



Título artículo / Títol article: Explosive type of moderate-resistance training induces functional, cardiovascular, and molecular adaptations in the elderly

Autores / Autors María Reyes Beltrán Valls, Ivan Dimauro, Andrea Brunelli, Eliana Tranchita, Emanuela Ciminelli, Paolo Caserotti, Guglielmo Duranti, Stefania Sabatini, Paolo Parisi, Attilio Parisi, Daniela Caporossi

Revista: AGE, v. 36, n. 2 (April 2014)

Versión / Versió: Preprint de l'autor

Cita bibliográfica / Cita bibliogràfica (ISO 690): BELTRÁN VALLS, M. R..[et al.]. Explosive type of moderate-resistance training induces functional, cardiovascular, and molecular adaptations in the elderly. AGE, v. 36, n. 2 (April 2014), pp. 759-772

url Repositori UJI: <http://repositori.uji.es/xmlui/handle/10234/123724>

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

3
4 Maria Reyes Beltran Valls^{1†}, Ivan Dimauro^{1†}, Andrea Brunelli¹, Eliana Tranchita², Emanuela Ciminelli², Paolo
5 Caserotti³, Guglielmo Duranti¹, Stefania Sabatini¹, Paolo Parisi¹, Attilio Parisi², Daniela Caporossi¹.

6
7 ¹Unit of Biology, Genetics and Biochemistry and ²Unit of internal Medicine, Department of Movement, Human
8 and Health Sciences, University of Rome "Foro Italico", Piazza Lauro De Bosis 15, 00135 Rome, Italy

9 ³Centre of Applied and Clinical Exercise Sciences, University of Southern Denmark, Campusvej 55, 5230
10 Odense M, Denmark

11
12 [†]These authors contributed equally to this research.

13 **Corresponding author:** Prof. Daniela Caporossi, Tel: +390636733212; Fax: +390632650767. Email:
14 daniela.caporossi@uniroma4.it

15 **Keywords:** Strength training, elderly, hemodynamic parameters, HSPs, thioredoxin system.

16
17 **Abstract**

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

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

1 **Introduction**

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

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

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

1

2 **Material and Methods**

3 *Study design*

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

18 Before (PRE) and after (POST) exercise intervention, strength, power and physical performances were assessed
19 by the same researcher on alternate days in both groups. Before the testing sessions, subjects included in the
20 study came to the laboratory to collect a blood sample at rest. The same day participants performed a
21 familiarization session with the climbing stairs and walking physical performance tests. In the first testing
22 session, physical performance was evaluated and following the subjects performed a familiarization session with
23 the resistance training exercises in order to get feedback regarding the correct technique and cadence of lifting.
24 Then, in an alternate day, they performed another familiarization session to become accustomed to the devices
25 and to learn the procedure of maximal strength and power tests. We verified that the performance of subjects that
26 would ultimately form the experimental and control groups was equated at the end of the familiarization session.
27 The second testing session included measurement of maximum dynamic strength by one maximum repetition
28 (1RM) and the third session included the assessment of maximum muscle power. Verbal encouragement was
29 given to maximize motivation and performance. At the end of the 3th month training period, each group
30 performed a familiarization session before the final tests. Then, participants had the blood sampling at least 3

1 days after the last testing session. Moreover, an evaluation of hemodynamic/cardiovascular response to maximal
2 exercise stress test and of the level of specific markers of cardiac and skeletal muscle damage such as, serum
3 concentration of myeloperoxidase (MPO), N-terminal pro-brain natriuretic peptide (NT-pro-BNP) and creatine
4 kinase (CK), as well as the total anti-oxidant status (TAS), pro- and anti-inflammatory cytokines (IL-4, IL-6, IL-
5 13, IL-15, TNF α and MIP-1b) was performed. Further, we analyzed in peripheral blood mononuclear cells
6 (PBMCs) the level of lipid peroxidation (4-hydroxynonenal [4-HNE]), the expression of antioxidant and stress-
7 induced proteins (thioredoxin reductase 1 [TrxR1], thioredoxin 1 [Trx1] and 2 [Trx2], heat shock protein 70
8 [Hsp70] and 27 [Hsp27]).

