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Effect of resistance training with different frequencies and detraining on muscular strength and oxidative stress biomarkers in older women

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Abstract The aim of this study was to compare the effect of resistance training (RT) performed with different frequencies followed by a detraining period on muscular strength and oxidative stress (OS) biomarkers in older women. Twenty-seven physically independent women (68.8±4.8 years, 69.1±14.3 kg, 156.0±6.5 cm, and 28.3±4.9 to kg.m^{-2}) were randomly assigned to perform a RT program for 2 or 3 days per week (G2X=13 vs. G3X=14) for 12 weeks followed by 12 weeks of detraining period. One repetition maximum (1RM) tests were used as measures of muscular strength (three exercises, three attempts for each exercise, 3–5 min of rest between attempts, and 5 min of rest between exercises). Advanced oxidized protein products (AOPP) and total radical-trapping antioxidant parameter (TRAP) were used as oxidative stress indicators. Both groups increased muscular strength after 12 weeks of training ($P<0.05$) in chest press (G2X=+11.9 % vs. G3X=+27.5 %, $P<0.05$), knee extension (G2X=+18.4 % vs. G3X=+16.7 %, $P>0.05$), and preacher curl (G2X=+37.6 % vs. G3X=+36.7 %, $P>0.05$). On the other hand, 12 weeks of detraining were not sufficient to eliminate the major effects produced by RT on muscular strength, although a significant decrease ($P<0.05$) has been observed for chest press (G3X=−9.1 % vs. G2X=−10.2 %, $P>0.05$), knee extension (G2X=−14.9 % vs. G3X=−12.1 %, $P>0.05$), and preacher curl (G2X=−20.5 % vs. G3X=−17.4 %, $P>0.05$). Pre- to post-training, both groups showed significant ($P<0.05$) increases in TRAP (G2X=+6.9 % vs. G3X=+15.1 %) with no statistical significant difference between the groups ($P>0.05$), and the scores remained elevated compared to pre-training after 12 weeks of detraining. AOPP was not changed by RT or detraining ($P>0.05$). The results suggest that a 12-week RT program with a frequency of 2 days per week may be sufficient to improve muscular strength and OS in older women and detraining for 12 weeks does not completely reverse the changes induced by RT.

Keywords Strength training · Aging · TRAP · AOPP

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Introduction

Aging is a natural process characterized by gradual muscle mass loss and reduction in the ability to generate muscular strength. This process has been hypothesized to be partially caused by the deleterious and cumulative effects of reactive oxygen species (ROS) occurring throughout the life span (Bouzid et al. 2014; Wang et al. 2013). Studies have shown that older individuals are under constant and increasing assault by ROS, as indicated by enhanced lipid peroxidation, protein oxidation, and decreased antioxidant capacity that occur with aging (Bouzid et al. 2014; Newman et al. 2003; Wanderley et al. 2013).

Oxidative stress (OS) contributes to endothelial dysfunction and large artery stiffening. Overproduction of ROS can affect endothelial function by suppressing nitric oxide synthesis and scavenging nitric oxide, decreasing its overall bioavailability. Moreover, OS due at least in part to the loss of the antioxidant effects of estrogen is a major contributor to the endothelial dysfunction observed in postmenopausal women (Moreau and Hildreth 2014).

The OS also has been associated with the loss of muscular strength with aging (Cesari et al. 2012; Howard et al. 2007). Older women are particularly at greater risk for muscular strength loss, since women have lower levels of muscular strength compared to men (Brady et al. 2014; Hughes et al. 2001). It is important to note that low levels of muscular strength have been shown to impact cardiovascular disease (Artero et al. 2011; Clark and Manini 2010; Ruiz et al. 2008).

Several resistance training (RT) studies show biomarkers of oxidative reactions and inflammatory processes to increase concurrently with an increase in biomarkers of antioxidant capacity pre- to post-training (de Gonzalo-Calvo et al. 2013; Gianni et al. 2004). Thus, RT may be an important exercise modality to improve the muscular strength and for increasing the body's antioxidant capacity by concomitantly increasing ROS and RNS generation (Hudson et al. 2008; Parise et al. 2005). However, these and other adaptations to RT may be dependent on the appropriate manipulation of training volume and intensity.

