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Effects of a 12-week time-of-day-specific concurrent resistance-aerobic training on muscle strength, bone mass and functional performance in elderly women

Effects of a 12-week time-of-day-specific concurrent resistance-aerobic training on muscle strength, bone mass and functional performance in elderly women

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Abstract

Diurnal strength fluctuations and the effects of time-of-day-specific training are well-documented in young adults but unclear in elderly women. This study investigated the effects of time-of-day-specific concurrent resistance-aerobic training on muscle strength, bone mass, and functional performance in elderly women. Twenty-six women (65-85 years) were randomized into morning (MTG), afternoon (ATG) and control (CON) groups. Training groups completed 12 weeks of concurrent resistance-aerobic training (3 sessions/week, factor of time). Outcomes included maximal knee extension torque (measured AM/PM, factor of test time), bone mineral density (DXA), and functional tests (30-s chair stand, Five Times Sit-to-Stand and Maximal gait speed). Analysis of strength confirmed a preserved diurnal rhythm, with morning performance deficit present (test time: $p=0.0129$). The group and time factors interaction approached significance ($p=0.0624$), further test showed that only ATG differed significantly from the CON, with broader improvement than MTG. The functional performance significantly improved in all tests ($p<0.0001$, $p=0.0002$, $p=0.0200$, respectively) compared to baseline, with no difference between groups. The bone mass parameters remained unchanged. The time-of-day-specific concurrent training improved strength and

functional performance in both training groups with afternoon training producing superior strength gains compared to morning training. Unchanged bone parameters suggest longer interventions might be needed for osteogenic effects.

Keywords: concurrent training; time-of-day-specific training, elderly women; muscle strength; bone mass

Ageing, characterized by a progressive decline in physiological systems and bodily functions,¹ is associated with osteosarcopenia, a decrease in bone mineral density and loss of skeletal muscle mass, which leads to increased risk of falls, fractures and loss of physical function in elderly population.² To prevent the muscle mass and bone mineral density loss and maintain a healthy ageing, regular exercise is recommended.³⁻⁵

Estrogen is the primary hormone that declines during menopause and significantly affects musculoskeletal health, leading to accelerated bone loss, while also negatively impacting joint, and muscle health. Research shows that over 70% of women experience musculoskeletal symptoms during the menopause transition, with 25% becoming disabled by them, which results in an increased risk of osteoporosis, sarcopenia, muscle weakness, and fractures .^{6,7} To counteract these changes, resistance training, with appropriate exercise intensity ($\geq 70\%$ of 1RM) and training period duration (24 weeks), is known to have impact on bone health improvement, increasing bone mineral density in post-menopausal women.^{5,8-10}

As both resting muscle tone and neuromuscular performance exhibit a diurnal variation,¹¹ the response to resistance training may depend on the time of day at which it is performed. Skeletal muscle strength typically peaks in the afternoon,¹¹ whereas a relative neuromuscular deficit is observed in the morning.¹² Although overall strength gains from morning and evening training appear to be comparable, evening training tends to preferentially improve performance later in the day, whereas morning training enhances performance in the morning and attenuates the amplitude of the diurnal strength rhythm.^{12,13}

Although current guidelines consistently advocate combined aerobic and resistance training to preserve musculoskeletal health in older adults,¹⁴⁻¹⁶ recent ICFSR global consensus recommendations¹⁷ specify that aerobic training should initially be performed in short bouts (~5–10 min) and progressively extended toward ~20–30 min per session, beginning at moderate intensity (approximately 40–60% Heart-Rate Reserve (HRR), or an equivalent perceived exertion) and progressing toward vigorous intensity (60–85% HRR) as tolerance and fitness improve. In parallel, progressive resistance training is recommended 2–3 times per week, targeting major muscle groups with approximately 6–10 exercises, starting with 1–2 sets and progressing to 2–3 sets of 8–12 repetitions, with intensity increased from ~50% 1RM toward ~70–80% 1RM and further adjusted based on perceived effort. However, these documents provide little guidance on whether the timing of such exercise influences its effectiveness. This represents a critical gap, because circadian variation in neuromuscular performance, endocrine responses and bone turnover suggests that identical exercise stimuli performed at different times of day may not be physiologically equivalent. Existing chrono-exercise studies have been conducted predominantly in young, healthy, often male populations,^{18,19} and have focused on performance outcomes rather than bone or functional status, rarely using concurrent resistance-aerobic protocols. Evidence in post-menopausal or older women, the very group at highest risk for osteosarcopenia and fracture is scarce and inconclusive.²⁰

Accordingly, the purpose of the study was to determine whether a 12-week time-of-day-specific concurrent resistance-aerobic training programme performed in the morning versus the afternoon differentially affects muscle strength, functional performance, and bone mass parameters in elderly women.