9

10 *Maximal exercise stress test*

11 Maximal exercise stress test was performed with a cycloergometer according to the following protocol: at first
12 the load was fixed at 30 Watt for 3 minutes, then the exercise load was increased by 10 Watt every minute until
13 reaching 85% of maximal heart rate (HR_{max}) (calculated through this formula: HR_{max} = 220 - age in years). If the
14 subjects were able to continue the test, they were encouraged to prolong the effort until exhaustion. A continuous
15 12-lead ECG recording was performed and blood pressure was assessed through a mercury sphygmomanometer
16 during the test and the following recovery.

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

21 It is defined as the ratio of metabolic rate (and therefore the rate of energy consumption) during a specific
22 physical activity to a reference metabolic rate, set by convention to 3.5 ml O₂·kg⁻¹·min⁻¹ (Jettè et al., 1990).

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

26

27 *Strength test*

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

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

5

6 *Power test*

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

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

24

25 *Physical performance tests*

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

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

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

9
10 *Explosive-type resistance training protocol*

11 The participant in the training group performed two days of explosive-type resistance training for 12 weeks
12 using the four RT machines used for the testing sessions (Technogym, Italy). Training sessions began with 10
13 minutes of specific neuromuscular warm-up. The first two weeks of training was performed at an intensity of 40-
14 50% 1RM (15 repetitions-4 sets) at moderate velocity to facilitate muscle adaptation. Thereafter, the specific
15 explosive training was initiated and each exercise was performed for 3-4 sets of 10-12 repetitions at 70% of
16 baseline 1RM with 2 minutes rest between sets and 3 minutes rest between exercises. Participants were
17 encouraged to perform the concentric part of each repetition at maximal intentional load acceleration while
18 performing the eccentric phase of each repetition with moderate speed. Approximately every two weeks,
19 resistance was incremented when a subject completed 12 repetitions for at least two of the total sets at a given
20 weight load while maintaining proper exercise technique (Baechle and Earle, 2008). The goal of this progression
21 was to induce volitional fatigue in the 10 to 12-repetition throughout the training program. The increase in
22 resistance was usually of 2.5 kg and before the increment the perceived fatigue was evaluated to consider also
23 the individual perception of the load. The EMRT was designed and supervised by expert strength and
24 conditioning coaches. Participants were continuously visually examined to verify correct technique and tempo of
25 the movement and were also verbally encouraged by the coaches.

26
27 *Blood sampling*

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

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

4

5 *Isolation of PBMCs and Western Blot analysis*

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

17

18 *Measurement of Myeloperoxidase and NT-proBNP*

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

21

22 *Cytokine profile*

23 The multiplex analysis methodology (Vignali, 2000) was utilized for cytokine profiling. In this study we
24 analyzed cytokines concentration in plasma by Bio-Plex system (Bio-Rad Laboratories, Milan, Italy) in
25 accordance with the manufacturer's instructions. The following cytokines were included: IL-4, IL-6, IL-13, IL-
26 15, TNF α and Macrophage inflammatory protein-1-beta (MIP-1b). Briefly, after thawing, plasma samples were
27 centrifuged at 10,000rpm for 10 min at 4°C to clear the samples of precipitate. The samples were then incubated
28 with 25 μ l of anti-cytokine conjugated beads in 96-well filter plates for 30 min at room temperature with shaking
29 and washed three times by vacuum filtration with 100 μ l of Bio-Plex wash buffer. Next, 25 μ l of diluted
30 detection antibody was added, and plates were incubated for 30 min at room temperature with shaking. After

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

6

7 *Total antioxidant status (TAS)*

8 Plasma TAS was determined spectrophotometrically, accordingly to Miller et al. (1993). This method is based
9 on the reactivity of plasmatic antioxidant compounds relative to a 1 mM Trolox® (vitamin E analogue) standard.
10 Briefly, 10 μ l of plasma or Trolox® standards (0.25 - 0.5 - 1.0 mM) were incubated in ABTS-metMyo-PBS
11 buffer and the absorbance at 734nm was monitored for 2 minutes. The reaction was started by the addition of
12 H₂O₂ (450 μ M) and variation of absorbance was then recorded. Sample Δ OD/min₇₃₄ were compared to those
13 obtained by using Trolox® standards.

