Explosive-type of moderate-resistance training induces functional, cardiovascular and molecular adaptations in the elderly

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Abstract

Current recommendations aimed at reducing neuromuscular and functional loss in aged muscle have identified muscle power as a key target for intervention trials, although little is known about the biological and cardiovascular systemic response in the elderly. This study investigated the effects of 12 weeks of low frequency, moderate intensity, explosive-type resistance training (EMRT) on muscle strength and power in old community-dwelling people (70–75 years), monitoring functional performance linked to daily living activities (ADL) and cardiovascular response, as well as biomarkers of muscle damage, cardiovascular risk, and cellular stress response.

The present study provides the first evidence that EMRT was highly effective in achieving a significant enhancement in muscular strength and power as well as in functional performance without causing any detrimental modification in cardiovascular, inflammatory and damage parameters. Moreover, trained elderly subjects showed an adaptive response both at systemic and cellular level by modulation of anti-oxidant and stress-induced markers such as myeloperoxidase (MPO), heat shock protein 70 (Hsp70) and 27 (Hsp27), and thioredoxin reductase 1 (TrxR1).

Introduction

Sarcopenia, the age-related loss of muscle mass and function, is accompanied by reduced ability to generate maximal muscle force (i.e. muscle strength), muscle power (the product of contractile force and movement velocity) and oxidative capacities, influencing negatively functional motor performance and self-sufficiency of the elderly (Narici and Maffulli, 2010; Aagaard et al., 2010; Fielding et al., 2011). Despite the causes behind this process are still under investigation, sarcopenia is considered to be a multi-factorial process, including biological conditions such as altered hormone secretion, increased oxidative stress or changes in circulating pro-inflammatory cytokines (Narici and Maffulli, 2010; Aagaard et al., 2010; Fielding et al., 2011).

Although muscle strength is considered an important determinant of functional limitation in older adults (Hairi et al., 2010), several studies indicated that muscle power is a stronger predictor than strength for daily motor activities such as fast-walking, stair-climbing, rising from a chair (Bean et al., 2003), and that peak muscle power was associated with functional limitations in older people (Bassey et al., 1992; Skelton et al., 1994; Izquierdo et al., 1999; Suzuki et al., 2001). Moreover, muscle power declines earlier and at a higher rate than strength (Izquierdo et al., 1999; Caserotti et al., 2008). Consequently, recent recommendations aimed at reducing age-related neuromuscular and functional loss have identified muscle power as a key target for intervention trials (Reid and Fielding, 2011). Explosive-type resistance training regimes are proposed to improve muscle power in older adults (Hakkinen et al., 2001; de Vos et al., 2005; Caserotti et al., 2008).

Increasing evidence suggests that chronic systemic inflammation and accumulating oxidative stress are related to the aging process and play a role in developing many chronic diseases' risk factors such as atherosclerosis, hypertension and insulin resistance (Stephens et al., 2009; Chung et al., 2009). Anti-inflammatory effects of regular exercise, including resistance training (RT), have been reported (Phillips et al., 2010), however, contradictory results have been found and they cannot be completely confirmed (Levinger et al., 2009). On the other hand, intense acute exercise can produce inflammation and excessive reactive oxygen and nitrogen species (Buford et al., 2009; Rietjens et al., 2007), possibly leading to increased damage to molecules if protective mechanisms are not enough stimulated. Nevertheless, a training regime at moderate intensity may be effective in inducing a specific adaptation response at both systemic and cellular levels. The primary purpose of this study was to examine the effect of a low frequency, moderate intensity, explosive-type resistance training (EMRT) on muscle strength, power and ADL performance, and whether this modulated the expression of antioxidant and/or stress-induced proteins as part of an integrated system of signaling critical to the support and mediation of physiological adaptation to RT.

Material and Methods

Study design

Eighty subjects were recruited from Rome community area by advertisements, by word of mouth and from social clubs. Before inclusion in the study, all participants underwent a scrupulous medical screening. Fifty-two subjects showing signs of cardiovascular, metabolic and pulmonary disease, orthopedic injury or joint disease, neurological or immunologic disease, or involved in resistance training into the past 12 months were excluded from the study. Twenty-eight volunteers were randomly assigned to either control or trained groups. Five subjects (2 from the experimental group and 3 from the control group) dropped out due to family or personal reasons. Therefore 13 subjects in the trained group and 10 subjects in the control group, with similar baseline characteristics (Table 1) and without any strength training background, successfully completed the study. The training group followed twelve weeks of explosive-type resistance training, two days per week on alternative days. The control group did not engage in physical training during the entire period of the experiment and maintained the usual lifestyle habits. Physical activity level was evaluated using the Modified Baecke Questionnaire for Older Adults (Voorrips et al., 1991). All subjects gave their informed consent prior to participation in the research. The study was approved by the Ethics Committee of the University of Rome "Foro Italico".