Materials and Methods

Participants

Twenty-six healthy elderly women were recruited and randomly assigned to one of three groups: a morning training group (MTG; n=10), an afternoon training group (ATG; n=10), and a control group (CON; n=6). Participants allocated to the training groups were distributed homogeneously based on age, body mass index (BMI) and baseline strength test of maximal voluntary isometric knee extension results across MTG and ATG. Participants were eligible for inclusion if they met the following criteria: i) female sex, ii) age 65-85 years, iii) no previous history of regular structured training or exercise meeting the minimum physical activity recommendations of the World Health Organization,²¹ defined

as ≥ 150 minutes per week of moderate-intensity activity or ≥ 75 minutes per week of vigorous-intensity activity, iv) BMI between 18.5 and 35.0 kg/m². Exclusion criteria were: i) current or recent acute infection, ii) physical or mental disabilities that could limit safe participation in the exercise intervention or completion of study assessments, iii) diagnosed malignant, cardiovascular, metabolic, or autoimmune disease, iv) clinical evidence of malnutrition, and v) current pharmacological treatments known to interfere with musculoskeletal or inflammatory responses to exercise. All participants underwent a pre-participation medical examination by a sports medicine physician to determine eligibility and obtain medical clearance for the study. Before enrolment, participants received detailed verbal and written information about the study design, procedures, and potential risks, and subsequently provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki (1964), as revised in 2013,²² and approved by the Ethics Committee of the Faculty of Physical Education and Sport, Comenius University, Bratislava, Slovakia (Protocol No. 33/2022).

Study design

A randomized, controlled trial was used to examine the effects of time-of-day-specific 12-week concurrent resistance-aerobic exercise programme with two supervised sessions and one home-based training per week on muscle strength, bone mass and functional performance in 26 elderly women. Previously inactive elderly women were recruited and randomly assigned to one of the three groups. Participants allocated to the groups were distributed homogeneously based on age, body mass index (BMI) and baseline strength test of maximal voluntary isometric knee extension results across MTG, ATG and CON. All outcomes were assessed during a three-week period at baseline (PRE) and after completion of the 12-week intervention (POST). The tests were performed under the supervision of two or three researchers with previous testing experience.

Training programme

Participants allocated to MTG and ATG completed a 12-week concurrent resistance-aerobic exercise programme, while the CON group was instructed to maintain usual daily activities. All supervised sessions were conducted at the Centre of Active Ageing, Faculty of Physical Education and Sport, Comenius University in Bratislava, Slovakia. Supervised sessions were conducted as group-based training, ensuring that all participants within the MTG (08:00 to 09:00) and ATG (16:00 to 17:00)

started at the same clock time. The selection of the training windows was based on both physiological and practical considerations. The afternoon window was chosen to align with the typical circadian peak in core temperature and neuromuscular performance¹¹ and the morning window represents the circadian nadir, while also selected to prioritize participant safety and maximize compliance.

Participants were instructed to perform their Home-Based Training (HBT) at the same time of day. Each supervised session lasted approximately 60 min and comprised a 10-min warm-up, 35 min of resistance exercise, 10 min of aerobic interval exercise, and a 5-min cool down.

During the first 2 weeks, participants attended two supervised familiarization sessions per week (Mondays and Fridays), focusing on familiarization with the programme and learning correct exercise technique with resistance loads of 12–10 Repetition Maximum (RM) for 1-2 sets per exercise. From week 3 onwards, the programme progressed to three sessions per week for 10 weeks, with supervised sessions on Mondays and Fridays and a HBT on Wednesdays. Supervised resistance training targeted major muscle groups and included deadlifts, chest presses/push-ups, split squats/squats, seated pulldowns, leg press, TRX rows/seated rows, knee extensions, overhead press) using 1-3 sets in the 12-6 RM range, progressing from higher- to lower-repetition, higher-load work over time. Exercises were organized in pairs with ~30 s rest between exercises and inter-set rest periods of approximately 2-3 minutes. HBT consisted of submaximal resistance exercise (2-3 sets of 10-12 repetitions) using bodyweight and resistance bands, guided by a standardized video template and supported by regular contact with the supervising trainer via WhatsApp, where participants sent a photo at the start of each session to verify compliance. Each HBT session lasted approximately 50 min and comprised a 10-min warm-up, 35 min of resistance exercise, and a 5-min cool down.

The aerobic component, which was included only in the supervised training sessions, consisted of high-intensity interval cycling on an air bike (Renegade AirBike PM3). After a brief warm-up, participants performed 5-8 intervals of 20 s at a Rating of Perceived Exertion (RPE) of approximately 12-16 on the 6-20 scale,²³ interspersed with 40 s of active recovery at RPE 8-10, followed by a short one-minute active cool down. The number of intervals and target RPE were progressively adjusted across the intervention to ensure an appropriate increase in training stimulus while maintaining safety.

Measurements and data collection

Before muscle strength and functional performance data collection, participants performed standardized preliminary warm-up (~5 min). All testing sessions were conducted indoors under

controlled environmental conditions (temperature: 22-25 °C). All the participants included in the study were instructed to avoid any caffeine intake during the testing, but not during the training intervention period.