Although several meta-analyses have shown a clear dose–response relationship between RT volume and muscular adaptations, the majority of studies included were not specific to older individuals (Krieger 2009, 2010; Rhea et al. 2003). The lack of specificity to older individuals may compromise the analysis of data because the older person displays an altered response to RT programs compared to the young, thereby limiting generalizability between these populations (Kosek et al. 2006). On the other hand, no study to date has investigated the effect of different RT volume on OS biomarkers in older women. Therefore, whether a dose–response exists between training volume and OS biomarkers is not known.

Considering that the adaptations to RT are dependent on adherence to training programs, temporal interruptions brought about for several reasons, such as travel, injury, or disease, may affect the adaptive responses obtained by training. Therefore, training interruption may lead to detraining, which can result in loss of important adaptations, such as reductions of muscle mass, bone mineral density and content, strength, power, muscular endurance, balance, and coordination (Bosquet et al. 2013; Correa et al. 2013; Lovell et al. 2010). Giving this information, we cannot rule out the possibility that OS biomarkers may also be affected by detraining.

The purpose of this study was to analyze the effect of RT performed with different frequencies followed by detraining on muscular strength and OS biomarkers in older women. On the basis of the information that the training volume may influence the responses from a RT program, our primary hypothesis was that higher frequency would cause greater changes than lower frequency of RT on muscular strength and OS biomarkers, resulting in improved OS modulation. A secondary hypothesis was that detraining would result in muscular strength and OS biomarkers returning toward pre-training values.

Methods

Experimental design

The study was performed over a period of 30 weeks, with 12 weeks dedicated to the RT program, 12 weeks to a detraining period, and 6 weeks used for data collection. Two weeks were used for anthropometric measurements, one repetition maximum tests (1RM), and blood samples at weeks 1–2 (pre-training), 15–16 (post-training), and 29–30 (detraining). The supervised RT program was performed during weeks 3–14, and the detraining period occurred during weeks 17–28. During the weeks 1, 15, and 29, three 1RM testing sessions were performed on nonconsecutive days, with an interval of 48 h between sessions. This 1RM testing protocol was used to ensure an accurate determination of muscular strength. Blood samples were obtained 7 days after the last 1RM session in each phase of this study for the determination of OS biomarkers (pre-training, post-training, and detraining).

Participants

Twenty-seven physically independent older women (≥60 years old) volunteered to participate in the study. Subject recruitment was carried out through newspaper and radio advertisements, and home delivery of flyers in residential neighborhoods. Written informed consent was obtained from all participants after a detailed description of study procedures was provided. Participants were randomly assigned into one of the two groups: a group performing RT twice a week (G2X) and a group performing RT three times a week (G3X). Prior to study participation, all subjects completed health history and physical activity questionnaires. Inclusion criteria were not being hypertensive (systolic blood pressure≤ 130 mmHg and diastolic blood pressure≤85 mmHg), not being diagnosed for diabetes, no known cardiac or renal dysfunction, not using inotropic or chronotropic drugs, nonsmoking, no hormonal replacement therapy, not involved in any regular systematic physical activity for more than once a week in the 6 months preceding the beginning of the study, and no musculoskeletal or other disorders that might affect the ability to complete the RT program or tests associated with study participation. Prior to the study, all participants underwent a diagnostic, graded treadmill exercise stress test with 12-lead electrocardiogram and excluded from the study if the cardiologist administering the test classified them as having contraindications for participating in physical exercise. The investigation was conducted according to the Declaration of Helsinki and approved by the local University Ethics Committee. All participants had an adherence to training sessions >85 % of the total sessions.

Anthropometry

Body mass was measured to the nearest 0.1 kg using a calibrated electronic scale (Filizola, model ID 110, São Paulo, Brazil), with participants wearing light workout clothing and no shoes. Height was measured with a stadiometer to the nearest 0.1 cm, with subjects not wearing shoes. Body mass index was calculated as body mass in kilograms divided by height in meters squared.

