

## Effects of high-load resistance training versus pyramid training system on maximal muscle strength in well-trained young men: a randomized controlled study

FRANCESCO FISCHETTI<sup>1</sup>, FRANCESCO CAMPOREALE<sup>2</sup>, GIANPIERO GRECO<sup>3</sup>

<sup>1,2,3</sup>Department of Basic Medical Sciences, Neuroscience and Sense Organs, School of Medicine, University of Bari, ITALY

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### Abstract:

Resistance training (RT) is the primary exercise intervention for increasing muscle strength in humans. However, to our knowledge, it is not clear whether it is better to train muscle strength with high-loads and low volumes or with high-intensity without drastic volume reductions. Therefore, this study aimed to investigate the effects of a high-load RT compared to Pyramid training system on muscular strength in experienced young men. Twenty participants (age:  $23.9 \pm 2.0$  years; body mass:  $75.6 \pm 9.4$  kg; height:  $1.77 \pm 0.05$  m; RT experience:  $4.1 \pm 3.4$  years) were pair-matched based on initial strength capacity and then randomly assigned to an experimental group ( $n = 10$ ) performing high-load RT (80-95% 1-RM, 3-min rest) twice a week interspersed with a Pyramid training weekly session, or a Pyramid training group ( $n = 10$ ) performing tri-weekly training sessions with Pyramid method alone (90-sec rest). Both groups trained for 8 weeks using a 3:1 loading structure. Measures pre-intervention and post-intervention included one-repetition maximum [1-RM] barbell bench press, barbell deadlift, lat pull-down, and standing barbell military press. Repeated-measures ANOVA and a paired t-test were used for statistical analyses ( $p < 0.05$ ). Significant 'Time x Group' interaction was found for all the outcome variables ( $p < 0.0001$ ) and the experimental group showed significantly greater improvements than Pyramid group ( $p < 0.0001$ ) in bench press (+13.1 vs. +3.7 kg), deadlifts (+19.3 vs. +5.3 kg), pull-down (+17.2 vs. +2.8 kg) and military press (+13.1 vs. +1.9 kg). These findings suggest that high-load RT is an effective method to promote positive short-term adaptations of muscular strength in well-trained young men. To use a combination of different RT systems over time may help to maintain interest in and motivation to perform RT by allowing a varied RT program.

**Key Words:** intensity; volume; 1-RM; periodization; mixed-method; advanced method.

### Introduction

Resistance training (RT) is an exercise modality that involves manipulation of program variables (e.g., number of sets, repetitions, and exercises; load; movement speed; and resting period) to promote a systematic increase in the overload placed upon the body to improve muscular fitness (Fleck & Kraemer, 2014; Ratamess et al., 2009). Thus, its prescription involves a number of variables of which training volume and intensity are key components (Fleck & Kraemer, 2014). Training volume is a summation of the total number of repetitions performed multiplied by the resistance used and is affected by the number of sets, repetitions, and exercises performed as well as training frequency. Intensity is expressed either as a percentage of the individual's one-repetition maximum (%1-RM) or as the repetition maximum (RM), which is the maximum weight the person can lift for a given number of repetitions of an exercise (e.g., 8-RM equals the maximum weight the person can lift for 8 repetitions) and is affected by the rest interval between sets and exercises and velocity of movement.

Some studies suggest that training volume is an important contributing factor for increasing muscular strength as some degree of volume is thought to be required for muscle growth (Marshall, McEwen, & Robbins, 2011; Radaelli et al., 2015; Rhea, Alvar, Ball, & Burkett, 2002; Rønnestad et al., 2007). The initial increase in strength is thought to be primarily from neural adaptations followed by larger contributions from muscle hypertrophy after the first few weeks of training (Moritani, 1979). In effect, many RT programs (i.e., classical periodization, daily undulating periodization) consist of a hypertrophy phase or day in an effort to provide a foundation from which to optimize strength after targeted training (Ratamess et al., 2009; Simão et al., 2012; Stone, O'Bryant, & Garhammer, 1981). However, given the aforementioned suggestion that muscle growth is a large contributor to strength gain after RT, it is perhaps surprising that little direct human evidence exists for this thesis (i.e., the change in size causing the change in strength).