Before (PRE) and after (POST) exercise intervention, strength, power and physical performances were assessed by the same researcher on alternate days in both groups. Before the testing sessions, subjects included in the study came to the laboratory to collect a blood sample at rest. The same day participants performed a familiarization session with the climbing stairs and walking physical performance tests. In the first testing session, physical performance was evaluated and following the subjects performed a familiarization session with the resistance training exercises in order to get feedback regarding the correct technique and cadence of lifting. Then, in an alternate day, they performed another familiarization session to become accustomed to the devices and to learn the procedure of maximal strength and power tests. We verified that the performance of subjects that would ultimately form the experimental and control groups was equated at the end of the familiarization session. The second testing session included measurement of maximum dynamic strength by one maximum repetition (1RM) and the third session included the assessment of maximum muscle power. Verbal encouragement was given to maximize motivation and performance. At the end of the 3^{th} month training period, each group performed a familiarization session before the final tests. Then, participants had the blood sampling at least 3

days after the last testing session. Moreover, an evaluation of hemodynamic/cardiovascular response to maximal exercise stress test and of the level of specific markers of cardiac and skeletal muscle damage such as, serum concentration of myeloperoxidase (MPO), N-terminal pro-brain natriuretic peptide (NT-pro-BNP) and creatine kinase (CK), as well as the total anti-oxidant status (TAS), pro- and anti-inflammatory cytokines (IL-4, IL-6, IL-13, IL-15, TNFα and MIP-1b) was performed. Further, we analyzed in peripheral blood mononuclear cells (PBMCs) the level of lipid peroxidation (4-hydroxynonenal [4-HNE]), the expression of antioxidant and stress-induced proteins (thioredoxin reductase 1 [TrxR1], thioredoxin 1 [Trx1] and 2 [Trx2], heat shock protein 70 [Hsp70] and 27 [Hsp27]).

Maximal exercise stress test

Maximal exercise stress test was performed with a cycloergometer according to the following protocol: at first the load was fixed at 30 Watt for 3 minutes, then the exercise load was increased by 10 Watt every minute until reaching 85% of maximal heart rate (HR_{max}) (calculated through this formula: HR_{max}= 220 - age in years). If the subjects were able to continue the test, they were encouraged to prolong the effort until exhaustion. A continuous 12-lead ECG recording was performed and blood pressure was assessed through a mercury sphygmomanometer during the test and the following recovery.

Assessment of metabolic equivalent (MET) energy expenditure also to be a simple, practical, and easily understood procedure to quantify the energy cost of activities, is routinely utilized to describe the functional capacity or aerobic power of an individual and to provide repertoire of activities in which subject can safely participate (Jettè et al., 1990).

It is defined as the ratio of metabolic rate (and therefore the rate of energy consumption) during a specific physical activity to a reference metabolic rate, set by convention to 3.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ (Jettè et al., 1990).

Heart Rate Recovery (HRR) was defined by the following formula: HRR= peak Heart Rate – HR_t, where "t" in our study corresponds to one minute after the cessation of exercise. It is an excellent indicator of cardiovascular health and seems to be associated to the aerobic fitness level (Cole et al., 1999).

Strength test

Upper and lower body extremity maximal strength was assessed by one repetition maximum (1RM) test. 1RM was estimated for leg-extension, leg-curl, low-row and chest-press using iso-inertial RT equipment (Technogym, Italy). After a general warm-up, the test was performed to find the heaviest load that the subject could lift 5

times (5RM) with the correct technique. The test started with a load that the subject could lift for 7-8 repetitions, according to the data obtained in the familiarization session. Subjects rested for 3 minutes, and then the load was increased by approximately 15% and lifted again until reaching the 5RM target which was generally obtained within 3 attempts. Subsequently 1RM was estimated from Baechle and Earle (2008).

