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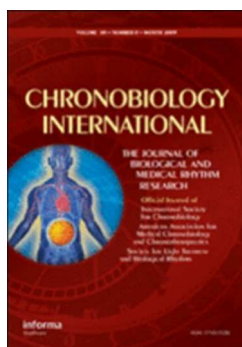
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**The effects of 12-week progressive strength training on strength, functional capacity, metabolic biomarkers, and serum hormone concentrations in healthy older women: morning versus evening training**

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

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1 **Title:** The effects of 12-week progressive strength training on strength, functional capacity, metabolic  
2 biomarkers, and serum hormone concentrations in healthy older women: morning versus evening  
3 training  
4

5 **Running Head:** Training at different times of the day in women  
6

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26

27 **ABSTRACT**

28 Previous findings suggest that performing strength training (ST) in the evening may provide  
29 greater benefit for young individuals. However, this may not be optimal for the older  
30 population. The purpose of this study was to compare the effects of a 12-week ST program  
31 performed in the morning vs. evening on strength, functional capacity, metabolic biomarker  
32 and basal hormone concentrations in older women. Thirty-one healthy older women  
33 (66±4years, 162±4cm, 75±13kg) completed the study. Participants trained in the morning  
34 (M) (07:30, n=10), in the evening (E) (18:00, n=10), or acted as a non-training control group  
35 (C) (n=11). Both intervention groups performed whole-body strength training with 3 sets of  
36 10–12 repetitions with 2–3 minutes rest between sets. All groups were measured before and  
37 after the 12-week period with; dynamic leg press and seated-row 6-repetition maximum (6-  
38 RM) and functional capacity tests (30-second chair stands and arm curl test, Timed Up and  
39 Go), as well as whole body skeletal muscle mass (SMM) (kg) and fat mass (FM-kg, FM%)  
40 assessed by bioelectrical impedance (BIA). Basal blood samples (in the intervention groups  
41 only) taken before and after the intervention assessed low-density lipoprotein (LDL-C), high-  
42 density lipoprotein (HDL-C), blood glucose (GLU), triglycerides (TG), high sensitive C-  
43 reactive protein (hsCRP) concentrations and total antioxidant status (TAS) after a 12h fast.  
44 Hormone analysis included prolactin (PRL), progesterone (P) estradiol (ESTR), testosterone  
45 (T), follicle stimulating hormone (FSH), and luteinizing hormone (LH). While C showed no  
46 changes in any variable, both M and E significantly improved leg press (+46±22% and  
47 +21±12%, respectively; p<0.001) and seated-row (+48±21% and +42±18%, respectively; p <  
48 0.001) 6-RM, as well as all functional capacity outcomes (p < 0.01) due to training. M were  
49 the only group to increase muscle mass (+3±2%, p < 0.01). Both M and E group significantly

( $p < 0.05$ ) 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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3 73 Strength training in older individuals has become one increasing research focus over the past  
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5 74 three decades in order to understand whether (and how) this form of exercise is efficacious to  
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7 75 reverse biological aging processes. In particular sarcopenia, which is defined by both loss of  
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9 76 skeletal muscle mass and muscle function (either muscle strength or functional capability), is  
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11 77 a major health issue in our aging society. Recent evidence suggests that the cause of  
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13 78 sarcopenia may be several factors including neural, hormonal and inflammatory changes,  
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15 79 along with or due to decreased physical activity connected with poor nutritional status  
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18 80 (Walston 2012). Studies in older individuals have shown increased muscular strength and  
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20 81 muscle fiber cross-sectional area (Frontera et al. 2003, Häkkinen et al. 1996, Sipilä and  
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22 82 Suominen 1995), improvements in tests of physical function (30-seconds chair stand, 30-  
23  
24 83 seconds arm curl) (Dias et al. 2015, Pinto et al. 2014), improved body composition including  
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26 84 lower body fat and increased upper and lower limb muscle mass (Binder et al. 2005, Galvão  
27  
28 85 and Taaffe 2005, Sillanpää et al. 2009).

31 86 More recently, improvements in metabolic biomarkers such as TG, HDL-C, LDL-C, GLU  
32  
33 87 (Martins et al. 2010, Tomeleri et al. 2016), as well as reduced levels of inflammatory  
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35 88 biomarker CRP (Ribeiro et al. 2015, Tomeleri et al. 2016) have also been observed. During  
36  
37 89 aging significant changes in endocrine function occurs and exercise appears to modify the  
38  
39 90 level of circulating hormones. In exercising older woman, levels of anabolic hormones  
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41 91 decrease (Ennour-Idrissi et al. 2015) and this may be connected with loss of muscle mass and  
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43 92 reduction in functional capacity. However, higher circulating estrogens have been linked with  
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45 93 risk of developing breast cancer (Key et al. 2002) and tumors (Pike et al. 1983). Hence, there  
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47 94 may be a competing need in older women to lower estrogen levels to reduce risk of disease  
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49 95 while increase levels to increase/maintain muscle mass.  
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54 96 While strength and muscle mass improvements are so robust that they are observed in almost  
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56 97 all strength training studies, the evidence for improved body composition and  
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3 98 metabolic/inflammatory biomarkers is weaker. For example, strength training of 8–16 weeks  
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5 99 did not improve body composition or blood lipid profiles in several studies (Elliot et al. 2002,  
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7 100 Hagerman et al. 2000, Joseph et al. 1999). The reason for the mixed findings is unclear, but  
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9 101 one possibility is that there were differences in the training programs used. Given that there is  
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11 102 no consensus on the optimal way to train older individuals (with particularly less studies  
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13 103 performed in women), different training variables should be assessed to determine their  
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15 104 potential role in influencing adaptations.