Anthropometry and bone mass analysis

Standing body height was measured to the nearest 0.1 cm using a digital free-standing stadiometer (InBody BSM 170, InBody Co., Ltd., USA). Whole-body and regional body composition, including bone parameters, were assessed by dual-energy X-ray absorptiometry (DXA; Hologic fan-beam bone densitometer, Discovery QDR series). All DXA scans were performed by a trained medical professional at University Hospital Ružinov, Bratislava, Slovakia following standardized manufacturer procedures.

From the DXA analyses, Bone Mineral Content (BMC; g), Bone Mineral Density (BMD; $\text{g}\cdot\text{cm}^{-2}$), and T-score for the whole body, Proximal Femur (PF), and Lumbar Spine (LS) were obtained. Regions of interest were defined according to the manufacturer's anatomical landmarks and analysed consistently across pre- and post-intervention assessments. Total body mass was derived from the DXA whole-body scan, and Body Mass Index (BMI) was calculated as total body mass (kg) divided by height squared (m^2).

Muscle strength

Lower-limb muscle strength was assessed as maximal voluntary isometric knee extension using a previously calibrated adjustable dynamometric chair with a knee dynamometer (ARS Dynamometry; S2P Ltd., Ljubljana, Slovenia), a method previously validated by Sarabon *et al.*²⁴ Participants were seated with the chair and lever arm individually adjusted to their anthropometry; the knee joint was fixed at the 60° of flexion (0° = full extension) and the hip at 90° of flexion. A padded support was positioned on the anterior shin ~4-5 cm proximal to the lateral malleolus to minimize discomfort, and the pelvis was secured with a strap to prevent extraneous movement. Each session began with two submaximal warm-up contractions (3-5 s at ~50% perceived maximal effort) separated by 30 s rest. Participants then performed two maximal bilateral knee-extension Maximal Voluntary Contraction (MVC) trials of 5 s duration, with 60 s rest between trials, while receiving standardized verbal encouragement. Peak torque was recorded, and the highest value was retained for analysis. Absolute MVC was expressed as peak bilateral torque, and relative knee extension torque was normalized to fat-

free mass derived from DXA. Strength testing was conducted twice at both PRE and POST: once in the morning (AM test; 08:00-09:00) and once in the afternoon (PM test; 16:00-17:00). The tests were performed on separate days with 1-3 rest days between sessions.

Functional performance

Functional lower-limb performance was assessed using the 30-s chair stand test and the Five Times Sit-to-Stand (5xSTS) test.^{25,26} Both tests were performed using a 45-cm plyobox placed against a wall to prevent movement. Participants began seated with their back upright, feet approximately shoulder-width apart and flat on the floor, and arms crossed over the chest. After a standardized demonstration and a single practice repetition, the 30-s chair stand test was administered by instructing participants to stand up fully and sit down again as many times as possible within 30 s, ensuring full contact with the box between repetitions. For the 5xSTS test, participants were instructed: “stand up and sit down 5 times as quickly as possible when I say ‘Go’.” Timing (to the nearest 0.1 s) started on the verbal cue “Go” and stopped when the participant’s buttocks contacted the box after the fifth stand. Only correctly executed stands were counted, and the score was the total number of valid stands completed in 30 s and the total time required to complete five valid repetitions. In both tests, participants were asked to fully extend the hips and knees in the standing position, avoid using their arms for assistance, and not contact the wall behind them. Standardized verbal encouragement was provided throughout.

Maximal gait speed was assessed over a 10-m distance using a photocell timing system (Microgate, Bolzano, Italy). A 12-m straight walkway was used, comprising a 1-m acceleration zone, a 10-m timed section, and a 1-m deceleration zone. Two pairs of infrared photocells were placed at the beginning and end of the 10-m section at approximately hip height. From a standing start behind the acceleration line and after the verbal cue “Go”, participants were instructed to walk, accelerate over the first m, and then walk “as fast and safely as possible” through the 10-m timed section, continuing into the deceleration zone. Timing was triggered when the leading leg broke the first beam and stopped when the second beam was interrupted. Time was recorded to the nearest 0.01 s, and gait speed ($\text{m}\cdot\text{s}^{-1}$) was calculated as 10 m divided by the recorded time. Each participant performed two trials, and the fastest time was retained for analysis.

Functional performance tests were integrated into the first and last supervised training sessions for the MTG and ATG, therefore the functional performance data were not available for the CON group.