Power test

As previously validated by Squadrone (Squadrone et al., 2012), muscle power was evaluated using a wireless inertial measurement unit (FreePower®, Sensorize, Italy) on a separate day than the 1RM test. The FreePower® contains a 3D accelerometer and a 3D gyroscope, therefore, during each lift a 3D linear acceleration and angular velocity are provided via Bluetooth to a laptop computer. The software complementary to the FreePower® stores and analyses the data of each lift to calculate linear displacement velocity and power parameters. The inertial measurement unit can be placed on the weight stack and on the centre of body mass by using a belt. Muscle power was evaluated during leg-extension, leg-curl, low-row and chest-press exercises using a load corresponding to the 70% of the 1RM. Participants performed 2 series of 6 repetitions with 3 minute rest between sets. They were encouraged to perform each repetition with maximal voluntary acceleration during the concentric part of the movement and to control speed during the eccentric phase. The lift producing maximum power was considered for data analysis.

Muscle power was evaluated also during countermovement jump (CMJ). Three standardized CMJ separated by 2 minute rest interval were performed. The inertial measurement unit of FreePower® was positioned approximately at the centre of body mass, placing the belt around the waist. Subjects started from a standing position with hands on their hips and were instructed to perform a fast downward movement up to 90º of knee flexion followed by an upward movement trying to jump as high as possible. The trial reporting maximum jump power was selected for further analysis.

Physical performance tests

Stair climbing time test: stair climbing ability was evaluated by the time employed in ascending a 12-stair flight that was measured using photocell chronometric devices (Ergo System, Globus, Italy). Subjects were instructed to ascend as quickly as possible touching every step without using the handrail. After 3 minutes rest, participants performed a stair climbing loaded test that consisted in repeating the stair climbing test while carrying one dumbbell in each hand with a total load corresponding to 12kg for women and 16 kg for men. For both tests,

stair climbing and loaded stair climbing, participants performed 3 trials separated by 1 minute rest and the fastest trial of each test was considered for further analysis.

Walking time test: speed was evaluated by the time employed in walking 6 meters at maximum velocity and was measured using a photocell chronometric device (Ergo System, Globus, Italy). Subjects were instructed to walk at maximum velocity, without running. After 3 minutes rest, participants performed a walking time loaded test in which subjects repeated the walking test carrying one dumbbell per hand with a total load corresponding to 12kg for women and 16 kg for men. For both tests, walking time and loaded walking time, participants performed 3 trials separated by 1 minute rest and the fastest trial of each test was considered for further analysis.

Explosive-type resistance training protocol

The participant in the training group performed two days of explosive-type resistance training for 12 weeks using the four RT machines used for the testing sessions (Technogym, Italy). Training sessions began with 10 minutes of specific neuromuscular warm-up. The first two weeks of training was performed at an intensity of 40-50% 1RM (15 repetitions-4 sets) at moderate velocity to facilitate muscle adaptation. Thereafter, the specific explosive training was initiated and each exercise was performed for 3-4 sets of 10-12 repetitions at 70% of baseline 1RM with 2 minutes rest between sets and 3 minutes rest between exercises. Participants were encouraged to perform the concentric part of each repetition at maximal intentional load acceleration while performing the eccentric phase of each repetition with moderate speed. Approximately every two weeks, resistance was incremented when a subject completed 12 repetitions for at least two of the total sets at a given weight load while maintaining proper exercise technique (Baechle and Earle, 2008). The goal of this progression was to induce volitional fatigue in the 10 to 12-repetition throughout the training program. The increase in resistance was usually of 2.5 kg and before the increment the perceived fatigue was evaluated to consider also the individual perception of the load. The EMRT was designed and supervised by expert strength and conditioning coaches. Participants were continuously visually examined to verify correct technique and tempo of the movement and were also verbally encouraged by the coaches.

Blood sampling

Before and after 12 weeks of intervention, fasted blood samples were drawn from the antecubital vein while subjects remained in reclined position. Samples in additives-free tubes (BD Biosciences, San Jose, CA, USA) were left at room temperature for coagulation for at least 1 hour and then centrifuged (2,500*rpm* ×10 min) for

serum separation. Blood sampled in EDTA tubes (BD Biosciences) were used for plasma collection by centrifugation of whole blood (2,500*rpm* ×10 min at 4ºC), and for PBMCs isolation. Serum, plasma and PBMCs samples were aliquoted and stored at -80°C for further analyses.