It is hypothesized instead that the changes in strength appear to be driven more so by the principle of specificity rather than the change in muscle size (Buckner et al., 2017; Mattocks et al., 2016). In support of this

contention, low-load (30% one repetition maximum [1RM]) and high-load (80% 1-RM) RT have shown similar changes in muscle growth between conditions but divergent changes in isotonic strength (Mitchell et al., 2012; Schoenfeld, Peterson, Ogborn, Contreras, & Sonmez, 2015). The authors noted that the high-load condition had a greater increase in 1RM strength than the low-load condition and this difference was attributed to the principle of specificity. The specificity principle states that the body's physiological and metabolic responses and adaptations to exercise training are specific to the type of exercise and the muscle groups involved. For example, the gains in muscular strength are specific to the exercised muscle groups, type and speed of contraction, and training intensity (Gibson, Wagner, & Heyward, 2018). Therefore, to optimize the strength gains the use of high-intensity–low-repetition resistance exercise has been suggested. Already DeLorme's classic work (1945) suggested that a resistance-training program using low repetition and high resistance favoured adaptations for strength. The mean optimal intensity for developing strength ranges between 70% to 80% 1-RM for intermediate lifters, and 80% to 100% 1-RM for advanced lifters (Kraemer & Ratamess, 2004; Ratamess et al., 2009).

There are a variety of methods to design RT programs. Each uses a different approach for prescribing sets, order of exercises, or frequency of workouts. For example, among the most commonly used methods is the pyramid training. It, because of its inherent characteristic of varying the resistance used and number of repetitions, permits exercise performance at higher intensities without necessarily causing a loss in volume, thus maintaining a favourable anabolic environment for increased muscle hypertrophy and thus strength gains. However, it might not be any more effective than performing multiple sets with the same weight. When training volume is equal, there is no difference between multiple sets and pyramiding for muscular strength, muscle damage, or hormonal responses (Charro, Aoki, Coutts, Araujo, & Bacurau, 2010; Ribeiro et al. 2017). Although the Pyramid system is widely used by practitioners, there is little scientific basis to support its actual effectiveness.

To our knowledge, the previous studies mentioned above highlight that the optimal training stimulus for developing muscular strength is controversial (Kraemer & Ratamess, 2004; Ratamess et al., 2009). Accordingly, it is not clear whether it is better to train muscle strength with high-loads and low volumes or with high-intensity without drastic volume reductions. Furthermore, one fundamental issue is that most studies have reported results for untrained subjects. It is well known that trained individuals respond differently than those who lack training experience (Peterson, Rhea, & Alvar, 2005). In addition, a “ceiling effect” makes it progressively difficult for trained individuals to increase muscular gain over time, thereby necessitating advanced RT protocols to elicit continual strength responses (Ratamess et al., 2009). Therefore, given the existing disputes and issues in the current literature, the purpose of this study was to investigate the effects of a high-load RT compared to Pyramid training system in experienced young men. We hypothesized that high-load RT would result in greater increases in muscular strength compared with Pyramid training. The rationale for this hypothesis is based on the principle of specificity since the high-load condition elicits a greater increase in 1-RM strength.

## Materials & Methods

### *Participants*

Subjects were 20 healthy male volunteers (age,  $23.9 \pm 2.0$  years; height,  $1.77 \pm 0.05$  m; weight,  $75.6 \pm 9.4$  kg; RT experience,  $4.1 \pm 3.4$  years) recruited from a fitness gym in Puglia (Italy). This sample size was justified by a priori power analysis in G\*power (Faul, Erdfelder, Lang, & Buchner, 2007) using a medium effect size, alpha of 0.05 and power of 0.80, which determined that 16 subjects were required for participation; the additional recruitment accounted for the possibility of dropouts. Subjects were required to meet the following inclusion criteria: 1) males between the ages of 18 to 35 years, 2) no existing musculoskeletal disorders, 3) claimed to be free from consumption of anabolic steroids or any other legal or illegal agents known to increase muscle size for the previous year, 4) experienced with RT, defined as consistently lifting weights at least three times per week for a minimum of 1 year.