Statistical analysis

Data are presented as mean \pm Standard Error of the Mean (SEM). All analyses and graphical outputs were performed using GraphPad Prism (version 8.0.1; GraphPad Software, San Diego, CA, USA). Distributions were evaluated for normality or lognormality using the Shapiro–Wilk and D’Agostino–Pearson tests. The level of statistical significance was set at $p < 0.05$. Outliers were identified using the ROUT method (Robust regression and Outlier removal) in GraphPad Prism, with the maximum False Discovery Rate (Q) set to 2%. All outcomes except relative knee extension torque were analysed using two-way Repeated-Measures Analysis Of Variance (RM-ANOVA) with time (PRE vs POST) as the within-subject factor and group (MTG, ATG, CON) as the between-subject factor. When significant interactions or main effects were detected, Sidak-adjusted multiple comparisons were used for post hoc testing. Relative knee extension torque was analysed using a mixed-design three-way RM-ANOVA with time (PRE vs POST) and test time (morning vs afternoon) as within-subject factors and group as the between-subject factor. In addition, a two-way RM-ANOVA (time \times group) was performed, with consolidated data combining the results of AM and PM test times, with Sidak-adjusted multiple comparisons. This study is a part of a bigger project involving both elderly males and females, for which an *a priori* power analysis was conducted using G*Power 3 software. Based on a Type I error (α) of 0.01 and a statistical power of 85% to detect changes in $VO_2\text{max}$, lean mass, fat mass, and aMT6s concentration, a target sample size of 20 participants per group was estimated. For the present analysis, which focuses specifically on elderly females, the final sample comprised 26 participants (MTG: $n = 10$, ATG: $n = 10$, CON: $n = 6$).

Results

Body mass and BMI remained largely stable across the intervention in all groups. In the MTG, mean body mass was 73.01 ± 3.10 kg at baseline and 72.43 ± 3.01 kg post-intervention, with corresponding BMI values of 27.41 ± 0.81 and 27.19 ± 0.75 $\text{kg}\cdot\text{m}^{-2}$. In the ATG, body mass was 68.46 ± 2.45 kg at baseline and 68.27 ± 2.48 kg post-intervention, and BMI was 26.88 ± 0.87 and 26.81 ± 0.89 $\text{kg}\cdot\text{m}^{-2}$, respectively. In the CON group, mean body mass was 69.64 ± 4.43 kg at the baseline and 69.01 ± 4.59 kg at the end of the study, with BMI values 27.46 ± 1.71 and 27.20 ± 1.75 $\text{kg}\cdot\text{m}^{-2}$, respectively. Overall, only minimal pre-to-post variations were observed.

Muscle strength

One participant from the CON group was excluded from the analysis due to missing data (CON, $n = 5$). Mean morning and afternoon values increased in MTG (+26% and +30%, respectively), ATG (+46% and +42%, respectively), and CON (+27% and +3%, respectively) (Figure 1). A three-way mixed design RM-ANOVA revealed that group \times time interaction approached statistical significance ($p = 0.0624$), and consequently, two-way mixed-design RM-ANOVA analysing factors of group and time showed a significant time \times group interaction ($p = 0.0488$). Sidak's post hoc tests confirmed no significant differences between the groups at baseline (PRE; $p > 0.05$). However, a significant difference was found between the ATG and CON groups ($p = 0.0315$), but not between any of the other pairs. A within-group analysis showed that, while the CON group remained unchanged ($p = 0.5515$), both training groups significantly increased their strength from PRE to POST (MTG: $p = 0.0029$; ATG: $p < 0.0001$). Furthermore, the ATG achieved significantly higher POST strength values than the CON group ($p = 0.0018$) and the MTG ($p = 0.0437$). No significant difference was found between the MTG and CON groups post-intervention ($p = 0.3413$). The three-way RM-ANOVA analysis also showed a significant main effect of test time ($p = 0.0129$) (Figure 1). On average, the afternoon values were higher than the morning values at both PRE and POST in MTG (+6% and +9%, respectively) and ATG (+9% and +7%, respectively). In the CON group, afternoon values exceeded morning values at PRE (+18%) but were slightly lower at POST (-4%).

Bone mass

The BMC values did not change significantly over time in any of the groups ($p = 0.2290$) (Table 1). For BMD and T-score, there was a trend towards a main effect of time (BMD: $p = 0.0529$; T-score: $p = 0.0627$) (Figure 2). Two participants were removed from the ATG in PF analysis ($n = 8$), one due to missing POST value data and one identified as a statistical outlier at baseline ($Q = 2\%$). The outlier was removed because its inclusion caused the data to fail normality testing; however, its exclusion did not alter the overall findings of the ANOVA regarding the training intervention (Table 1). BMC, BMD, and T-scores of PF and LS did not change significantly over time in any of the groups ($p > 0.05$ for all).

Functional performance

In the training groups, functional performance improved from PRE to POST in the 30-s chair stand test, the 5xSTS, and the maximal gait speed test (Figure 3a–c). Two-way RM-ANOVA showed significant main effects of time for these outcomes ($p < 0.0001$, $p = 0.0002$, $p = 0.0200$, respectively), indicating

overall gains in functional performance, but no significant group \times time interactions ($p \geq 0.69$ for all), indicating that the magnitude of improvement did not differ between the MTG and ATG.

Discussion

In this study, we investigated whether a 12-week, time-of-day-specific, concurrent resistance-aerobic training programme influences adaptations in bone mass, muscle strength, and functional performance in previously inactive elderly women. Our primary objective was to compare responses to morning versus afternoon training. Overall, the intervention improved lower-limb strength relative to the control, and functional performance significantly increased in both training groups, with afternoon training associated with the most pronounced changes.