Isolation of PBMCs and Western Blot analysis

Humans PBMCs were purified from whole blood by Ficoll gradient (Sigma-Aldrich, Milan, Italy), using a standard technique (English and Andersen, 1974). The cells were then lysed in lyses buffer (RIPA) and their protein content was determined using the BCA assay (Sigma-Aldrich). For immunoblotting analysis, similarly to Towbin et al. (1979), aliquots of cell extract were electrophoresed on an SDS-PAGE and transferred onto a polyvinylidene fluoride membrane (PVDF, Amersham Biosciences, Milan, Italy). Membranes were blocked with 5% nonfat dry milk and exposed to the following antibodies: Hsp27 (1: 2,000), TrxR1 (1: 2,000), Trx1 (1: 500), Trx2 (1: 500), TrxR2 (1: 500) (Santa Cruz Biotechnology, CA, USA); β-actin (1: 3,000; Sigma-Aldrich); Hsp70 (1: 1,000; Stressgen, Florence, Italy); 4-Hydroxynonenal (HNE) (1: 1,000; Abcam, Cambridge, UK). All immunoblots were visualized with horseradish peroxidase-conjugated secondary antibody followed by detection with enhanced chemiluminescence (Amersham Biosciences). Bands were quantified by Image J software. The expression of β-actin was used as a normalizing control.

Measurement of Myeloperoxidase and NT-proBNP

Commercial ELISA tests were purchased for the assessment of MPO and NT-proBNP (EIAab Science Co.Ltd) levels in serum. ELISA tests were performed according to the manufacturer's protocol.

Cytokine profile

The multiplex analysis methodology (Vignali, 2000) was utilized for cytokine profiling. In this study we analyzed cytokines concentration in plasma by Bio-Plex system (Bio-Rad Laboratories, Milan, Italy) in accordance with the manufacturer's instructions. The following cytokines were included: IL-4, IL-6, IL-13, IL-15, TNFα and Macrophage inflammatory protein-1-beta (MIP-1b). Briefly, after thawing, plasma samples were centrifuged at 10,000*rpm* for 10 min at 4°C to clear the samples of precipitate. The samples were then incubated with 25 µl of anti-cytokine conjugated beads in 96-well filter plates for 30 min at room temperature with shaking and washed three times by vacuum filtration with 100 µl of Bio-Plex wash buffer. Next, 25 µl of diluted detection antibody was added, and plates were incubated for 30 min at room temperature with shaking. After

three filter washes, 50 µl of streptavidin-phycoerythrin was added, and the plates were incubated for 10 min at room temperature with shaking. Finally, plates were washed by vacuum filtration three times, beads were suspended in Bio-Plex assay buffer, and samples were analyzed on a Bio-Rad 96-well plate reader using the Bio-Plex Suspension Array System and Bio-Plex Manager software 4.1 TM (Bio-Rad Laboratories, Segrate, Mi, Italy).

Total antioxidant status (TAS)

Plasma TAS was determined spectrophotometrically, accordingly to Miller et al. (1993). This method is based on the reactivity of plasmatic antioxidant compounds relative to a 1 mM Trolox® (vitamin E analogue) standard. Briefly, 10 µl of plasma or Trolox® standards (0.25 - 0.5 - 1.0 mM) were incubated in ABTS-metMyo-PBS buffer and the absorbance at 734nm was monitored for 2 minutes. The reaction was started by the addiction of H_2O_2 (450 µM) and variation of absorbance was then recorded. Sample $\Delta OD/min_{734}$ were compared to those obtained by using Trolox® standards.

Creatine Kinase (CK)

Plasma CK activity was determined spectrophotometrically, according to manufactory recommendations, by manual procedure using a commercial test kit (Greiner Diagnostic GmbH, Bahlingen-Gremany). Briefly, plasma was incubated in Hexokinase-Glucose 6 Phosphate-G6P Dehydrogenase buffer for 3 minutes and then NADPH production was followed at 340 nm for a further 3 minutes.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics 18 (IBM Corporation). After testing whether data were normally distributed (*Shapiro-Wilk test*), an analysis of variance (ANOVA) with repeated measures for time (pre-training and at 12 weeks) and group (trained and control) was performed. In the case of non-homogeneity of variances revealed by the Mauchly's sphericity test ($p < 0.05$), the *Greenhouse-Geisser* correction was used to assess significant main effects. Whenever significant main effects were observed, *Bonferroni's* post hoc correction was used to aid interpretation of these interactions. The delta percentage was calculated trough the standard formula: Change (%) = [(post-test score-pre-test score)/pre-test score] ×100.

