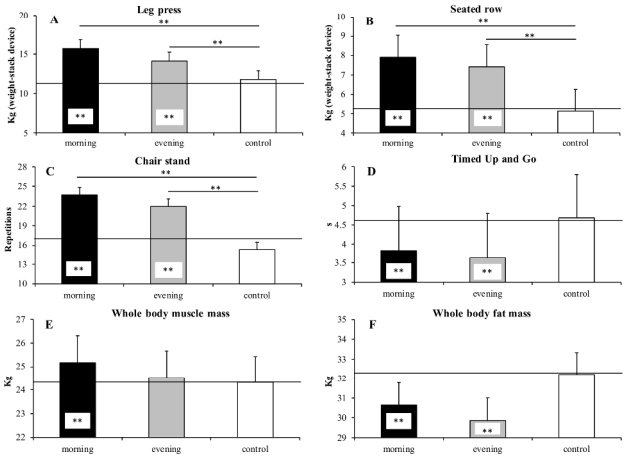

Figure 2 Changes in leg press (A) and seated row (B) 6RM strength, chair stand (C) and Timed Up and Go (D) performance, and whole-body muscle mass (E) and fat mass (F) over the study duration. Stars within the bars = within-group differences, Stars above bars = between-group differences, The horizontal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

209x297mm (300 x 300 DPI)

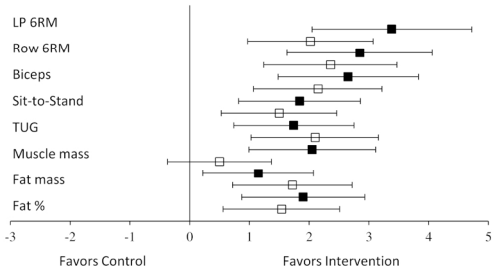

Figure 3 Effect size plot (Hedge's g) showing training-induced changes in maximum strength, functional capacity tests and body composition adjusted for control group changes. Closed squares=morning group; Open squares=evening group

583x825mm (72 x 72 DPI)

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

	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)
					Pre-training					
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					
Morning	15.0±2.5**$$	8.1±1.3**$$	24±5**$$	29±2.4**$$	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**$$	7.3±1.4**$$	22±5**$$	26±4**$$	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; $0.05, $$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

	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 (mlU/ml)	LH (mlU/ml)	PRL (ng/ml)	P (ng/ml)	ESTR (pg/ml)	T (ng/ml)	ALD (ng/ml)
Pre-training														
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