The efficacy of resistance training in elderly women is well-established, with studies confirming that 12-week programmes,^{20,27} or even interventions as short as 6 weeks,²⁸ significantly improve skeletal muscle strength, mass, and quality. Consistent with these findings, we observed a significant improvement in knee extension torque across the training groups. Our findings indicate that the timing of the exercise session modulates the magnitude of the adaptive response. While the MTG showed respectable average improvements (+26% in the morning performance and +30% in the afternoon), the ATG demonstrated significantly greater relative gains, increasing by approximately 46% and 42% in AM and PM tests, respectively. This outcome differs from previous findings in elderly women,²⁰ which reported no meaningful differences in strength gains between morning and afternoon resistance training over 12 weeks, although morning training was suggested to be more favourable for hypertrophic adaptations. Our findings suggest that afternoon training may provide greater improvements in maximal knee extensor strength than morning training and no training.

Regarding the time-of-day effect, our data support the existence of a diurnal rhythm in muscle strength. This is consistent with previous literature identifying an afternoon/evening peak in neuromuscular performance,¹¹ and associated morning performance deficit,¹² since afternoon torque values generally exceeded morning values across all groups. However, the adaptation to training in our cohort contrasted with the “temporal specificity” observed in younger populations. In younger adults, morning training typically reduces the morning neuromuscular deficit (reducing the diurnal amplitude), while afternoon training effectively improves same timed performance.^{13,18,19} In contrast, our results suggest a broader adaptation in elderly women. The ATG participants improved their performance markedly in

the morning as well, defying the expectation that the afternoon training preserves the diurnal gap.^{13,18,19} Conversely, the MTG did not demonstrate the expected specific enhancement of morning performance. Regarding bone mass, DXA analyses revealed no statistically significant changes in bone parameters over the course of the study. Whole-body BMC, BMD and T-scores remained stable across the groups, with no meaningful alterations observed in site-specific parameters at the PF or LS. This indicates that the applied training stimulus may not have been sufficient to induce measurable skeletal adaptations within the 12-week intervention. This aligns with earlier research showing that both the intensity and duration of resistance training are critical determinants of bone adaptation. Moderate-to-high intensity resistance exercise ($\geq 70\%$ of 1RM) has been shown to significantly increase BMD in older adults and postmenopausal women.^{8,9} Compared to lower-intensity or non-resistance interventions, protocols that combine high-intensity strength training with impact or weight-bearing activities generally elicit more pronounced osteogenic responses.^{5,29} Longer-term exercise interventions (≥ 24 weeks) are generally associated with greater increases in BMD, whereas shorter programmes often fail to produce detectable changes.⁹ This supports the notion that bone remodelling is a slow process, and that a minimum loading period of approximately 24 weeks may be required to elicit significant mineral accrual. On the other hand, very prolonged interventions (> 1 year) may be accompanied by a reduction in skeletal responsiveness to a given mechanical stimulus, potentially attenuating training effects unless new or more varied loading modalities are introduced.⁸ Training frequency is also critical; programmes with three sessions per week typically yield larger osteogenic adaptations than those with fewer weekly sessions.^{8,9} In our study, participants completed three sessions per week, but the HBT was performed using only bodyweight and resistance-band exercises, whereas only the two supervised sessions provided high-intensity mechanical loading. Thus, the effective frequency of high-magnitude loading was likely limited to two sessions per week. The combination of a relatively short intervention duration (12 weeks) and suboptimal high-load frequency probably contributed to the absence of significant changes in bone parameters in the present cohort.

Our training programme led to significant improvements in all functional performance tests: 30-s chair stand test, 5xSTS and maximal gait speed test, with no significant difference between the MTG and ATG. These findings are consistent with previous studies showing that relatively short resistance training interventions can enhance lower-limb function in older women, with improvements reported after 8 weeks for sit-to-stand performance,³⁰ and as little as 6 weeks of twice-weekly resistance training for the 30-s chair stand test.²⁸ Importantly, better performance in these tests has been closely linked to

greater independence in daily life. Higher 30-s chair stand scores are associated with lower dependency in Activities of Daily Living (ADL),³¹ while slower 5xSTS performance predicts subsequent disability and loss of instrumental ADL in older adults and an inability to complete the test has been identified as a marker of increased fall risk.³² Similarly, higher 10-m gait speed is associated with better ADL function³³ and has been shown to mediate the relationship between sarcopenia and dependency in ADL, suggesting that improvements in gait speed may attenuate the adverse functional consequences of sarcopenia.³⁴