RESULTS

Baseline and exercise stress-induced cardiovascular parameters

Anthropometric and physical activity level of experimental (TRAINED) and control (CONTROL) groups are shown in Table 1. Unless differently stated, no gender differences were found concerning all parameters analyzed ($p > 0.05$). No significant differences between groups were reported for all baseline characteristics of participants.

There was not a significant interaction time × group for resting heart rate (HR) nor for blood pressure. HR, systolic (SBP) and diastolic blood pressure (DBP) remained unchanged after the training period for both groups ($p > 0.05$) (Table 2). On the other hand, in response to maximal exercise stress test, a significant group × time interaction was found for the peak cycling intensity achieved ($p = 0.002$). Training group compared to control group improved the peak watts ($p = 0.004$). Training group improved the peak load reached from PRE to POST protocol by 16% ± 3.5 (PRE *vs.* POST: 96 W ± 7 *vs.* 112 W ± 8, $p = 0.001$). The peak load achieved by the control group remained unchanged ($p = 0.51$). In addition, also peak heart rate reached during the test showed group × time interaction ($p = 0.009$). The training group in comparison with the control group reached a higher peak heart rate after training ($p = 0.008$). The training group increased the peak heart rate by 4.7% (PRE *vs.* POST: 130 ± 2 *vs.* 136 ± 2 bpm, $p = 0.04$) while it remained unchanged in the control group ($p = 0.1$) (Table 2). Further, maximal blood pressure attained during the cycloergometer test was not affected by the training period in any of the groups neither for SBP, ($p = 0.2$) nor for DBP ($p = 0.3$). Similarly, at any time the estimated exercise capacity (MET) did not change for both groups ($p = 0.1$). HRR showed a tendency to increase in the training group ($p = 0.06$) (Table 2).

Muscle power, muscle strength and functional motor capabilities

Twelve weeks of EMRT induced significant time × group interaction in peak power in all upper and lower body exercises and in the countermovement jump (CMJ) ($p = 0.001$ and $p = 0.004$ respectively). Following training, peak power was higher in the exercise group compared to the control group for all five exercises ($p < 0.05$) (Fig. 1A and Table 3). In addition, peak power increased after training compared to baseline within experimental group. The peak muscle power increased by 36.0% ± 5.2 in leg extension ($p = 0.001$), 28.0% ± 5.4 in leg curl ($p = 0.001$), 34.0% ± 3.4 in chest press ($p = 0.001$), 28.0% ± 4.5 in low row ($p = 0.001$), 18.0% ± 4.3 in the CMJ ($p = 0.004$). There were not changes within the control group in the power output of the five exercises (range from -2% to -5%) ($p > 0.05$).

A significant time × group interaction was found for peak strength of the four training exercises ($p = 0.001$). Maximal strength improved in the exercise group compared to the control one in all four exercises ($p < 0.05$) (Fig. 1B and Table 3). Maximal strength post-training was higher compared to pre-test values in the training group. The mean increase in strength was 16.0% ± 1.1 ($p = 0.001$) in leg extension, 15.0% ± 1.5 ($p = 0.001$) in leg curl, 20% ± 1.9 ($p = 0.001$) in chest press, 15.0% ± 1.2 ($p = 0.001$) in low row. Maximal strength did not change in the control group from PRE to POST protocol, with values almost unchanged (range from -1.7% to 0.6%) ($p > 0.05$).

The evaluation of functional capacity through daily living motor tasks resulted in significant time × group interaction in 6m walking test ($p = 0.001$), 6m walking loaded ($p = 0.002$), stairs climbing ($p = 0.01$) and stairs climbing loaded ($p = 0.008$). The training group decreased the time to perform these four exercises compared to the control group ($p < 0.05$) (Fig. 1C and Table 3). Time in the PRE tests was reduced compared to the POST tests within training group in the 6m walking test by 9.0% ± 1.6 ($p = 0.001$), 6m walking with loads by 10.0% ± 2.1 ($p = 0.001$), stairs climbing by 8.0% ± 3.1 ($p = 0.04$) and stairs climbing with loads by 12.0% ± 2.5 ($p = 0.006$). The control group did not change the performance time of each test, with a slightly increase in the percentage of change (0-4%) ($p > 0.05$).