Upon completion of testing, the participants were pair-matched based on initial strength capacity and then randomly assigned to an experimental group ( $n = 10$ ) performing high-load RT twice a week interspersed with a Pyramid training weekly session, or a Pyramid training group ( $n = 10$ ) performing tri-weekly training sessions with Pyramid method alone. For randomization, we used the method of randomly permuted blocks using Research Randomizer, a program published on a publicly accessible official website ([www.randomizer.org](http://www.randomizer.org)). All participants received a complete explanation in advance about the purpose of the experiment, its contents, and safety issues based on the Declaration of Helsinki. The study was conducted from March to May 2018.

### *Experimental design*

In this randomized controlled trial study design with experimenter blinding the participants visited the gym weight room 30 times over the course of 8 week: two familiarization sessions, two pre-testing visits, 24 training visits (three training sessions per week), and two post-testing visits. The two pre-testing visits occurred in the first week (week 1) and the two post-testing visits occurred in the last week (week 8). During previsit 1, the participants filled out an informed consent. After confirming that they did not meet any exclusion criteria,

height and body mass was measured using a standard stadiometer and an electronic scale. Next, the first session of 1RM testing for the maximal strength assessment was completed. Previsit 2 occurred 48 h after previsit 1. During the second previsit, participants completed 1RM testing. After, a total of 24 mixed-methods strength training sessions consisting of high-load RT twice a week interspersed with a Pyramid training weekly session were performed by experimental group, whereas the Pyramid training group performed the same number of sessions using only the Pyramid method. There were 48 hours of rest between training sessions for eight consecutive weeks. The first post-testing visit was performed 48 to 72 h after the final training session at the same time of day as previsit 1. The second post-testing visit was after 48 h apart the first posttesting visit and at the same time of day as previsit 2. In both visits the 1-RM tests were carried out and completed, and researchers collected final data for statistical analysis.

#### *Testing procedures*

*Familiarization sessions.* The familiarization process was conducted over two non-consecutive days with an interval of 48 h. During each familiarization session, participants performed a workout similar to the testing sessions. Familiarization sessions are needed for an accurate assessment of 1RM load, which may vary from two to three sessions in well-conditioned individuals (Cronin & Henderson, 2004). Both sessions were planned for the participants to become familiar with the equipment and correct execution (appropriate posture, lifting, and breathing technique, use of a constant range of motion, and movement speed) for resistance exercises. These processes were designed to facilitate the estimation of initial loads and subsequent increments in the 1RM (Haff & Dumke, 2018).

*Maximal Strength Assessments.* After the familiarization process, maximal dynamic strength was evaluated using the 1-RM test assessed for four different exercises in the following order: 1) Barbell bench press, 2) Barbell deadlift, 3) Lat pull-down, and 4) Standing barbell military press. 1RM was determined using a validated testing procedure and defined as the heaviest weight a participant could lift once with a proper lifting technique, without compensatory movements (Haff & Dumke, 2018). A general warm-up before testing that consisted of light cardiovascular exercise lasting approximately 5 to 10 min. Testing for each exercise was preceded by a specific warm-up set (6–10 repetitions), with approximately 50% of the estimated load used in the first attempt of the 1RM, during which the subjects were instructed to perform the movement over 2 s (1 s for eccentric and 1 s for concentric phase). The testing procedure was initiated 2 minutes after the warm-up set. The subjects were instructed to try to accomplish 2 repetitions with the load in 3 attempts in all exercises tested. The rest period was 3–5 minutes between each attempt and 5 minutes between exercises. The 1RM was recorded as the last resistance lifted in which the subject was able to complete only 1 repetition. The technique for each exercise was standardized and continuously monitored to ensure reliability. All 1RM testing sessions were supervised by 2 experienced researchers graduates in sport sciences to maximize safety and test reliability. Verbal encouragement was given throughout each test. Each testing session ended with ~5 min. of cool-down activities including stretching. Two 1RM sessions for all four exercises were performed separated by 48 hours. The highest load achieved among the two sessions was used for analysis in each exercise. The ICC values ( $r$ ) between tests were 0.94, 0.91, 0.89, and 0.92 for bench press, barbell deadlift, lat pull-down, and standing barbell military press, respectively ( $p < 0.05$ ).