There are limitations to the current study, including relatively small sample size, which is a consequence of the difficult process of recruiting eligible healthy participants from the elderly population. Specifically, the limited number of participants in the CON group and the further reduction in the PF DXA analysis ($n = 8$) may have reduced statistical power. This limitation increases the risk of Type II error, whereby true physiological effects might have been underestimated or remained undetected. Furthermore, we relied on participants' adherence to the instructions regarding the maintenance of their usual daily activity and nutritional intake, which was not strictly monitored. The HBT sessions likely provided less mechanical loading than the supervised sessions, even though this hybrid approach increases feasibility. The 12-week intervention period proved to be sufficient for improving muscular adaptations and functional capacity, although a longer intervention involving more frequent resistance training sessions might be needed to conclusively determine the effect of training on bone mass. It is also important to note that functional tests were not performed in the CON group. Therefore, we cannot strictly isolate the training effect from the potential test-retest improvements in these measures. One of the limitations was the absence of a greater familiarization period before the strength tests. This introduced a confounding learning effect which likely caused the CON group's improvement between the first PRE AM test and the PRE PM test (+18%). As the two tests were performed at maximum three days apart, this performance increase could not have been influenced by lifestyle changes, but rather by the participants learning the test execution. It is reasonable to assume that a similar learning effect occurred in the MTG and ATG groups, implying that baseline strength levels were likely underestimated, particularly in the PRE AM tests. This is particularly relevant given that older subjects are reported to need multiple familiarization sessions to achieve consistent strength measurements.^{35,36} Additionally, although individual chronotypes were recorded, they were not factored into the participant stratification or the current analysis. Given that morningness-eveningness preference may influence responsiveness to time-of-day-specific training, this interaction will be

addressed in our future investigation. Furthermore, habitual caffeine intake was not controlled. While caffeine can negate the morning strength deficit in young highly trained men,³⁷ this effect has not been investigated in older adults. Moreover, studies in elderly indicate that caffeine does not augment maximal voluntary strength, suggesting its impact on our strength outcomes was likely negligible.^{38,39}

Conclusions

In conclusion, a 12-week time-of-day-specific concurrent resistance-aerobic training programme was found to significantly improve muscle strength and functional performance in previously inactive elderly women. Notably, afternoon training produced superior strength gains compared to morning training, suggesting that it provides a more effective stimulus for muscular adaptation. However, bone mass parameters remained unchanged, indicating that a longer intervention or higher-frequency loading is necessary to achieve osteogenic effects. While afternoon sessions appear to be optimal for increasing strength, larger sample sizes are required in future studies to confirm these time-specific adaptations.

List of Abbreviations

5xSTS, Five Times Sit-to-Stand

ADL, activities of daily living

ANOVA, analysis of variance

ATG, afternoon training group

BMC, bone mineral content

BMD, bone mineral density

BMI, body mass index

CON, control group

DXA, dual-energy X-ray absorptiometry

HBT, home-based training

HRR, heart-rate reserve

LS, lumbar spine

MTG, morning training group

MVC, maximal voluntary contraction

PF, proximal femur

RM-ANOVA, repeated measures analysis of variance

RM, repetition maximum

RPE, rating of perceived exertion

SEM, standard error of the mean

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Contributions

Conceptualization, organization and supervision, Viktor Oliva, Milan Sedliak and Ľudmila Oreská; participants' recruitment, Viktor Oliva, MSt, Ľudmila Oreská, Milan Sedliak; protocol design and implementation, Viktor Oliva, Michal Štrelecký, Milan Sedliak; examinations and data acquisition, Viktor Oliva, Michal Štrelecký, Milan Sedliak; data analysis, Gabriela Šašiovová, Barbora Kundeková;

visualization, Gabriela Šašiovová, Barbora Kundeková, manuscript preparation, Gabriela Šašiovová, Barbora Kundeková and Viktor Oliva; review, editing and validation, Gabriela Šašiovová, Barbora Kundeková, Ľudmila Oreská, Milan Sedliak, Michal Střelecký, Monika Okuliarová, Michal Zeman, Zdenko Kilinger, Juraj Payer and Viktor Oliva. All authors reviewed and edited the manuscript, provided substantial intellectual contributions, and approved the final version for publication. The authors take full responsibility for the content of this work.

Conflict of interests

The authors declare no financial, personal, or other conflicts of interest.

Ethics approval

The study was conducted in accordance with the Declaration of Helsinki (1964), as revised in 2013, and approved by the Ethics Committee of the Faculty of Physical Education and Sport, Comenius University, Bratislava, Slovakia (Protocol No. 33/2022). Informed consent was obtained from all subjects involved in the study prior to participation.

Availability of data and materials

All data generated or analysed during this study are included in the article. Further inquiries can be directed to the corresponding author.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