14

15 *Creatine Kinase (CK)*

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

20

21 *Statistical analyses*

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

29

30 **RESULTS**

1 *Baseline and exercise stress-induced cardiovascular parameters*

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

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

20

21 *Muscle power, muscle strength and functional motor capabilities*

22 Twelve weeks of EMRT induced significant time \times group interaction in peak power in all upper and lower body
23 exercises and in the countermovement jump (CMJ) ($p = 0.001$ and $p = 0.004$ respectively). Following training,
24 peak power was higher in the exercise group compared to the control group for all five exercises ($p < 0.05$) (Fig.
25 1A and Table 3). In addition, peak power increased after training compared to baseline within experimental
26 group. The peak muscle power increased by $36.0\% \pm 5.2$ in leg extension ($p = 0.001$), $28.0\% \pm 5.4$ in leg curl (p
27 $= 0.001$), $34.0\% \pm 3.4$ in chest press ($p = 0.001$), $28.0\% \pm 4.5$ in low row ($p = 0.001$), $18.0\% \pm 4.3$ in the CMJ (p
28 $= 0.004$). There were not changes within the control group in the power output of the five exercises (range from -
29 2% to -5%) ($p > 0.05$).

1 A significant time \times group interaction was found for peak strength of the four training exercises ($p = 0.001$).
2 Maximal strength improved in the exercise group compared to the control one in all four exercises ($p < 0.05$)
3 (Fig. 1B and Table 3). Maximal strength post-training was higher compared to pre-test values in the training
4 group. The mean increase in strength was $16.0\% \pm 1.1$ ($p = 0.001$) in leg extension, $15.0\% \pm 1.5$ ($p = 0.001$) in
5 leg curl, $20\% \pm 1.9$ ($p = 0.001$) in chest press, $15.0\% \pm 1.2$ ($p = 0.001$) in low row. Maximal strength did not
6 change in the control group from PRE to POST protocol, with values almost unchanged (range from -1.7% to
7 0.6%) ($p > 0.05$).

8 The evaluation of functional capacity through daily living motor tasks resulted in significant time \times group
9 interaction in 6m walking test ($p = 0.001$), 6m walking loaded ($p = 0.002$), stairs climbing ($p = 0.01$) and stairs
10 climbing loaded ($p = 0.008$). The training group decreased the time to perform these four exercises compared to
11 the control group ($p < 0.05$) (Fig. 1C and Table 3). Time in the PRE tests was reduced compared to the POST
12 tests within training group in the 6m walking test by $9.0\% \pm 1.6$ ($p = 0.001$), 6m walking with loads by $10.0\% \pm$
13 2.1 ($p = 0.001$), stairs climbing by $8.0\% \pm 3.1$ ($p = 0.04$) and stairs climbing with loads by $12.0\% \pm 2.5$ ($p =$
14 0.006). The control group did not change the performance time of each test, with a slightly increase in the
15 percentage of change ($0-4\%$) ($p > 0.05$).

16

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

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

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

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

PBMCs expression of Hsp70, Hsp27 and Trx

The analyses of HSPs in PBMCs revealed a significant time \times 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 $32.0\% \pm 6.0$ and $51.4\% \pm 12.5$, 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 \times 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 \times 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