Muscular strength

Maximal dynamic strength was determined as the 1RM of the chest press, knee extension, and preacher curl, performed in the order listed. Testing for each exercise was preceded by a warm-up set (six to ten repetitions), with approximately 50 % of the estimated resistance used in the first attempt of the 1RM test. The 1RM testing procedure was initiated 2 min after the warm-up set. For each 1RM attempt, subjects were instructed to try to complete two repetitions. If a subject completed one or two repetitions, the resistance used in the second 1RM attempt was increased by 3–10 %. If the first attempt was not successful, the resistance used in the second 1RM attempt was decreased by 3–10 %. This same procedure was used to perform a third 1RM attempt. The rest period was 3 to 5 min between 1RM attempts of an exercise and 5 min between exercises. The 1RM was recorded as the heaviest resistance lifted in the three 1RM attempts of an exercise in which the subject was able to complete a complete repetition as normally defined for that exercise (Amarante do Nascimento et al. 2013). Three 1RM testing sessions were separated by 48 h. This same procedure was used during each of the 3-day periods pre-training, post-training, and after detraining on which 1RMs were determined. To help ensure 1RM reliability, exercise technique for each exercise was standardized and continuously monitored during all testing sessions. To ensure subject safety, two experienced researchers supervised all 1RM testing sessions. Standardized verbal encouragement was given during testing sessions. The greatest resistance achieved in the three sessions pre-training, post-training, and after detraining was used for statistical analysis. During all sessions, participants were allowed to drink water whenever necessary and were encouraged to remain hydrated throughout testing sessions. This 1RM testing procedure resulted in an ICC≥0.97 pre-training, post-training, and after detraining for all three exercises tested.

Oxidative stress indicators

Blood was collected from the antecubital vein with participants in the sitting position after a 12-h fasting period. After collection, tubes containing ethylenediamine tetra-acetic acid plus samples were centrifuged at 3.000*g* for 5 min at 4 °C for plasma separation.

Advanced oxidation protein products (AOPP) were determined by a semi-automated method (Witko-Sarsat et al. 1996) for assessing the oxidant capacity in micromoles per liter (μmol L^{-1}), equivalent to chloramine-T. The antioxidant capacity was determined by evaluation of the total radical-trapping antioxidant potential (TRAP) using the chemiluminescence method with induction time of 2.2-azobis (2-amidinopropane) and calibrated with analogue TROLOX vitamin E (Repetto et al. 1996). This method detects water-soluble and soluble antioxidants in the plasma. TRAP values were expressed in micromoles Trolox equivalents.

Resistance training program

Supervised RT was performed in the morning during the 12 weeks of training. RT was based on recommendations for older populations to improve muscular strength and endurance (American College of Sports Medicine 2009; Garber et al. 2011). All participants were individually supervised by physical education professionals throughout each training session to ensure that the study's training protocol was followed and to ensure subject safety. Participants performed RT using a combination of machines and free weights (preacher curl).

The G2X performed RT twice a week (Tuesdays and Thursdays), while G3X performed three sessions a week (Mondays, Wednesdays, and Fridays). Both training groups performed the same RT program composed of eight exercises performed in the following order: chest press, horizontal leg press, seated row, knee extension, preacher curl, leg curl, triceps pushdown, and seated calf raise. In each, exercise was performed one set of 10–15 repetitions maximum. Participants were instructed to inspire during the eccentric muscle action and exhale during the concentric muscle action in each repetition of all exercises and to maintain a ratio of 1:2 for the concentric and eccentric muscle actions, respectively. Participants rested for 2 to 3 min between each exercise. The load used for each exercise was adjusted according to the subject's ability and improvements in exercise capacity throughout the study to assure that the subjects were exercising with as much resistance as possible while maintaining proper exercise technique. Progression was planned so that when 15 repetitions within a given exercise were completed on two consecutive training sessions, the weight was increased 2–5 % for the upper limb exercises and 5–10 % for the lower limb exercises (American College of Sports Medicine 2009). At the end of each session, one flexibility exercise was performed for all major muscle groups. Subjects were asked not to perform any other type of physical exercise during the entire study period.

Detraining period

Participants from both groups were requested not to perform any type of physical exercise for 12 weeks after the post-training measurements. In addition, they were encouraged to maintain their regular daily living activities throughout this period and not to change their nutritional habits.

Statistical analyses

Normality of all data was checked using the Shapiro-Wilk's test. Levene's test was used to analyze the homogeneity of variance. Baseline difference comparisons were analyzed with an independent *t* test. Two-way analysis of variance (ANOVA) for repeated measures was used for within- and between-group comparisons. In variables where sphericity was violated as indicated by Mauchly's test, the analyses were adjusted using a Greenhouse-Geisser correction. When an *F* ratio was significant, Bonferroni's post hoc test was applied to identify mean differences. The effect size (ES) for training was calculated as post-training mean minus pre-training mean divided by pooled standard deviation of pre- and post-training, while the detraining ES was calculated as detraining mean minus post-training mean divided by pooled standard deviation of detraining and post-training. The ES magnitude was evaluated by the scale suggested by Cohen where an ES of 0.20–0.49 was considered small, 0.50–0.79 moderate, and ≥0.80 large (Cohen 1988). For all statistical analyses, significance was accepted at $P<0.05$. The data were stored and analyzed using STATISTICA software version 10.0 (STATSOFT Inc., Tulsa, OK, USA).