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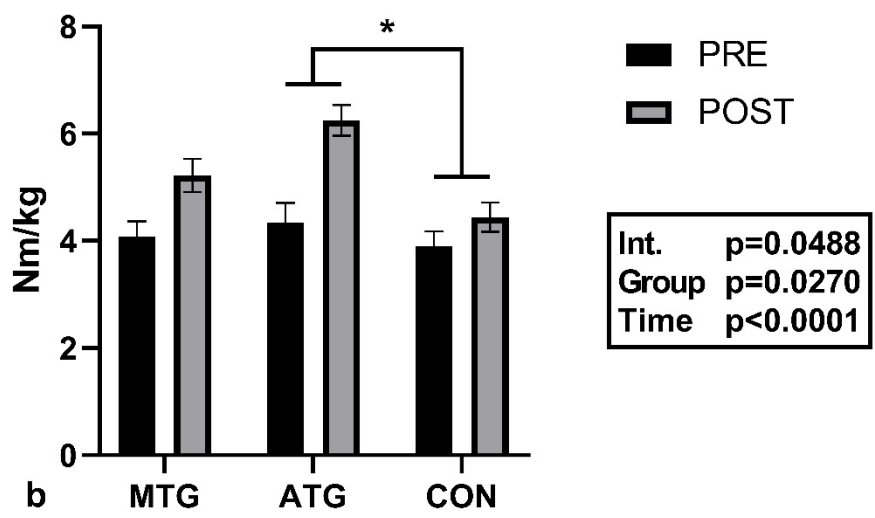
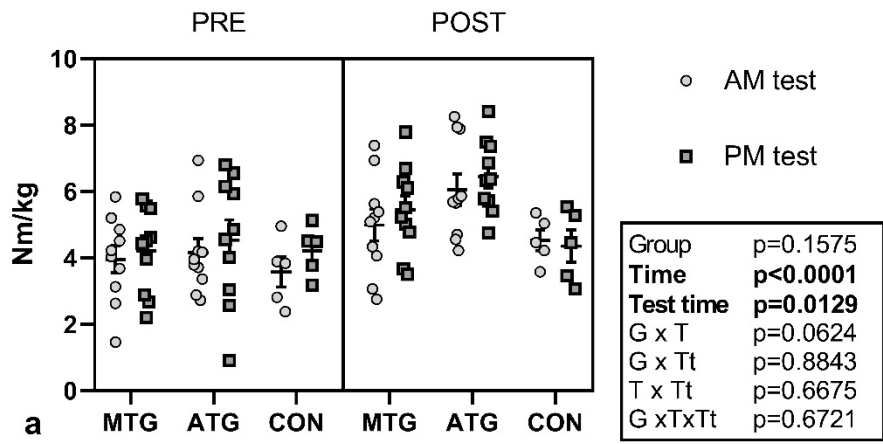


Figure 1. Relative knee extension torque. a. Three-way repeated measures ANOVA analysis for the factors of time, test time and group. b. Two-way repeated measures ANOVA analysis for the factors of time and group. Values are expressed as a mean \pm SEM. PRE – before intervention, POST – after intervention. MTG – morning training group (n=10), ATG – afternoon training group (n=10), CON – control group (n=5). Results of the three and two-way repeated measures ANOVA analyses are listed in the tables on the right side of the figures. Results of Sidak post hoc analysis are represented by the lines in the corresponding graph.