#### *Strength training program*

Supervised RT was performed during the afternoon hours in the gym weight room. The protocol was based on scientific knowledge of training methods to improve muscular strength (Haff & Triplett, 2015). Both groups trained for 8 weeks (i.e., 2 mesocycles) using a 3:1 loading structure, that is volume and intensity is increased for 3 weeks followed by an “unload” week. Thus, the fourth and eighth week (or microcycle) the experimental group reduced intensity of 10% and Pyramid group volume of 50%. These unload weeks, which create a marked variation in workload, were used to reduce overtraining potential and allow for adaptation or “supercompensation”. It is known that periodization allows an appropriate balance of training loads, fatigue management and the reduction of overtraining potential, and adequately staging and timing of the peak (Bompa & Buzzichelli, 2018).

All participants were personally supervised by physical education professionals graduates in sport sciences to help ensure consistent and safe exercise performance. Both groups performed RT using a combination of free weights and machines. The sessions were performed 3 times per week on Mondays, Wednesdays, and Fridays. Researchers adjusted the loads of each exercise according to the subject’s abilities and improvements in exercise capacity throughout the study to ensure that the subjects were exercising with as much resistance as possible while maintaining proper exercise technique. On Wednesday experimental group performed complementary exercises to those performed the other two days (mostly smaller muscle groups and mono-articular exercises). Prior to each training session, all subjects participated in a 10-minute warm-up period which included light cardiovascular exercise (10 min.). Each training session ended with ~5 min. of cool-down activities including stretching. Both training programs are shown in detail in table 1.

Table 1. Training schedule for both training group.

Experimental group (3 d·wk <sup>-1</sup> )		Pyramid training group (3 d·wk <sup>-1</sup> )			
	Monday/Friday	Wednesday	Monday	Wednesday	Friday
<b>Week</b>	High-load (80-95% 1RM)	Pyramid method		Pyramid method	
<b>1</b>	4 x 5	3 x 12-10-8		3 x 12-10-8	
<b>2</b>	5 x 3	3 x 12-10-8		3 x 10-8-6	
<b>3</b>	6 x 2	3 x 10-8-6		4 x 12-10-8-6	
<b>4</b>	4 x 5	3 x 10		2 x 10	
<b>5</b>	4 x 5	3 x 12-10-8		3 x 12-10-8	
<b>6</b>	5 x 3	3 x 12-10-8		3 x 10 -8-6	
<b>7</b>	6 x 2	3 x 10-8-6		4 x 12-10-8-6	
<b>8</b>	4 x 5	3 x 10		2 x 10	
Rest	set/exercise ~3 min.	set/exercise ~90 sec.		set/exercise ~90 sec.	
	<b>Exercises</b>		<b>Exercises</b>		
	-Barbell bench press	-Row machine	-Barbell bench press -Incline Dumbbell Bench Press	-Barbell deadlift	-Lat pull-down
	-Barbell deadlift	-Squat	-Cable crossover	-Leg extension	-Low-Pulley Seated Row
	-Lat pull-down	-Incline Dumbbell Fly	-EZ barbell french press	-Leg curl	-Single arm dumbbell row
	-Standing barbell military press	-Barbell Biceps Curl	-Rope push-down	-Military press	-Barbell Biceps Curl
		-Alternate dumbbell curl	-Parallel dips	-Lateral Shoulder Raise	-Alternate dumbbell curl
		-Lateral Shoulder Raise		-Upright Row	-Rope curl
		-Upright Row			
		-Rope push-down			
		-Parallel dips			

Experimental group: combined High-load and Pyramid training methods. Both groups used a 3:1 loading structure (mesocycle). The fourth and eighth week (or microcycle) represent the unload weeks: experimental group reduced intensity of 10% and Pyramid group volume of 50%. All exercises were prescribed as sets x repetitions.