Circulating MPO, NT-ProBNP, CK, TAS and cytokines

The evaluation of serum MPO as a marker of oxidative stress and inflammation showed a time × group interaction ($p = 0.008$). Training group decreased MPO concentration compared to the control group ($p = 0.005$). Training group showed a reduction of MPO concentration from PRE to POST protocol by 16.0% ± 3.0 (PRE *vs.* POST: TRAINED, 86.8 ± 6.4 *vs.* 74.5 ± 3.2 $pg \cdot mL^{-1}$, $p < 0.05$). Otherwise, control group did not change MPO concentration ($p > 0.05$) (Fig. 2A). In relation to the serological marker of cardiac damage NT-proBNP, we did not find any time × group interaction ($p = 0.62$) (Fig. 2B). There were not changes at any time and in any group analyzed ($p > 0.05$).

Similarly, the plasma marker of muscle damage CK did not show a change at any time and in any group observed ($p > 0.05$) (Fig. 2C). Likewise, there was not a time × group interaction for total antioxidant capacity (TAS) ($p > 0.05$). No differences were found within group and between the groups ($p > 0.05$) (Fig. 2D).

The evaluation of serum cytokines did not show a time × group interaction of IL-4, IL-6, IL-13, IL-15, MIP-1b and TNFα ($p > 0.05$). The pattern of change of these cytokines was not different between PRE and POST intervention for both training group and control group (Table 4).

PBMCs expression of Hsp70, Hsp27 and Trx

The analyses of HSPs in PBMCs revealed a significant time × group interaction for Hsp70 ($p = 0.006$) and Hsp27 ($p = 0.01$) (Fig. 3A and 3B). PRE levels of the HSPs were not different between training and control group. After EMRT, the training group showed a decrease in the expression of Hsp70 ($p = 0.003$) and Hsp27 ($p = 0.006$) compared to the control group. Whereas changes within the training group showed a decreased expression of both Hsp70 and Hsp27 from PRE to POST intervention period by 32.0% ± 6.0 and 51.4% ± 12.5, respectively (PRE *vs.* POST: Hsp70/β-actin ratio, 0.5 ± 0.05 *vs.* 0.3 ± 0.03; Hsp27/β-actin ratio, 0.3 ± 0.08 *vs.* 0.1 ± 0.04, $p < 0.05$), the control group did not show any change of their expression from PRE to POST intervention ($p > 0.05$).

In relation to lipid peroxidation marker, no significant time × group interaction was found for the levels of 4-HNE adducts in PBMCs ($p > 0.05$). The pattern of change in 4-HNE was not different between PRE and POST intervention for both training group and control group ($p > 0.05$) (Fig. 3C and 3D).

The thioredoxin antioxidant system revealed a time × group interaction for TrxR1 levels indicating that this protein was modulated by the training protocol ($p < 0.05$). Indeed, EMRT caused a significant decrease of TrxR1 from PRE to POST period in the training group (PRE *vs.* POST: TrxR1/β-actin ratio, 0.61 ± 0.06 *vs.* 0.28 ± 0.07, $p < 0.05$), while we did not find any change of Trx1 and Trx2 expression ($p > 0.05$) (Fig. 3E, 3F, and 3G). The protein levels of all thioredoxin components were unchanged in the control group ($p > 0.05$). Since the expression of TrxR2 is very low and the processing of the samples did not allow the enrichment with the mitochondrial fractions, we failed to detect its expression by means of western blot analysis.

Discussion

Our study confirms that combination of high-movement acceleration and moderate intensity RT is effective in increasing upper and lower body power, strength and functional motor performance in well-functioning older adults even with short training frequency. The trained group significantly increased upper- and lower-limbs muscle power, with improvements ranging from 28 to 36%. Muscle strength was also significantly increased although the effects of training were lower than those obtained on muscle power (from 15 to 20%). These results are in agreement with previous studies showing similar enhancement in functional and performance tests after explosive-type resistance training (Henwood and Taffe, 2005; Larsen et al., 2011). The most important power result from a functional point of view was given by the significant increase in CMJ power output, a multi-joint

motor task that represents the lower limbs muscle power relative to one's own body weight. Since CMJ is a measure of maximal skeletal muscle power during coupled stretch-shortening muscle action (SSC) (Caserotti et al., 2008), the improvement in maximal CMJ power is highly expected to transfer into relevant functional outcomes. Despite the fact that the testing of strength and power was performed on the same machines used for the training protocol, the significant improvement in lower limb muscle power in the CMJ test confirms that power was improved independently of the choice of testing exercise. In addition, familiarization sessions were completed before testing in both groups to minimize the learning effect on the results.