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18 105 One aspect of training that may influence the efficacy of the training stimulus or sport  
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20 106 performance, which has not been extensively investigated, is the time-of-day when the  
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22 107 training occurs (Vitale and Weydahl, 2017). It is already known that hormone concentrations  
23  
24 108 exhibit circadian rhythmicity and vary throughout the day (Kraemer et al. 2001) along with  
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26 109 body temperature (Bailey and Heitkemper, 2001) and strength performance (Sedliak et al.  
27  
28 110 2009). Since strength performance is greater in the evening compared to morning and that  
29  
30 111 e.g. testosterone concentrations are higher in the morning (i.e. during the more immediate  
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32 112 recovery period), it has been hypothesized that evening training may be more efficacious than  
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34 113 morning training. Few studies have investigated this hypothesis during short-term training  
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36 114 (Sedliak et al. 2009, Sedliak et al. 2017), but of those there is some evidence to suggest that  
37  
38 115 muscle hypertrophy may be greater and/or systematic following evening training in young  
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40 116 individuals (Küüsmaa et al. 2016). Furthermore, it was also recently shown that not only  
41  
42 117 strength performance and hormonal concentrations vary throughout the day but also rating of  
43  
44 118 perceived exertion (RPE) and mood states were affected after high intensity interval training  
45  
46 119 performed at different times of the day (Vitale et al. 2017). Here, especially the evening types  
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48 120 were more fatigued with less energy and higher RPE during morning training sessions (Vitale  
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50 121 et al. 2017). However, considering the clear evidence that older individuals have greater  
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52 122 vitality and/or are less fatigued in the mornings compared to the evening (Wanigatunga et al.  
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3 123 2017), this hypothesis may not hold for older populations. It is important to determine  
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5 124 possible implications of performing strength training at various times during the day, as this  
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7 125 could enhance national and international recommendations for strength training in older  
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9 126 individuals.

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12 127 Therefore, the present study aimed to determine the effects of time-of-day on adaptations  
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14 128 from 12-week progressive strength training program in a group of older women. This age and  
15  
16 129 sex population may derive the greatest health-enhancing benefit from strength training, and it  
17  
18 130 is important to optimize prescribed training methods for older women. We hypothesized that  
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20 131 the morning training group would enhance strength and functional performance to a greater  
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22 132 extent than the evening training group, while the differences in body composition,  
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24 133 biochemical and hormonal outcomes between the groups will be similar.  
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## 30 135 **MATERIALS AND METHODS**

### 32 136 **Study design**

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35 137 Thirty-one elderly women were allocated into a morning (n=10) or an evening (n=10)  
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37 138 training group or a non-training control group (n=11). Morning (07:30) and evening (18:00)  
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39 139 training groups performed a 12-week progressive strength-training program with a frequency  
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41 140 of 2 days per week, while the control group continued their normal daily activities. Seven  
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43 141 days before and seven days after the experiment, evaluations consisting of anthropometric  
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45 142 measures, tests of 6-repetition maximum (6-RM), functional capacity tests, body composition  
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47 143 assessment by bioelectrical impedance (BIA), and blood draws for biochemical analyses were  
48  
49 144 performed. For strength and functional capacity tests, the morning training group was tested  
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51 145 in the morning hours (from 07:30), and the evening group was tested in the evening hours  
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53 146 (from 18:00) to match their training times. The study was conducted between February and  
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56 147 June.  
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5 149 **Participants**

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7 150 Participant recruitment was carried out through newspaper advertisements and personal  
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9 151 meetings. All potential participants completed a questionnaire focused on health history and  
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11 152 physical activity. Inclusion criteria for this study were as follows: female sex, at least 60  
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13 153 years old, physically independent, no orthopedic and cardiac problems pass a medical  
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15 154 evaluation, no medication affecting blood pressure or cholesterol, glucose or hormonal  
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17 155 concentrations. At the beginning of the experiment 40 women enrolled to the study, but 4 did  
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19 156 not pass the medical examination and were excluded. Therefore, 36 women were randomized  
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21 157 to the morning training group (M: n=12), evening training group (E: n=12), and the control  
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23 158 group (C: n=12) (Fig 1). After the initial evaluation process, and following intervention or  
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25 159 control period there were 31 women who completed the study (age:  $66 \pm 4$  years, height:  $162$   
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27  $\pm 4$  cm, body mass:  $75 \pm 13$  kg). **None of the participants in this study were sarcopenic.**  
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29 160 **Sarcopenia was calculated as skeletal muscle mass index by the standard equation: muscle**  
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31 161 **mass/(height<sup>2</sup>). Cut-off point that may indicate sarcopenia in older woman according to**  
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33 162 **European working group on sarcopenia was  $7.2 \text{ kg/m}^2$  (Bahat et al. 2016).** The reasons for  
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35 163 withdrawal from the study were different, for instance: personal reasons, lack of time, did not  
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37 164 meet the inclusion criteria (Fig. 1). All participants read and signed informed consent where  
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39 165 detailed description of study design, training program and evaluation process was listed prior  
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41 166 to the study. This study was performed according to the Declaration of Helsinki, and the local  
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43 167 University Ethic Committee approved this experiment.  
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48 16949  
50 170 **6-RM muscular strength**