Results

There were no significant differences at pre-training between the two groups for any variable investigated ($P>0.05$). The anthropometric characteristics of the participants are presented in Table 1.

Table 1 General characteristics of the sample at baseline

Variables	G2X (n=13)	G3X (n=14)	P value
Age (years)	68.9±5.0	68.7±4.8	0.91
Body mass (kg)	66.7±13.9	71.3±14.8	0.82
Height (cm)	155.6±7.2	156.3±7.2	0.48
Body mass index Kg.m^{-2})	27.4±4.8	29.1±5.0	0.88

Data are presented as mean and standard deviation

G2X group that performed resistance training twice a week, *G3X* group that performed resistance training three times a week

Changes in muscular strength at the different time points of the study are presented in Table 2. There was a significant group by time interaction (P<0.05) for 1RM chest press, in which the G3X showed a greater increase after training than G2X (G3X=+27.5 %, ES=2.43 vs G2X=+11.9 %, ES=0.62), and both groups showed significant reductions (P<0.05) after detraining (G3X= −9.1 %, ES=−0.80 vs G2X=−10.2 %, ES=−0.59). No significant effects for group by time interaction or for main effect of group were observed for the 1RM in knee extension and preacher curl (P>0.05). However, a significant main effect of time (P<0.05) was observed with both groups showing similar increase after training (knee extension, G2X=+18.4 %, ES=0.83 vs G3X=+16.7 %, ES=0.67; preacher curl, G2X=+37.6 %, ES=1.80 vs G3X=+36.7 %, ES=2.13) and reductions after detraining (knee extension, G2X=−14.9 %, ES=−0.82 vs G3X=−12.1 %, ES=−0.52; preacher curl, G2X= −20.5 %, ES=−1.52 vs G3X=−17.4 %, ES=−1.29).

Figure 1 displays the OS biomarkers by group at different time points of the study. No significant effect for group by time interaction or for main effect of group or time was found for AOPP (P>0.05). On the other hand, a main effect of time was observed for TRAP (P<0.001), in which both groups similarly increased after training (G2X=+6.9 %, ES=0.44 vs G3X=+ 15.1 %, ES=0.94) and remained elevated after the detraining period.

Discussion

The main and novel finding of the present study was that RT improves OS by increasing antioxidative biomarkers in older women (mean age 68 years) independent of the typical training frequencies of two and three sessions per week. Additionally, a 12-week detraining period results in a decrease in antioxidant biomarkers compared to post-training, but antioxidant biomarkers remained elevated compared to pre-training. To the best of our knowledge, this is the first study designed to compare the effects of RT frequencies of two versus three times per week followed by a detraining period on OS in older women.

To date, few studies have investigated the RT effect on OS biomarkers, and our outcomes agree with some previous investigations. Rowinski et al. (2013) concluded that OS increases with age when comparing "65-69-year-old group and a 90-year-old one"., but physical activity can reduce OS biomarkers and induce an adaptive increase in erythrocyte antioxidant enzyme activity regardless of age. Parise et al. (2005) showed that in older individuals (mean age 71 years), 12 weeks of RT with three sessions per week increased muscle

Table 2 Muscular strength (kg) at pre- and post-training according to resistance training frequencies

	G2X (n=13)	G3X (n=14)	Effects	F	P value
Chest press					
Pre-training	35.7±6.6	37.1±3.8	Group	2.70	0.11
Post-training	42.2±8.0[a]	47.3±4.6[a]	Time	111.87	<0.001
Detraining	37.9±6.6[ab]	43.0±6.2[ab]	Interaction	7.36	<0.01
Knee extension					
Pre-training	41.3±9.3	46.1±10.5	Group	1.67	0.20
Post-training	48.9±9.1[a]	53.8±12.5[a]	Time	37.70	<0.001
Detraining	41.6±8.7[b]	47.3±12.4[b]	Interaction	0.15	0.85
Preacher curl					
Pre-training	17.0±3.3	17.7±2.5	Group	0.69	0.41
Post-training	23.4±3.8[a]	24.2±3.6[a]	Time	160.00	<0.001
Detraining	18.6±2.5[ab]	20.0±2.9[ab]	Interaction	0.46	0.63