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

8 The increase in muscle strength and power observed in the trained group is highly relevant for health-related
9 fitness benefits even in well-functioning subjects since those help to maintain high level of physical reserve
10 capacity and to keep distance from disability, prolonging independence and quality of life (LaRoche et al.,
11 2011). Indeed, an important finding from this study is the improvement in muscle power and strength related to
12 the time reduction of walking and climbing stairs motor tasks, both with and without carrying loads. Particularly,
13 the improvement in the performance of walking loaded and climbing stairs loaded, which simulates the ADL
14 activity of carrying groceries, points out the transference from the improvement of upper-limbs muscle power to
15 this functional tasks. On the other hand, the control group showed a tendency to decrease muscle strength, power
16 and also the physical performance activities. Despite this data did not get statistical significance, represent the
17 progressive decline in muscle mass, muscle function and motor performance that is known to occur during aging
18 (Mitchell WK et al., 2012). Our results support that a resistance training program using moderate-to-heavy (70%
19 1RM) with maximal intentional acceleration of the load (explosive type) is able to improve both strength and
20 power resulting in higher prescription relevance due to the optimal combination for hypertrophy and neural
21 adaptation effects.

22 Physical exercise provides many health benefits, however, the occurrence of adverse events and elevation in
23 various biochemical markers has been demonstrated acutely following different exercise protocols (Thompson,
24 1996; Mergener et al., 2009). Moreover, considering that ageing is associated with a decrease in the systemic
25 defence response in combination with a chronic low-grade inflammation and oxidative stress (Ungvari et al.,
26 2004), moderate training protocol could potentially produce adverse effects such as oxidative injury to cardiac
27 and skeletal muscle in this age group.

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

Ivan 30/8/13 10:44

Eliminado: of

Ivan 30/8/13 10:45

Eliminado: testing

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

3 Exercise stress test has been validated as a predictor of risk in older adults and as an instrument for estimating
4 their cardiovascular fitness and diagnosing cardiovascular disease (Weiner et al., 1995; Goraya et al., 2000). Our
5 results showed that brachial blood pressure and HR at rest were unchanged following the training protocol.

6 Additionally, the peak exercise capacity reached during the maximal exercise stress test was increased
7 significantly after training. It occurred without ECG trace of pressure overload, heart wall thickening or
8 significant ST depression (>2 mm) (data not shown). Moreover, subjects did not have any signals of arrhythmic
9 events and any symptoms like palpitations, chest pain, and shortness of breath, headache, or nausea after the test.

10 On the other hand, HRR, a valid predictor of cardiovascular risk (Jouven et al., 2005), indicates a normal-
11 conditioned heart even after reaching higher peak intensity. There are few data evaluating the effect of RT on
12 HRR in sedentary elderly, however, their conclusions support our results (Messinger-Rapport et al., 2003).

13 Therefore, based on these findings, we may conclude that EMRT protocol did not affect negatively the
14 hemodynamic/cardiovascular response to exertion, indeed it could improve the aerobic fitness levels of the
15 participants, as demonstrated from the positive trend close to significance of HRR in the trained group.

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

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

28 It should be noted that, to date, there have not been reports documenting the effects of EMRT on cellular
29 antioxidant capacity especially in those cells such as PBMCs, that are critical component in the immune system.

30 Moreover, especially in untrained people, exercise can trigger the signals of oxidative stress in blood. This may

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

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

10 To evaluate if endogenous and/or exogenous radicals generated by exercise training induced both a persistent
11 accumulation of lipid peroxidation and/or a modulation of antioxidants, 4-HNE and the expression of
12 Thioredoxin system proteins were analyzed. The paucity of the human studies that investigate the modulation of
13 4-HNE following a period of training makes difficult any comparison and even more if it refers to an elderly
14 population. Similarly to others human studies, where the subjects recruited and the type of training were
15 different (Fauzi et al., 2007; Venojarvi et al., 2008), 4-HNE levels were similar to baseline values without
16 differences between groups. However, we found that the protein expression of TrxR1, one of the most abundant
17 enzymes of the thioredoxin system, was decreased. The thioredoxin system plays an essential role in cell
18 function and protection by limiting oxidative stress directly via its antioxidant effects, and also indirectly by
19 protein-protein interactions with key signaling molecules. As already suggested by Jones et al. (2006), we
20 hypothesize that the decreased expression of TrxR1 may be important for the maintenance of redox control and
21 to trigger physiological adaptations following a training period.