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52 171 Maximal dynamic strength was assessed using the 6-RM (modified 1-RM test) (Ribeiro et al.  
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54 172 2017) test for the horizontal leg press and seated-row exercises. Testing was preceded by a

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3 173 warm-up set which consisted of 8-12 repetitions, with approximately 70% of the estimated  
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5 174 load used in the first attempt of the 6-RM. After 2 minutes of rest the testing procedure  
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7 175 began. The participants were instructed to perform 6 repetitions with the highest possible load  
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9 176 within 3-4 attempts in both exercises. Two experienced coaches supervised the testing to  
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11 177 ensure reliability and safety of the participants. The duration of the concentric and eccentric  
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13 178 phase was 2 seconds, respectively and was controlled by the coaches. The 6-RM was  
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15 179 recorded when the last successful repetition with a given load was lifted and the participant  
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17 180 was not able to accomplish the next repetition. Inter-correlation coefficient (ICC) for the leg-  
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19 181 press and seated row 6-RM was 0.95 and 0.78, respectively. Participants were verbally  
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21 182 encouraged throughout each test. Rest intervals between attempts were 3-4 minutes and 7  
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23 183 minutes between exercises.  
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### 29 185 **Functional capacity tests**

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31 186 Testing procedures followed the standard SFT protocol (Rikli and Jones, 2013). A battery of  
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33 187 tests was used to examine functional capacity of the participants. A 30-second chair-stand test  
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35 188 measured the number of times that a participant can stand upright from a chair and sit down.  
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37 189 On a signal, participants stood up to a full standing position from a chair and then returned to  
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39 190 the fully seated position (stand up and sit down = 1 repetition) and they continued to  
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41 191 complete as many full stands as possible in 30 seconds. The chair (seat height = 43cm) was  
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43 192 positioned against a wall and safely secured. Time was taken using stopwatch and the total  
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45 193 number of complete repetitions was recorded. If the participant had completed only the stand  
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47 194 phase when the time elapsed, this repetition was counted.

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50 195 The arm curl test measured the number of arm curls with a 2.3 kg dumbbell in the dominant  
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52 196 hand. Participants sat on the (same) chair while holding the dumbbell with palm facing  
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54 197 towards the body with the arm beside the chair. During the curls, the upper arm and elbow  
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3 198 joint were positioned and maintained near to the body. If necessary, the tester assisted in  
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5 199 maintaining the upper arm in the correct position. On a signal, participants began to flex  
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7 200 (with gradually turning the palm – flexion with supination) and extend the elbow of the  
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9 201 dominant hand, over the entire range of motion (the arm must be fully flexed and then fully  
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11 202 extended at the elbow), as many times as possible in 30 seconds. Again, if the participants  
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13 203 had only raised the arm, but not fully lowered the arm when time elapsed then that repetition  
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15 204 was counted.

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18 205 A Timed Up-and-Go (TUG) test was used to determine the amount of time required to stand  
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20 206 up from a chair, walk 2.4 meters, turn around a cone, return and sit down on a chair. Time  
21  
22 207 during the tests was taken by stopwatch similarly as in the original SFT and previous  
23  
24 208 research. Participants completed 2 to 3 attempts from each test, and the best results were  
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26 209 taken to further analysis. The ICCs for the all SFT tests were high 0.93 to 0.98 (Milanović et  
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28 210 al. 2013).

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### 32 33 212 **Anthropometry**

34  
35 213 Body height was measured to the nearest 0.1 cm with a stadiometer attached to the scale.  
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37 214 Height measurements were performed while the participants were standing barefoot. Body  
38  
39 215 mass was evaluated to the nearest 0.1 kg using the InBody device which was used to  
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41 216 determine muscle mass and percentage fat mass analysis (see below) (Biospace Co., Seoul,  
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43 217 Korea). All measures were performed in the morning after overnight fast.

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### 47 48 219 **Body composition measurements**

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50 220 Whole-body muscle mass, fat mass, whole-body fat %, as well as leg and arm muscle mass  
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52 221 was measured using the InBody 720 device (Biospace Co., Seoul, Korea). InBody 720  
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54 222 measures body composition by passing multiple frequencies at 5, 50, 250, 500, and 1000 kHz  
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3 223 and reactance in mean frequencies (5, 50 and 250 kHz). Overall body impedance values were  
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5 224 calculated by summing the segmental impedance values that were analyzed separately with a  
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7 225 tetrapolar 8-point tactile electrode system. The measurement procedures were similar to a  
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9 226 previous study (Esco et al. 2015). Briefly, before the participants stood on the device their  
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11 227 soles and palms were wiped with an electrolyte tissue. According to the examiner's  
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13 228 instructions, the participants gripped the handles with the palm, fingers, and thumb in contact  
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15 229 with the hand electrodes. The participants' soles were also in contact with the foot electrodes.  
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17 230 According to the manufacturer's guidelines, the participants held their arms and legs in such  
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19 231 a position that they would not come into contact with any other body part during the  
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21 232 measurement. The arms were positioned at approximately 20° away from the trunk, and legs  
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23 233 were positioned 45° apart. Before the measurement began, the participants were instructed  
24  
25 234 not to move. The duration of the analysis was approximately 2-3 minutes per participant.  
26  
27 235 Test-retest reliability of this device was performed in previous study with good interclass  
28  
29 236 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**

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37 239 Venous blood was collected in the morning after a 12-hour fast in a standard manner from an  
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39 240 antecubital vein. After separation of serum, samples were stored at -80°C until further  
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41 241 analyses. Samples were assessed by immunoassay for total antioxidant status (TAS), high  
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43 242 sensitivity C-reactive protein (hsCRP), triglycerides (TG), blood glucose (GLU) and total  
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45 243 cholesterol (TC) concentration using a discrete photometric Clinical Chemistry Analyzer  
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47 244 Biolis 24i Premium (Tokyo Boeki Machinery, Tokyo, Japan).  
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49 245 High-density lipoprotein (HDL-C), and small dense low-density lipoprotein (sdLDL-C)  
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51 246 cholesterol were determined by detergent-based isolation and enzyme-linked colorimetric  
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3 247 detection (Direct HDL cholesterol and direct sdLDL-C cholesterol; Randox Laboratories,  
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5 248 Crumlin, UK).