Data are expressed as mean and standard deviation

G2X group that performed resistance training twice a week, *G3X* group that performed resistance training three times a week

[a]P<0.05 vs. pre-training

[b]P<0.05 vs. post-training

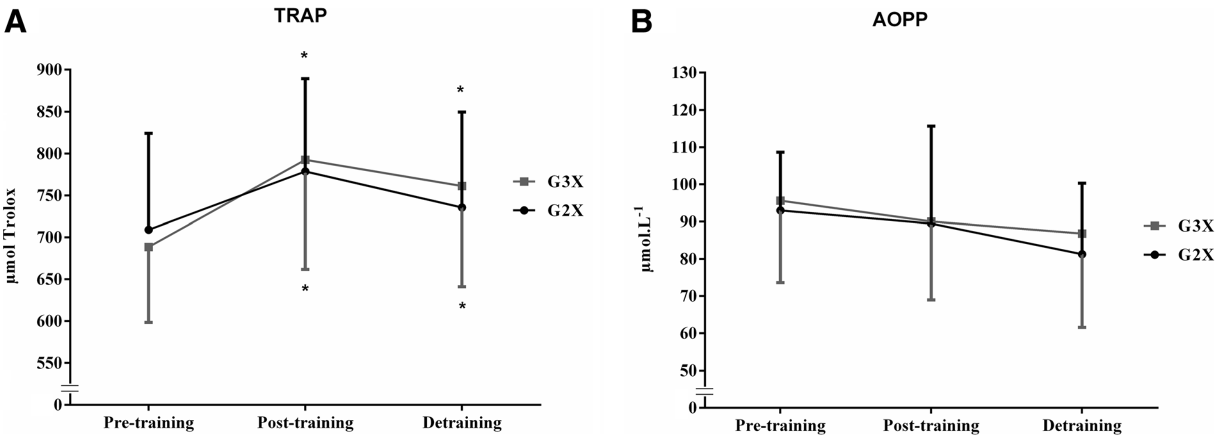

Fig. 1 Advanced oxidized protein products (*AOPP*) (**a**) and total radical-trapping antioxidant parameter (*TRAP*) (**b**) at pre-training, post-training, and detraining in older women according to resistance training frequencies. *G2X* group that performed resistance training twice a week ($n=13$), *G3X* group that performed resistance training three times a week ($n=14$). There is no group by time interactions. *$P<0.05$ vs. pre-training. Data are expressed as mean and standard deviation

antioxidant capacity. Our results, however, do not agree with Venojarvi et al. (2013) who showed that 12 weeks of aerobic and RT performed three times per week did not result in modifications of OS in overweight middle-aged men (40–65 years).

Several techniques are available for the assessment of OS biomarkers, such as TRAP and AOPP which were analyzed in the current study, but also include other biomarkers of oxidation such as thiobarbituric reactions species (TBARS), malondialdehyde (MDA), lipoperoxidation by chemiluminescence induced by *terc-butil* hydroperoxides (CL), and other antioxidant biomarkers such as catalase activity (CAT), superoxide dismutase activity (SOD), glutathione peroxidase (GPx), glutathione oxidized (GSSG), glutathione reduced (GSH) and glutathione total (GSH/GSSG), and ferric-reducing antioxidant power (FRAP).

The TRAP assay is useful in getting a global picture of relative antioxidant activities in different fluids and how they change in clinical conditions. Furthermore, TRAP has been the most widely used among antioxidant defense indicators (Venturini et al. 2012). However, the potential mechanisms for the increase in TRAP are not yet fully elucidated, although the OS modulation caused by RT may be related to some known mechanisms.

Physical exercise can increase the synthesis of ROS through the activation of the electron transport chain, the synthesis of lactic acid, catecholamines, and inflammation factors that contribute to the production of reactive oxygen species. Additionally, anaerobic exercise, such as RT, can increase the synthesis of xanthine oxidase and NADPH oxidase enzymes both of which affect OS modulation (McHugh et al. 1999). In response to this process, the antioxidant system adapts with adjustments favorable to the endogenous antioxidant system, thus increasing the body's defense capacity.