The increase in muscle strength and power observed in the trained group is highly relevant for health-related fitness benefits even in well-functioning subjects since those help to maintain high level of physical reserve capacity and to keep distance from disability, prolonging independence and quality of life (LaRoche et al., 2011). Indeed, an important finding from this study is the improvement in muscle power and strength related to the time reduction of walking and climbing stairs motor tasks, both with and without carrying loads. Particularly, the improvement in the performance of walking loaded and climbing stairs loaded, which simulates the ADL activity of carrying groceries, points out the transference from the improvement of upper-limbs muscle power to this functional tasks. On the other hand, the control group showed a tendency to decrease muscle strength, power and also the physical performance activities. Despite this data did not get statistical significance, represent the progressive decline in muscle mass, muscle function and motor performance that is known to occur during aging (Mitchell WK et al., 2012). Our results support that a resistance training program using moderate-to-heavy (70% 1RM) with maximal intentional acceleration of the load (explosive type) is able to improve both strength and power resulting in higher prescription relevance due to the optimal combination for hypertrophy and neural adaptation effects.

Physical exercise provides many health benefits, however, the occurrence of adverse events and elevation in various biochemical markers has been demonstrated acutely following different exercise protocols (Thompson, 1996; Mergener et al., 2009). Moreover, considering that ageing is associated with a decrease in the systemic defence response in combination with a chronic low-grade inflammation and oxidative stress (Ungvari et al., 2004), moderate training protocol could potentially produce adverse effects such as oxidative injury to cardiac and skeletal muscle in this age group.

To evaluate if our training modality was safe in the present population, at rest and during maximal exercise stress test, hemodynamic/cardiovascular parameters such as ECG trace, SBP, DBP, HR and METs were

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analyzed before and after the training period. Moreover, several systemic markers related to cardiac/skeletal muscle damage and inflammation (i.e. MPO, NT-pro-BNP, CK, cytokines and TAS) were investigated.

Exercise stress test has been validated as a predictor of risk in older adults and as an instrument for estimating their cardiovascular fitness and diagnosing cardiovascular disease (Weiner et al., 1995; Goraya et al., 2000). Our results showed that brachial blood pressure and HR at rest were unchanged following the training protocol. Additionally, the peak exercise capacity reached during the maximal exercise stress test was increased significantly after training. It occurred without ECG trace of pressure overload, heart wall thickening or significant ST depression (>2 mm) (data not shown). Moreover, subjects did not have any signals of arrhythmic events and any symptoms like palpitations, chest pain, and shortness of breath, headache, or nausea after the test. On the other hand, HRR, a valid predictor of cardiovascular risk (Jouven et al., 2005), indicates a normal-conditioned heart even after reaching higher peak intensity. There are few data evaluating the effect of RT on HRR in sedentary elderly, however, their conclusions support our results (Messinger-Rapport et al., 2003). Therefore, based on these findings, we may conclude that EMRT protocol did not affect negatively the hemodynamic/cardiovascular response to exertion, indeed it could improve the aerobic fitness levels of the participants, as demonstrated from the positive trend close to significance of HRR in the trained group.

Myeloperoxidase is a marker of inflammation and oxidative stress and, recently, it has been proposed as a useful risk marker and a diagnostic tool in acute coronary syndromes (Loria et al., 2008). Although the increase of MPO is not likely to be specific to cardiac diseases, the significant reduction of MPO levels following the training period and absence of changing of NT-pro-BNP, another objective marker of heart failure (Sheikhani et al., 2011), suggests that EMRT is devoid of inducing cardiovascular risk in this specific age group.

Furthermore, we did not find any modulation of serum CK as indicator of the integrity of skeletal muscle. The above expands the knowledge that RT (Ferri et al., 2006) does not induce detrimental effects at muscle level in our study population. A further confirmation of the safety of our motor intervention is obtained analyzing the results of the TAS and the circulating cytokines. In agreement with Stewart et al. (2007), we failed to reveal any effect of the exercise intervention on resting concentration of circulating TAS and cytokines. Despite the differences between training protocols and inconclusive results about the effect of RT, we believe that the total volume of the EMRT was insufficient to modify these parameters as a result of the low frequency of training.