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7 249 Total cholesterol, HDL-C and sdLDL-C were analyzed using commercial kits (Randox  
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9 250 Laboratories, Crumlin, UK). Total antioxidant status (TAS) was assessed by the Trolox-  
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11 251 equivalent antioxidant capacity assay performed with the kit supplied by Randox (Randox  
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13 252 Laboratories, Crumlin, UK). Briefly, the test was based on the formation of blue-green cation  
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15 253 radical of ABTS (2,2-Azino 3-ethyl benzthiazoline sulfonate) in the presence of  
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17 254 metmyoglobin and hydrogen peroxide. LDL-C concentration was estimated using the  
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19 255 Friedewald, Levy, and Fredrickson equation (Friedewald et al. 1972).

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22 256 Intra-assay variability (%) was  $\leq 3.0$  (sdLDL-C),  $\leq 1.3$  (HDL-C),  $\leq 2.2$  (GLU),  $\leq 2.5$  (TG),  $\leq 2.8$   
23  
24 257 (CRP),  $\leq 3.1$  (TAS). Sensitivity was: 0.025 mmol/l (sd-LDL), 0.04 mmol/l (HDL-C), 0.1  
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26 258 mmol/l (GLU), 0.26 mmol/l (TG), 0.007 mmol/l (hsCRP).

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30 259 Quantification of hormones was performed using ELISA (Enzyme-Linked Immunosorbent  
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32 260 Assay). All analyzes were performed on the DIAREADER ELX800 G (Dialab, GMBH,  
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34 261 Wiener Neudorf, Austria) with measuring range from 400 nm to 750 nm for reading 24, 48 or  
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36 262 96-well plates. ELISA assays (Dialab, Wiener Neudorf, Austria) were performed according  
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38 263 to the manufacturer's instructions. The color intensity was inversely proportional to the  
39  
40 264 concentration of hormones in the sample. The absorbance was determined according to the  
41  
42 265 manufacturer's instructions on a microplate ELISA reader - GloMax®-Multi+ Detection  
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44 266 System (Promega Corporation, Madison, USA). Seven basal hormone levels were analyzed  
45  
46 267 using commercially available assays: follicle stimulating hormone (FSH) (Dialab, Wiener  
47  
48 268 Neudorf, Austria), luteinizing hormone (LH), prolactin (PRL) (NovaTec, Immundiagnostica  
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50 269 GMBH, Dietzenbach, Germany), progesterone (P) (Dialab, Wiener Neudorf, Austria),  
51  
52 270 estradiol (ESTR) (NovaTec, Immundiagnostica GMBH, Dietzenbach, Germany), aldosterone  
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54 271 (ALD) (NovaTec, Immundiagnostica GMBH, Dietzenbach, Germany), and testosterone (T)

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3 272 (Dialab, Wiener Neudorf, Austria). Intra-assay variability (%) was  $\leq 6.4$  (FSH),  $\leq 9.2$  (LH),  
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5 273  $\leq 3.5$  (PRL),  $\leq 4.0$  (P),  $\leq 9.0$  (ESTR),  $\leq 10.0$  (ALD),  $\leq 7.0$  (T). Sensitivity was 1.0 mIU/ml  
6  
7 274 (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  
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9 275 (ALD), 0.1 ng/ml (T).

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### 12 13 277 **Strength training program**

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15 278 Supervised strength training was performed during the morning (07:30) and evening hours  
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17 279 (18:00). Four weeks before the first pre-training measures and analyses, the participants  
18  
19 280 completed 6 familiarization sessions with a frequency of 1-2 days per week in order to learn  
20  
21 281 the correct exercise techniques. During these sessions only exercise technique (exercises used  
22  
23 282 in training program) with light loads was performed. During the entire study, the participants  
24  
25 283 were personally supervised by qualified instructors to ensure safety and consistency during  
26  
27 284 training sessions. Participants trained two times per week, on Mondays and Thursday.  
28  
29 285 Whole-body strength training program comprised of 8 exercises in the following order:  
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31 286 dumbbell bench press, horizontal leg press, seated row, knee extension, lat pull-down, leg  
32  
33 287 curl, machine chest fly, and seated calf raise. The participants performed 3 sets of 10-12  
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35 288 repetition maximums. The same load was kept from set 1 to set 3, and participants always  
36  
37 289 finished the prescribed repetition range, which ended with concentric failure in the final set.  
38  
39 290 Rest periods between sets were 2-3 minutes and 3 minutes between exercises. Participants  
40  
41 291 were constantly instructed to inhale during the eccentric phase and exhale during the  
42  
43 292 concentric phase. Tempo during the lifting was approximately 1 second for concentric and 2  
44  
45 293 seconds for eccentric phase. External load was gradually increased in the following manner:  
46  
47 294 for upper body exercises  $\sim 3-5\%$  and for lower body exercises  $\sim 5-8\%$  every second week  
48  
49 295 (after 4 training sessions) (Ribeiro et al. 2015). Participation in the training program was  
50  
51 296 sufficient, with all participants participating in  $>90\%$  of the total training sessions. In  
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3 297 addition, each participant received a 25g dose of whey isolate protein after each training  
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5 298 session to ensure a similar anabolic effect during the immediate period after strength training.  
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7 299 The Control group was asked to maintain similar physical activity as they did before the  
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9 300 study. All groups were also assessed by International Physical Activity Questionnaire (IPAQ)  
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11 301 to assess physical activity performed external to the intervention.  
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### 15 303 **Statistical analyses**