22 Currently, there is not unquestionable explanation to the paradox of physical activity, which is doubtless
23 beneficial for individuals at all ages but simultaneously could be potentially harmful if excessive free radicals are
24 produced. Although further studies are required to better clarify the potential role of antioxidant and stress-
25 proteins modulation during exercise training in older adults, the strength of our research is the combined analysis
26 of molecular, muscular and performance parameters in older adults. The findings of the present investigation
27 demonstrate that in the absence of clinical disease, 12 week of EMRT could be well tolerated by old adults (70-
28 75 years). It was highly effective in eliciting a significant enhancement in muscular and functional performance,
29 which was absent of detrimental changes of cardiovascular, inflammatory, pro- and anti-oxidants parameters.
30 Moreover, trained elderly subjects showed an improvement in cardiovascular health as well as an adaptive

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

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

6

7 **Acknowledgments**

8 This work was supported by grants from the University of Rome 'Foro Italico' (Research 2009) to D. C. The
9 Lazio Regional Municipality (Agreement CRUL-Lazio n. 12650/2010) supported the post-doc scholarship to ID.

10 Each author of this study further declares no relationships with the companies or manufacturers who will benefit
11 from the results of the present study.

12 The authors of this article declare no conflicts of interest.

13

14 **References**

15 Aagaard P, Suetta C, Caserotti P, Magnusson SP, Kjaer M (2010). Role of the nervous system in sarcopenia and
16 muscle atrophy with aging: strength training as a countermeasure. *Scand J Med Sci Sports* 20:49-64.

17 Baechle TR, Earle RW. *Essentials of Strength Training and Conditioning*, 3th edition (2008). Human Kinetics,
18 Champaign IL.

19 Bassey EJ, Fiatarone MA, O'Neill EF, Kelly M, Evans WJ, Lipsitz LA (1992). Leg extensor power and functional
20 performance in very old men and women. *Clin Sci (Lond)* 82:321-7.

21 Bean JF, Leveille SG, Kiely DK, Bandinelli S, Guralnik JM, Ferrucci L (2003) A comparison of leg power and leg
22 strength with the in CHIANTI study: which influences mobility more? *J Gerontol: Med Sci* 58A:728-733.

23 Buford TW, Cooke MB, Willoughby DS (2009) Resistance exercise-induced changes of inflammatory gene
24 expression within human skeletal muscle. *Eur J Appl Physio* 107:463-71.

25 Caserotti P, Aagaard P, Larsen JB, Puggard L (2008) Explosive-heavy resistance training in old and very old adults:
26 changes in rapid muscle force, strength and power. *Scand J Med Sci Sports* 18:773-782.

27 Chung HY, Cesari M, Anton S et al. (2009) Molecular inflammation: underpinnings of aging and age-related
28 diseases. *Ageing Research Reviews* 8:18-32.

29 Cole CR, Foody JM, Blackstone EH, Lauer MS (2000) Heart rate recovery after submaximal exercise testing as a
30 predictor of mortality in a cardiovascularly healthy cohort. *Ann Intern Med* 132:552-5.

1 de Vos NJ, Singh NA, Ross DA, Stavrinou TM, Orr R, Fiatarone Singh MA (2005) Optimal load for increasing
2 muscle power during explosive resistance training in older adults. *J Gerontol A Biol Sci Med Sci* 60:638-47.

3 English D, Andersen BR (1974) Single-step separation of red blood cells. Granulocytes and mononuclear leukocytes
4 on discontinuous density gradients of Ficoll-Hypaque. *J Immunol Methods* 5:249-52.

5 Fauzi F, Budin SB, Azwan SS & Yuen LK (2007) The Effect of 5-Week Exercise Program on Oxidative Stress and
6 Response to Acute Exercise among Sedentary Subjects. *Jurnal Sains Kesihatan Malaysia* S2: 39-52.

7 Fehrenbach E, Passet F, Niess AM, Pohla H, Weinstock C, Dickhut H (2000) HSP expression in human leukocytes
8 is modulated by endurance exercise. *Med Sci Sports Exerc* 32:592-600.

9 Ferri A, Narici M, Grassi B, Pousson M (2006) Neuromuscular recovery after a strength training session in elderly
10 people. *Eur J Appl Physiol* 97:272-279.