It should be noted that, to date, there have not been reports documenting the effects of EMRT on cellular antioxidant capacity especially in those cells such as PBMCs, that are critical component in the immune system. Moreover, especially in untrained people, exercise can trigger the signals of oxidative stress in blood. This may

be related to increasing lipid peroxidation and to the modulation of antioxidant and/or stress-induced proteins (i.e. Hsp70, Hsp27, 4-HNE, TrxR1, Trx1 and Trx2).

It is known that regular exercise induces long-term cellular adaptations modulating the expression of specific stress-induced proteins at rest (Fehrenbach et al., 2000). Actually, when we analyzed the training-related-effects on HSPs, we found that their basal expression in the trained group at rest displayed a unique pattern, different from that of non-trained individuals. Similarly to Fehrenbach et al. (2000), Hsp70 and Hsp27 in PBMCs were significantly diminished in trained compared with untrained persons. This suggests that the organism may become accustomed to certain repeated exercise-stressor during the training period that may translate into physiological adaptations affecting the expression of HSP at basal state.

To evaluate if endogenous and/or exogenous radicals generated by exercise training induced both a persistent accumulation of lipid peroxidation and/or a modulation of antioxidants, 4-HNE and the expression of Thioredoxin system proteins were analyzed. The paucity of the human studies that investigate the modulation of 4-HNE following a period of training makes difficult any comparison and even more if it refers to an elderly population. Similarly to others human studies, where the subjects recruited and the type of training were different (Fauzi et al., 2007; Venojarvi et al., 2008), 4-HNE levels were similar to baseline values without differences between groups. However, we found that the protein expression of TrxR1, one of the most abundant enzymes of the thioredoxin system, was decreased. The thioredoxin system plays an essential role in cell function and protection by limiting oxidative stress directly via its antioxidant effects, and also indirectly by protein-protein interactions with key signaling molecules. As already suggested by Jones et al. (2006), we hypothesize that the decreased expression of TrxR1 may be important for the maintenance of redox control and to trigger physiological adaptations following a training period.

Currently, there is not unquestionable explanation to the paradox of physical activity, which is doubtless beneficial for individuals at all ages but simultaneously could be potentially harmful if excessive free radicals are produced. Although further studies are required to better clarify the potential role of antioxidant and stress-proteins modulation during exercise training in older adults, the strength of our research is the combined analysis of molecular, muscular and performance parameters in older adults. The findings of the present investigation demonstrate that in the absence of clinical disease, 12 week of EMRT could be well tolerated by old adults (70-75 years). It was highly effective in eliciting a significant enhancement in muscular and functional performance, which was absent of detrimental changes of cardiovascular, inflammatory, pro- and anti-oxidants parameters. Moreover, trained elderly subjects showed an improvement in cardiovascular health as well as an adaptive

response both to systemic and cellular level of antioxidant and stress-induced markers compared with untrained subjects.

Given the difficulties to motivate individuals to take part in a vigorous training program, we believe that healthy elderly subjects participating in supervised low-frequency EMRT can effectively improve muscle strength and power, benefiting both systemic and cellular levels.

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Figure Legends

Figure 1. Percent change in power (A), strength (B) and functional tests (C) after 12 weeks EMRT in TRAINED group (grey bars) and CONTROL group (black bars). Data is expressed as mean ± SEM. *$p < 0.05$, significantly different from control group value.

Figure 2. MPO (A) NT-pro BNP (B) CK (C) and TAS (D) levels evaluated in TRAINED and CONTROL groups at rest, before (PRE) and after (POST) training period. Data is expressed as mean ± SEM. *$p < 0.05$, significantly different within group and between groups values.

Figure 3. Modulation of Hsp70 (A), Hsp27 (B), 4-HNE (C and D), TrxR1 (E), Trx1 (F) and Trx2 (G) in PBMCs from both healthy TRAINED and CONTROL groups at rest, before (PRE) and after (POST) a training period. Protein expression was measured as the ratio between the optical density (OD) of marker protein and the OD of β-actin. In each panel, images show immunoblot results from the same representative subject, while the histograms represent the mean ± SEM. *$p < 0.05$, significantly different within group and between groups values.