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18 304 Analysis of covariance (ANCOVA) with repeated measures was used for comparison of all  
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20 305 three groups (3 group×2 time) and the two training groups (2 group×2 time) using baseline  
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22 306 values as covariate. One-way ANOVA was used to assess between-group differences at  
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24 307 baseline and post-training in all three training groups. Hedge's *g* estimates were employed,  
25  
26 308 where small (<0.3), medium (0.3–0.8), and large (>0.8) effect sizes were identified to  
27  
28 309 determine the magnitude of the training-induced changes between the three training groups  
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30 310 (Hopkins 2012). Pearson product moment correlation (*r*) determined relationships between  
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32 311 pre-training value and changes during the training. Alpha was set at 0.05. Descriptive  
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34 312 statistics and statistical methods were calculated using statistical software IBM SPSS 22.  
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## 38 314 **RESULTS**

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41 315 Results of the IPAQ showed no significant differences between groups at any point (from  
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43 316 pre- to post-training) during the study. All measured (absolute values) maximum strength,  
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45 317 functional capacity and body composition data are shown in table 1.

### 46 318 **Muscular strength and functional capacity**

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49 319 Time ( $p < 0.01$ ) and time×group ( $p < 0.01$ ) interactions were found in leg press 6-RM, seated  
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51 320 row 6-RM, biceps curls and chair stand functional capacity test. M significantly improved leg  
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53 321 press and seated row 6-RM ( $+46\pm 23\%$  and  $+48\pm 22\%$ , respectively;  $p < 0.01$ , Figure 2A and  
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3 322 2B), chair stand ( $+35\pm 32\%$ ,  $p < 0.01$ , Figure 2C), biceps curl ( $+30\pm 22\%$ ,  $p < 0.01$ ) and TUG  
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5 323 ( $-17\pm 11\%$ ,  $p < 0.01$ , Figure 2D). Similarly, E significantly improved leg press and seated  
6  
7 324 row 6-RM ( $+21\pm 12$  and  $+43\pm 18\%$ , respectively;  $p < 0.01$ , Figure 2A and 2B), chair stand  
8  
9 325 ( $+34\pm 33\%$ ,  $p < 0.01$ , Figure 2C), biceps curl ( $+36\pm 21\%$ ,  $p < 0.01$ ) and TUG ( $-20\pm 9\%$ ,  $p <$   
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11 326  $0.01$ , Figure 2D). Improvements in both training groups were significantly larger compared to  
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13 327 the control group ( $p < 0.01$ ) except for TUG where no significant difference between the  
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15 328 groups was observed (Figure 2A-D). No significant differences in 6-RM strength and  
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17 329 functional capacity tests between M and E group were observed.

### 330 **Body composition**

331 A significant time $\times$ group interaction ( $p < 0.01$ ) was found in measures of whole-body muscle  
332 mass where only M significantly ( $+3\pm 2\%$ ,  $p < 0.01$ ) increased muscle mass from pre- to post-  
333 training (Figure 2E). However, M and E both significantly decreased fat mass ( $-6\pm 5\%$  and  $-$   
334  $8\pm 4\%$ , respectively;  $p < 0.01$ , Figure 2F) and body fat % ( $-6\pm 5\%$  and  $-5\pm 3\%$ , respectively;  $p$   
335  $< 0.01$ ) from pre- to post-training. No significant differences between all the three groups in  
336 measures of body composition were recorded.

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

### 340 **Biomarker and hormone concentrations**

341 Table 2 shows results (absolute values) of the metabolic and inflammatory biomarkers and  
342 hormone level after the training. Significant difference between pre- to post-training in M  
343 and E were found in sdLDL-C where both groups increased their levels ( $79\pm 84\%$  and  
344  $31\pm 50\%$ , respectively;  $p < 0.05$ ). Both M and E groups significantly decreased the level of  
345 the GLU ( $-4\pm 6\%$  and  $-8\pm 10\%$ , respectively;  $p < 0.05$ ), where the decrease in GLU level was  
346 significantly greater for E compared to M ( $p < 0.05$ ). Only E significantly decreased the level

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3 347 of TG ( $-17\pm 25\%$ ,  $p < 0.01$ ) from pre- to post-training which differed significantly ( $p < 0.01$ )  
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5 348 compared to M ( $+16\pm 27\%$ ,  $p < 0.01$ ). No other significant increases or decreases in  
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7 349 biochemical or inflammatory parameters were observed. Similarly, no significant changes in  
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9 350 the hormone level, except for ESTR ( $+16\pm 19\%$ ,  $p < 0.05$ ) in M were observed.