The oxidant biomarker investigated in our study was AOPP because it involves a relatively simple methodology and is a commonly used marker of OS. AOPP is a reliable marker to estimate the degree of damage to proteins, the intensity of OS and inflammation. Oxidative damage to proteins is caused by action of free radicals and various other oxidizing compounds (de Gonzalo-Calvo et al. 2013). It has been suggested that AOPP measures highly oxidized proteins, especially albumin.

The AOPP is derived not only from oxidation-modified albumin but also from fibrinogen and lipoproteins. Physiologically, AOPP is formed in small quantities during the entire life span, and its synthesis increases with age. Given this information, despite the relatively short study duration, the absence of changes of this oxidant biomarker throughout the experimental period suggests that RT can possibly attenuate the deleterious effects of the aging process.

Our results indicate also that increased RT volume due to increased training frequency from two to three times a week only positively affected chest press strength with increased training frequency having no effect on knee extension and preacher curl strength. To date, only a few studies have investigated the effects of different resistance training frequencies in seniors ranging in age from 60 to 79 years, and the results of these studies are not in agreement (DiFrancisco-Donoghue et al. 2007; Farinatti et al. 2013; Taaffe et al. 1999). For example, the results observed by both DiFrancisco-Donoghue et al. (2007) and Taaffe et al. (1999) indicated that programs with high and low frequencies produce similar changes in muscular strength. However, the training frequencies compared in these previous studies were one and two sessions per week. Farinatti et al. (2013) compared one, two, and three training sessions per week and observed that in two exercises (seated dumbbell curl and knee extension), higher training frequencies resulted in greater strength improvement, while in two other exercises (bench press and standing calf raise), muscular strength was improved equally with one, two, and three sessions per week. Collectively, the results of the studies to date suggest that strength increases caused by different frequencies may be exercise-dependent and warrant further investigation.

Detraining did not significantly affect TRAP or AOPP compared to post-training, indicating that the 12-week detraining period did not reverse this benefit promoted by RT. Concerning muscular strength, our findings are consistent with previous results reported in literature, indicating that detraining results in decreased muscular strength in older individuals, although after detraining, strength can still be greater than prior to training over shorter (4 weeks), similar (12 weeks), or longer (24 weeks) detraining periods (Correa et al. 2013; Fatouros et al. 2006; Lovell et al. 2010). A more detailed comparison with findings from the literature is not simple, due to the possible effects of training intensity, the effect on training volume due to number of sets and repetitions per set, type of exercise, length of training periods, and length of detraining periods. It is important to note that in our study, although muscular strength has been significantly reduced after 12 weeks of detraining compared to post-training, the values found were still greater after detraining compared to pre-training in two of the exercises investigated (chest press and preacher curl).

Single-set training has been recommended as a starting point for beginners engaged in RT (American College of Sports Medicine 2009; Cadore et al. 2014). This recommendation is largely based on studies showing that single-set protocols are as effective as multiple sets for improvements in muscular strength and body composition outcomes in this population (Cannon and Marino 2010; Radaelli et al. 2013). Our results add to these guidelines indicating that a single set program also can increase antioxidant OS biomarkers in older women. However, future studies are needed to replicate these findings.

Our investigation has some limitations. The absence of additional OS biomarkers is a weakness. Group sizes were relatively small, and we thus cannot rule out the possibility of a type II error. The lack of a non-trained control group, although not essential to answer the research question, can be considered a limitation as well, because it impedes the ability to draw conclusions on the effect of training per se on the dependent variables. Moreover, results cannot be extrapolated to other populations. Finally, we did not control or evaluate daily physical activity level, especially during the detraining period, although the older women were asked to maintain their regular daily living activities throughout this period and not to change their nutritional habits.

The practical application of our results from an OS standpoint suggests that a flexible approach to training frequency in program design can be used and training frequency can be tailored to lifestyle preferences and individual response to the respective training programs. Furthermore, from a clinical point of view, in order to prevent losses of the adaptations induced by RT in important variables, such as muscular strength and antioxidant capacity, due to detraining, older women should avoid physical inactivity periods.

Conclusions

The results of the present study suggest that a 12-week RT program with a frequency of 2 days per week may be sufficient to improve muscular strength and OS in older women, whereas detraining for 12 weeks does not completely reverse the changes induced by RT compared to pre-training.

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