11 Fielding RA, Vellas B, Evans WJ et al. (2011) Sarcopenia: an undiagnosed condition in older adults: prevalence,
12 etiology and consequences. International working group on sarcopenia. *J Am Med Dir Assoc* 12:249-256.

13 Goraya TY, Jacobsen SJ, Pellikka PA, et al. (2000) Prognostic value of treadmill exercise testing in elderly persons.
14 *Ann Intern Med* 132:862-870.

15 Hairi NN, Cumming RG, Naganathan V et al. (2010) Loss of muscle strength, mass (sarcopenia) and quality
16 (specific force) and its relationship with functional limitations and physical disability: the concord health and
17 ageing in men project. *J Am Geriatr Soc* 58:2055-2062.

18 Hakkinen K, Kraemer WJ, Newton RU, Alen M (2001) Changes in electromyographic activity, muscle fibre and
19 force production characteristics during heavy resistance/power strength training in middle-aged and older men
20 and women. *Acta Physiol Scand* 171:51-62.

21 Henwood TR, Taaffe DR (2005) Improved physical performance in older adults undertaking a short-term
22 programme of high-velocity resistance training. *Gerontology* 51:108-15.

23 Izquierdo M, Aguado X, Gonzalez R, López JL, Häkkinen K (1999) Maximal and explosive force production
24 capacity and balance performance in men of different ages. *Eur J Appl Physiol Occup Physiol* 79:260-7.

25 Jetté M, Sidney K, Blümchen G (1990) Metabolic equivalents (METs) in exercise testing, exercise prescription, and
26 evaluation of functional capacity. *Clin Cardiol* 13:555-65.

27 Jones DT, Pugh CW, Wigfield S, Stevens M, Harris AL (2006) Novel thioredoxin inhibitors paradoxically increase
28 hypoxia-inducible factor-1 expression but decrease functional transcriptional activity, DNA binding, and
29 degradation. *Clin Cancer Res* 12:5384-5394.

1 Joven X, Empana JP, Schwartz PJ (2005) Heart-rate profile during exercise as a predictor of sudden death. *N Engl*
2 *J Med* 352:1951-8.

3 LaRoche DP, Millett ED, Kralian RJ (2011). Low strength is related to diminished ground reaction forces and
4 walking performance in older adults. *Gait Posture* 33:668:672.

5 Larsen HA, Caserotti P, Puggaard L, Aagaard P (2011) Stair-ascent performance in elderly women: effect of
6 explosive strength training. *JAPA* 19:117-136.

7 Levinger I, Goodman C, Peake J et al. (2009) Inflammation, hepatic enzymes and resistance training in individuals
8 with metabolic risk factors. *Diabet Med* 26:220-7.

9 Loria V, Dato I, Graziani F, Biasucci LM (2008) Myeloperoxidase: a new biomarker of inflammation in ischemic
10 heart disease and acute coronary syndromes. *Mediators Inflamm* 135625.

11 Mergener M, Martins MR, Antunes MV et al. (2009) Oxidative stress and DNA damage in older adults that do
12 exercises regularly. *Clin Biochem* 42:1648-53.

13 Messinger-Rapport B, Pothier Snader CE, Blackstone EH (2003) Value of exercise capacity and heart rate recovery
14 in older people. *J Am Geriatr Soc* 51:63-8.

15 Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A (1993) A novel method for measuring antioxidant
16 capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci (Lond)* 84:407-
17 412.

18 Mitchell WK, Williams J, Atherton P, Larvin M, Lund J, Narici M (2012). Sarcopenia, dynapenia, and the impact of
19 advancing age on human skeletal muscle size and strength; a quantitative review. *Front Physiol* 3:1-18.

20 Narici MV, Maffulli N (2010). Sarcopenia: characteristics, mechanisms and functional significance. *Br Med Bull*
21 95:139-59.

22 Phillips MD, Flynn MG, McFarlin BK, Stewart LK, Timmerman KL (2010) Resistance training at eight-repetition
23 maximum reduces the inflammatory milieu in elderly women. *Med Sci Sports Exerc* 42:314-25.