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## 12 352 **DISCUSSION**

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15 353 The main aim of this study was to compare effects of training performed at different times of  
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17 354 the day in a group of older women on multiple variables; including maximum strength,  
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19 355 functional capacity, and basal biomarker and hormonal concentrations. The results show that  
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21 356 morning and evening training groups significantly improved maximum strength, functional  
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23 357 capacity, body composition, as well as some biomarker concentrations. **The findings partially**  
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25 **support our hypothesis that similar changes in body composition and blood markers between**  
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27 **the groups would be observed, but there was little evidence to support our hypothesis that**  
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29 **morning training would be more beneficial for strength and functional capacity improvement.**  
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33 361 The observed improvements in strength and functional capacity tests in M and E are not  
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35 362 surprising since participants had no regular strength training or any other physical activity. A  
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37 363 large number of studies have demonstrated increased maximum strength in older individuals  
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39 364 after initiating progressive strength training (Frontera et al. 2003, Häkkinen et al. 1996). In  
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41 365 the present study, both M and E significantly improved leg press (46% and 21%;  $p < 0.001$ )  
42  
43 366 and seated-row (48% and 42%;  $p < 0.01$ ) 6-RM. No significant differences were observed  
44  
45 367 when comparing M and E groups in both tests, however, M demonstrated larger increases  
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47 368 (twofold) compared to E in the leg-press 6-RM ( $\sim 46\pm 21\%$  vs.  $\sim 21\pm 12\%$ ). Despite non-  
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49 369 significance, effect size values favor M compared to E ( $g = \sim 3.4$  and  $\sim 2.1$ , respectively). It  
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51 370 could be speculated that M gained more in the leg press due to higher quality training, since it  
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55 371 has been shown that older individuals have greater vitality in the mornings (Wanigatunga et

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3 372 al. 2017). Nevertheless, these larger gains did not translate into greater improvements in  
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5 373 functional capacity.  
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7 374 Functional capacity performance significantly improved in both M and E over 12 weeks of  
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9 375 training. Comparable results in functional capacity have also been reported (Hanson et al.  
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11 376 2009, Pedersen et al. 2017, Turpela et al. 2017). Interestingly, we did not observe significant  
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13 377 relationships ( $r = -0.1$ – $-0.4$ ,  $p > 0.05$ ) between the changes in leg press or seated row 6-RM  
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15 378 strength and changes in any functional capacity test. The importance of increasing maximum  
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17 379 strength for improving functional capacity seems questionable since only one study has  
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19 380 shown a statistically significant relationship (Santos et al. 2017), while most others have not  
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21 381 (Moura et al. 2017, Turpela et al. 2017). Hence, it would seem likely that the effect of  
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23 382 possibly more efficient strength training in the morning versus evening will have little or no  
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25 383 effect on the overall magnitude of functional capacity improvement in older individuals.  
26  
27 384 Collectively, the results of all of the aforementioned 6-RM strength tests and functional  
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29 385 capacity tests indicate that training time is not a major factor influencing performance in  
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31 386 favor of morning or evening training over 12 weeks in previously untrained older women.  
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33 387 Many previous strength training studies have shown positive effects on body composition  
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35 388 over 8 to 18 weeks in older women (Bouchard et al. 2009, Santos et al. 2017, Sipilä and  
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37 389 Suominen 1995, Tomeleri et al. 2016). In the present study, both M and E significantly  
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39 390 decreased whole-body fat mass (kg) ( $-6 \pm 5\%$  and  $-8 \pm 4\%$ , respectively;  $p < 0.01$ ) and body fat  
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41 391 percentage ( $-6 \pm 5\%$  and  $-5 \pm 3\%$ , respectively;  $p < 0.01$ ). However, only M significantly  
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43 392 increased whole body muscle mass in the present study (kg) (M:  $+3.4\%$ ,  $p < 0.01$ ; E:  $+0.7\%$ ).  
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45 393 This increase in muscle mass may help to explain the larger gains in leg press 6-RM, but  
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47 394 correlation analyses suggest that this influence was small and not statistically significant ( $r =$   
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49 395  $0.373$ ,  $p > 0.05$ ,  $n=20$ ). The reason why only the M group significantly increased muscle  
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51 396 mass is not clear. But this finding is in contrast to the trends found in young individuals by  
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3 397 Kūusmaa et al. (2016), while several papers by Sedliak and colleagues (2009, 2017) have  
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5 398 shown equal hypertrophy in young men. This is a potentially important finding for practical  
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7 399 applications when aiming to counteract age-related loss of muscle mass and should be  
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9 400 explored further.