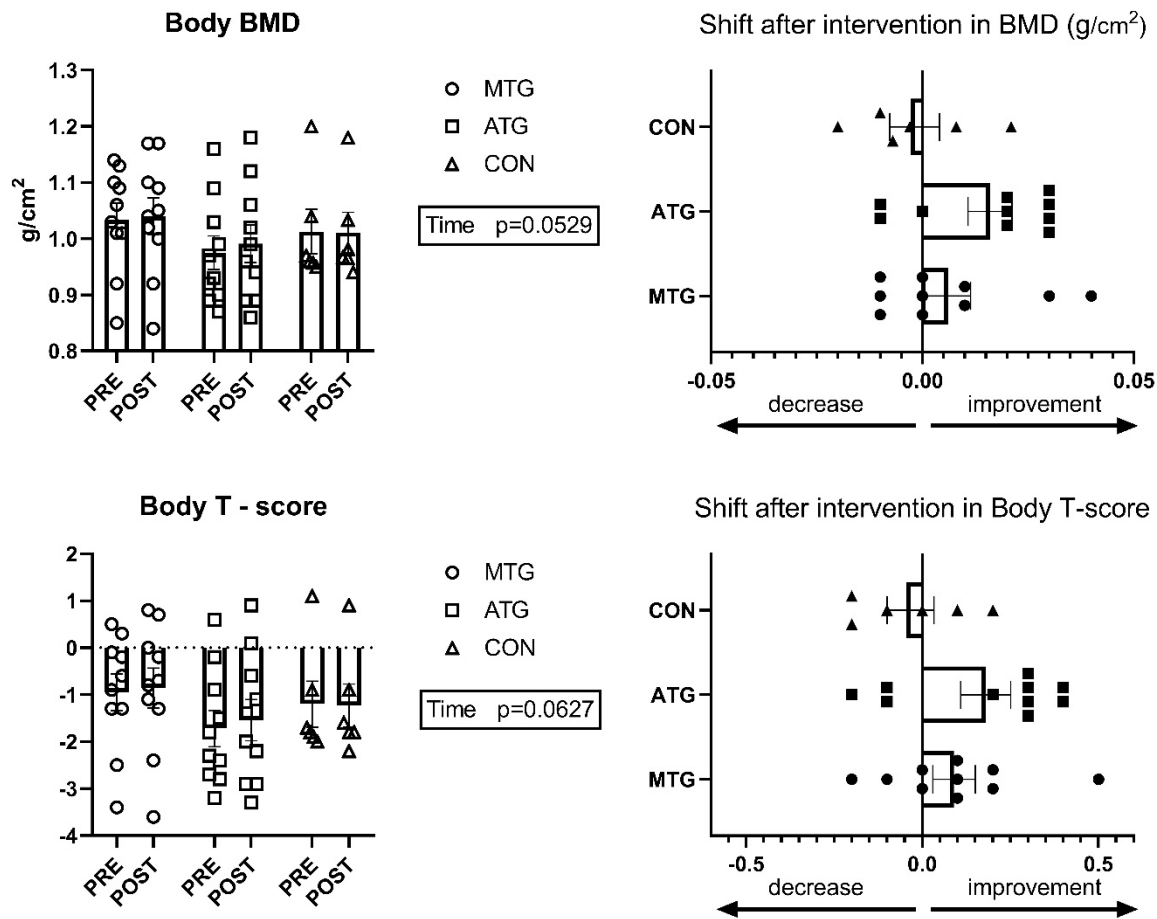


Figure 2. Body bone mineral density and T-score measured by DXA scan. Values are expressed as a mean \pm SEM. MTG – morning training group (n=10), ATG – afternoon training group (n=10), CON – control group (n=6), PRE – before intervention, POST – after intervention, BMD – bone mineral density. Two-way repeated measures ANOVA analysis results are displayed in the tables.

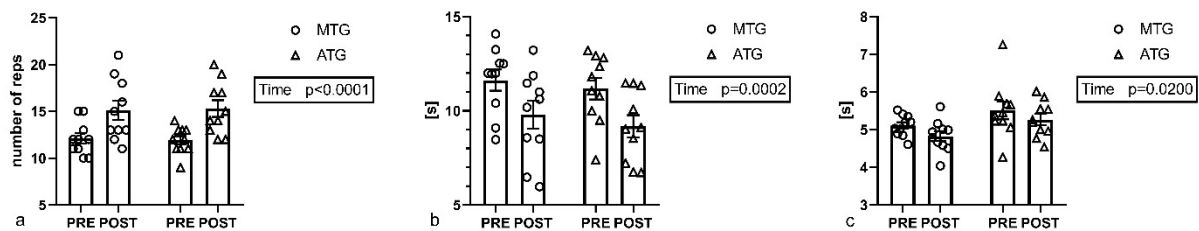


Figure 3. Functional tests. a. 30-s chair stand test from Senior fitness test battery. b. 5 Times Sit-to-Stand Test. c. maximal gait speed test. MTG – morning training group (n=10), ATG – afternoon

training group (n=10), PRE – before intervention, POST – after intervention. Values are expressed as a mean \pm SEM. Two-way repeated measures ANOVA analysis results are displayed in the tables.

Table 1. Bone composition parameters measured by DXA scan.

PARAMETER	MORNING TRAINING GROUP			AFTERNOON TRAINING GROUP			CONTROL GROUP		
	PRE	POST	n	PRE	POST	n	PRE	POST	n
BMC (g)	2017 \pm 76.63	2012 \pm 78.43	10	1820 \pm 63.81	1831 \pm 71.43	10	1884 \pm 81.92	1898 \pm 85.79	6
BMD	1.034 \pm 0.03	1.040 \pm 0.03	10	0.975 \pm 0.03	0.991 \pm 0.03	10	1.013 \pm 0.04	1.011 \pm 0.04	6
T-score	-0.95 \pm 0.39	-0.86 \pm 0.43	10	-1.72 \pm 0.39	-1.54 \pm 0.44	10	-1.20 \pm 0.49	-1.23 \pm 0.46	6
PF BMC (g)	33.53 \pm 1.1	33.44 \pm 1.1	10	28.46 \pm 0.7	28.32 \pm 0.77	8	30.49 \pm 1.18	30.67 \pm 2.78	6
PF BMD	0.864 \pm 0.03	0.868 \pm 0.03	10	0.770 \pm 0.01	0.773 \pm 0.01	8	0.826 \pm 0.02	0.816 \pm 0.02	6
PF T-score	-0.63 \pm 0.25	-0.60 \pm 0.27	10	-1.41 \pm 0.11	-1.39 \pm 0.11	8	-0.93 \pm 0.18	-1.03 \pm 0.15	6
LS BMC (g)	57.69 \pm 4.1	58.38 \pm 4.02	10	56.55 \pm 4.39	57.42 \pm 4.65	10	47.03 \pm 7.26	46.79 \pm 7.31	6
LS BMD	0.985 \pm 0.06	0.983 \pm 0.06	10	0.956 \pm 0.07	0.969 \pm 0.07	10	0.948 \pm 0.05	0.95 \pm 0.05	6
LS T-score	-0.55 \pm 0.51	-0.59 \pm 0.51	10	-0.84 \pm 0.63	-0.71 \pm 0.64	10	-0.95 \pm 0.46	-0.95 \pm 0.49	6