24 Reid KF, Fielding RA (2011) Skeletal Muscle Power: A Critical Determinant of Physical Functioning in Older
25 Adults. *Exerc. Sport Sci. Rev* 40:4-12.

26 Rietjens SK, Beelen M, Koopman R et al. (2007) A single session of resistance training induces oxidative damage
27 in untrained men. *Med Sci Sports Exerc* 39: 2145-2151.

28 Sheikhan H, Babae Beygi MA, Daryanoosh F, Jafari B (2011) Alteration of plasma brain natriuretic peptide level
29 after acute moderate exercise in professional athlete. *Int Cardiovasc Res J* 5:148-150.

1 Skelton DA, Greig CA, Davies JM, Young A (1994). Strength, power and related functional ability of healthy
2 people aged 65-89 years. *Age Ageing* 23:371-7.

3 Squadrone R, Rodano R, Preatoni E (2012) Comparison of velocity and power output data derived from an inertial
4 based system and an optical encoder during squat lifts in a weight room setting. *J Sports Med Phys Fitness*
5 52:40-6.

6 Stephens SW, Khanolkar MP, Bain SC (2009) The biological relevance and measurement of plasma markers of
7 oxidative stress in diabetes and cardiovascular disease. *Atherosclerosis* 202:321-329.

8 Stewart LK, Flynn MG, Campbell WW et al. (2007) The influence of exercise training on inflammatory cytokines
9 and C-reactive protein. *Med Sci Sports Exerc* 39:1714-9.

10 Suzuki T, Bean JF, Fielding RA (2001) Muscle power of the ankle flexors predicts functional performance in
11 community dwelling older women. *J Am Geriatr Soc* 49:1161-1167.

12 Thompson PD (1996) The cardiovascular complications of vigorous physical activity. *Arch Intern Med* 156:2297-
13 2302.

14 Towbin H, Staehelin T, and Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to
15 nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A* 76:4350-4354.

16 Ungvari Z, Csiszar A, Kaley G (2004) Vascular inflammation in aging. *Herz* 29:733-740.

17 Venojärvi M, Aunola S, Puhke R, Marniemi J, Hämäläinen H, Halonen JP, Lindström J, Rastas M, Hällsten K,
18 Nuutila P, Hänninen O, Atalay M (2008) Exercise training with dietary counselling increases mitochondrial
19 chaperone expression in middle-aged subjects with impaired glucose tolerance. *BMC Endocr Disord* 27;8:3.

20 Vignali DA (2000) Multiplexed particle-based flow cytometric assays. *J Immunol Methods* 21;243:243-55.

21 Voorrips LE, Ravelli AC, Dongelmans PC, Deurenberg P, Van Staveren WA (1991) A physical activity
22 questionnaire for the elderly. *Med Sci Sports Exerc* 23:974-9.

23 Weiner DA, Ryan TJ, Parsons L, et al. (1995) Long-term prognostic value of exercise testing in men and women
24 from the Coronary Artery Surgery Study (CASS) registry. *Am J Cardiol* 75:865-870.

25
26
27
28
29
30

1 **Figure Legends**

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

5
6 **Figure 2.** MPO (A) NT-pro BNP (B) CK (C) and TAS (D) levels evaluated in TRAINED and CONTROL
7 groups at rest, before (PRE) and after (POST) training period. Data is expressed as mean \pm SEM. * $p < 0.05$,
8 significantly different within group and between groups values.

9
10 **Figure 3.** Modulation of Hsp70 (A), Hsp27 (B), 4-HNE (C and D), TrxR1 (E), Trx1 (F) and Trx2 (G) in PBMCs
11 from both healthy TRAINED and CONTROL groups at rest, before (PRE) and after (POST) a training period.
12 Protein expression was measured as the ratio between the optical density (OD) of marker protein and the OD of
13 β -actin. In each panel, images show immunoblot results from the same representative subject, while the
14 histograms represent the mean \pm SEM. * $p < 0.05$, significantly different within group and between groups
15 values.

16
17
18
19
20