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11 401 The results in metabolic and inflammatory biomarkers are somewhat contradictory in our  
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13 402 study compared to others' findings. Both M and E significantly improved GLU from pre- to  
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15 403 post-training (M:  $-4\pm 6\%$ , E:  $-8\pm 10\%$ ;  $p < 0.05$ ), and the improvement in E group was  
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17 404 significantly higher compared to M ( $p < 0.05$ ). Tomeleri et al. (2016, 2017) found reductions  
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19 405 in glucose level after 8- and 12-weeks of resistance training from 6% to 20 %, respectively.  
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21 406 Improved basal glucose concentrations may have been due to improvements in insulin  
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23 407 sensitivity brought about by loss of fat (Boden 2002). Studies have observed significant  
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25 408 relationships between changes in body fat and changes in glucose concentration (Tomeleri et  
26  
27 409 al. 2016), however, in our study we did not observe such a relationship. Further, it is difficult  
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29 410 to attribute that fat loss would be a major factor in reduced glucose concentration since both  
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31 411 M and E lost fat mass to a similar extent. It neither seems likely that muscle hypertrophy  
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33 412 would play such an important role considering that M increased muscle mass more than E,  
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35 413 but E reduced glucose concentration more than M. Regardless of the possible mechanisms, an  
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37 414 important finding from a general health perspective is that a significant relationship between  
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39 415 baseline glucose level and changes during the training was observed ( $r = -0.491$ ,  $p < 0.05$ ,  
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41 416  $n=20$ ). Thus, those individuals with higher basal glucose concentration gain the most benefit  
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43 417 from strength training, regardless of whether training is performed in the morning or evening.  
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45 418 Only E significantly decreased TG ( $-17\%$ ,  $p < 0.01$ ) while M actually showed an increase in  
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47 419 TG ( $+16\%$ ,  $p < 0.01$ ). Strength training may decrease lipid concentrations by the ability of  
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49 420 skeletal muscle to use fat stores during physical activity (Mann et al. 2014). However, the  
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51 421 results in M are hard to explain, particularly given the muscle mass results, and we can only  
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3 422 speculate what mechanism(s) may be responsible for this result (e.g. dietary intake, intra-  
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5 423 individual differences, and daily/seasonal variation in TG, synthesis of tissue/hormones from  
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7 424 cholesterol). Once again correlation analyses between baseline values and changes during  
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9 425 training revealed a negative relationship ( $r = -0.677$ ,  $p < 0.01$ ,  $n=20$ ). Therefore, individuals  
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11 426 with higher initial levels benefit most from beginning strength training.

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13 427 Small dense low-density lipoprotein is a new emerging risk factor associated with  
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15 428 cardiovascular diseases because it is more atherogenic than LDL-C. sdLDL-C can be used as  
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17 429 a predictor of future CVD and other conditions associated with dislipidemia (Ivanova et al.  
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19 430 2017). Our study is the first to examine the effects of strength training on sdLDL-C  
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21 431 concentration, and it is difficult to explain why both groups increased the level of sdLDL-C  
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23 432 and whether strength training is the cause of such change.

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25 433 Except for estradiol in M ( $+16 \pm 19\%$ ,  $p < 0.05$ ) no significant changes in basal hormone  
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27 434 concentrations were observed from pre- to post-training. These results match findings in the  
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29 435 younger as well as in the older population (Häkkinen et al. 2000, Sallinen et al. 2006).  
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31 436 However, this result should be interpreted with caution because it is unknown whether this  
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33 437 change is due to greater production or lower uptake of ESTR in M, and therefore, it is unclear  
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35 438 whether this is a positive effect related to strength training.

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37 439 This study has some limitations that should be mentioned; 1) It was not possible to  
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39 440 objectively control physical activity during daily living despite participants being instructed  
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41 441 to avoid any exhaustive activities or beginning new exercises that could potentially affect  
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43 442 results of the study. Instructions were also provided regarding nutritional intake. 2) Sample  
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45 443 size in the present study may not have been sufficient to determine statistical significance in  
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47 444 some biomarkers, since the pattern of change suggested improvements in both M and E for  
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49 445 HDL-C (M:  $+3 \pm 12\%$ , E:  $+10 \pm 12\%$ ) and TAS (M:  $+10 \pm 12\%$ , E:  $+14 \pm 11\%$ ), and E only for  
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51 446 hsCRP (M:  $+0.02 \pm 47\%$ , E:  $-4 \pm 41\%$ ). 3) Our strength training program was focused on  
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3 447 “hypertrophic” type of training. Therefore, it is not known whether manipulation of other  
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5 448 program variables (i.e. shorter rest-interval, higher number of repetitions) may have led to  
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7 449 greater benefit on metabolic and inflammatory biomarker concentrations. Nevertheless, one  
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9 450 strength of the study was that both training groups received a 25 g-dose of whey isolate  
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11 451 protein after each training session. Hence, both groups had similar conditions for immediately  
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13 452 post-training anabolic effects.  
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## 16 454 **CONCLUSIONS**

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18 455 This study showed that improvements in maximum strength, functional capacity and some  
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20 456 metabolic biomarkers in previously untrained older woman occur regardless of whether  
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22 457 training was performed in the morning or evening. However, morning trainers gained more in  
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24 458 leg press 6-RM and whole-body muscle mass compared to evening trainers, which appears in  
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26 459 contrast to some (limited) findings in young individuals. An important finding, which  
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28 460 requires further detailed study, was that these greater gains in maximum strength and muscle  
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30 461 mass did not influence the changes in functional capacity performance, fat loss, or blood  
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32 462 profile of these women.  
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## 47 468 **DECLARATION OF INTEREST**

48  
49 469 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

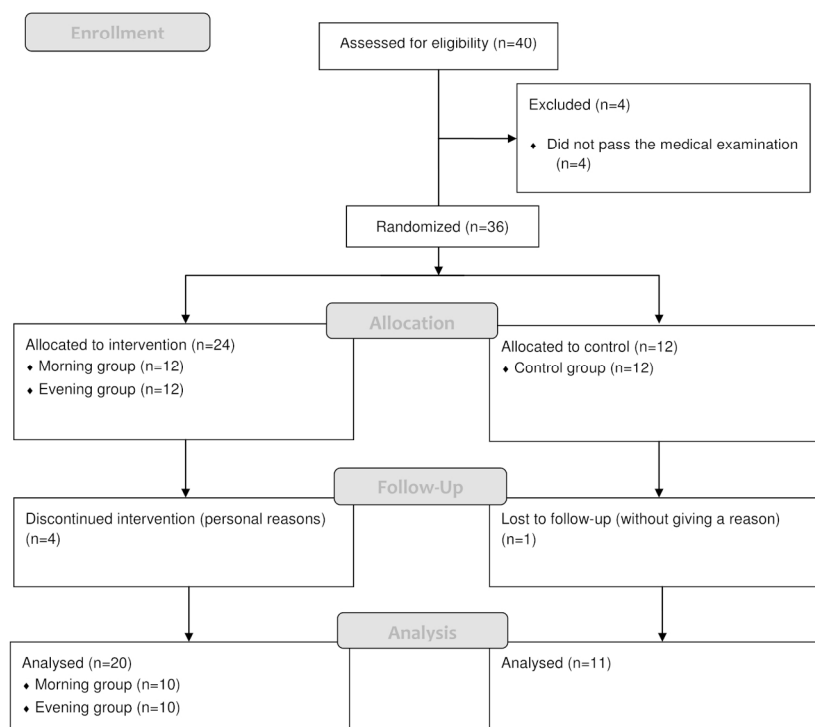


Figure 1 Consort flow diagram showing subjects enrollment in this experiment

599x776mm (72 x 72 DPI)



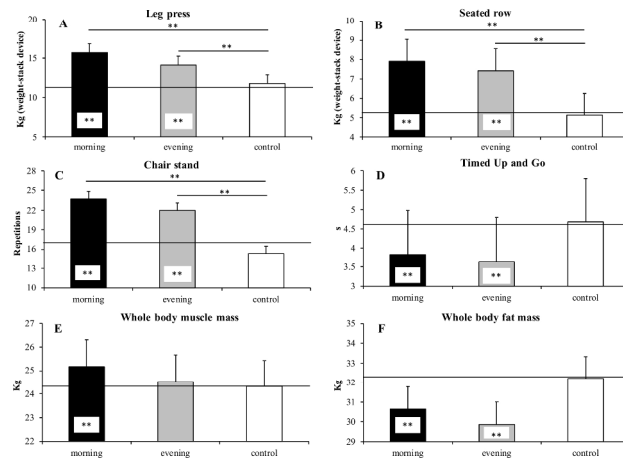


Figure 2 Changes in leg press (A) and seated row (B) 6RM strength, chair stand (C) and Timed Up and Go (D) performance, and whole-body muscle mass (E) and fat mass (F) over the study duration. Stars within the bars = within-group differences, Stars above bars = between-group differences, The horizontal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

209x297mm (300 x 300 DPI)

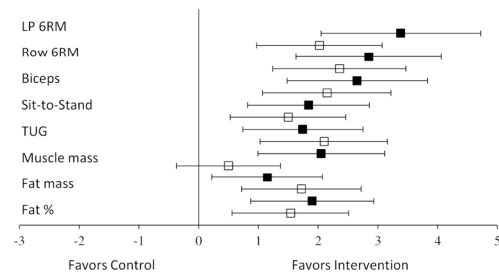


Figure 3 Effect size plot (Hedge's g) showing training-induced changes in maximum strength, functional capacity tests and body composition adjusted for control group changes. Closed squares=morning group; Open squares=evening group

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

	<b>Pre-training</b>									
	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)
<b>Morning</b>	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
<b>Evening</b>	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
<b>Control</b>	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
	<b>Post-training</b>									
<b>Morning</b>	15.0±2.5** <sup>SS</sup>	8.1±1.3** <sup>SS</sup>	24±5** <sup>SS</sup>	29±2.4** <sup>SS</sup>	3.8±0.9**	24.9±0.9**	30.5±7.3**	39.5±5.7**	13.0±1.1	4.9±0.6
<b>Evening</b>	14.9±3.0** <sup>SS</sup>	7.3±1.4** <sup>SS</sup>	22±5** <sup>SS</sup>	26±4** <sup>SS</sup>	4.0±1.1**	25.3±4.6	33.3±13.5**	40.4±7.0**	13.4±1.7	5.1±1.2
<b>Control</b>	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; <sup>§</sup>0.05, <sup>SS</sup>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)
<b>Morning</b>	2.6±0.8	0.4±0.2	1.5±0.5	5.8±0.4 <sub>#</sub>	1.4±0.4 <sub>#</sub>	2.0±1.2 <sub>##</sub>	1.2±0.8	36.6±3.4	16.4±6.8	15.0±11.4 <sub>#</sub>	7.4±3.3 <sub>#</sub>	11.9±2.4	0.07±0.01	5.1±2.9
<b>Evening</b>	3.4±1.3	0.7±0.6	1.5±0.4	5.6±0.9 <sub>#</sub>	1.7±1.0 <sub>#</sub>	2.5±1.8 <sub>##</sub>	1.3±0.1	36.1±5.3	15.6±8.3	11.4±7.4 <sub>##</sub>	10.1±9.0 <sub>#</sub>	12.3±3.4	0.08±0.03	5.5±3.0
	Post-training													
<b>Morning</b>	3.0±0.9	0.8±0.4 <sub>*</sub>	1.5±0.4	5.6±0.6 <sub>*</sub>	1.6±0.4 <sub>**</sub>	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 <sub>*</sub>	0.07±0.02	5.0±2.3
<b>Evening</b>	3.6±1.4	0.8±0.5 <sub>*</sub>	1.7±0.4	5.1±0.5 <sub>**</sub>	1.3±0.7 <sub>***</sub>	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; <sub>#</sub>0.05, <sub>##</sub>0.01 pre-training difference between the M and E group; <sub>†</sub>0.05, <sub>‡</sub>0.01 = difference compared to the M group