#### Statistical analyses

All analyses were performed using SAS JMP® Statistics (Version <14.1>, SAS Institute Inc., Cary, NC, USA, 2018) and the data are presented as group mean values and standard deviations. Normality of all variables was tested using Shapiro-Wilk test procedure. Levene's test was used to determine homogeneity of variance. A multivariate analysis of variance (MANOVA) was used to detect differences between the study groups in all baseline variables. Training-related effects were assessed by 2-way analyses of variance (ANOVA) with repeated measures (group x time). When 'Time x Group' interactions reached the level of significance, group-specific post hoc tests (i.e., paired t-tests) were conducted to identify the significant comparisons and, next, Cohen's *d* effect size was made.

The effect size was identified to provide a more qualitative interpretation of the extent to which changes observed were meaningful. Cohen's *d* was calculated as post-training mean minus pre-training mean divided by pooled SD before and after training, and interpreted as small, moderate and large effects defined as 0.20, 0.50, and 0.80, respectively (Cohen, 1988). Partial eta squared ( $\eta^2_p$ ) was used to estimate the magnitude of the difference within each group and interpreted using the following criteria (Cohen, 1988): small ( $\eta^2_p < 0.06$ ), medium ( $0.06 \leq \eta^2_p < 0.14$ ), large ( $\eta^2_p \geq 0.14$ ). The reliabilities of the sit and reach and vertical jump test measurements were assessed using intraclass correlation coefficients; scores from 0.8 to 0.9 were considered as good, while values above > 0.9 were considered as high (Vincent & Weir, 2012). Percentage changes were calculated as [(posttraining value – pretraining value)/pretraining value] x 100.

We accepted  $p \leq 0.05$  as our criterion of statistical significance, whether a positive or a negative difference was seen (i.e., a 2-tailed test was adopted).

## Results

All participants received the treatment conditions as allocated. All twenty subjects completed the training program, and none reported any training-related injury. Both groups did not differ significantly at baseline either in anthropometric characteristics and maximal strength measures ( $p > 0.05$ ).

Significant main effects of ‘time’ were observed for bench press ( $F_{1,18} = 267.4, p < 0.0001, \eta_p^2 = 0.94$ ), deadlift ( $F_{1,18} = 236.6, p < 0.0001, \eta_p^2 = 0.93$ ), lat pull-down ( $F_{1,18} = 112.1, p < 0.0001, \eta_p^2 = 0.86$ ) and military press ( $F_{1,18} = 83.1, p < 0.0001, \eta_p^2 = 0.82$ ). Significant ‘Time x Group’ interaction was found for bench press ( $F_{1,18} = 83.7, p < 0.0001, \eta_p^2 = 0.82$ ), deadlift ( $F_{1,18} = 76.6, p < 0.0001, \eta_p^2 = 0.81$ ), lat pull-down ( $F_{1,18} = 58.1, p < 0.0001, \eta_p^2 = 0.76$ ) and military press ( $F_{1,18} = 46.3, p < 0.0001, \eta_p^2 = 0.72$ ). The post hoc analysis revealed that the experimental group showed significantly greater improvements in maximal strength from pre- to post-testing for all dependent variables ( $p < 0.0001$ ) than pyramid training group ( $p < 0.05$ ). A significant main effect of ‘Group’ was only detected for lat pull-down ( $F_{1,18} = 6.5, p = 0.0203, \eta_p^2 = 0.26$ ). Pre- and post- intervention results for all outcome measures are presented in Table 2.

Table 2. Changes after 8-weeks of training measured by 1-RM test. Data are expressed as mean ( $\pm$ SD).

Variables	Experimental group (n = 10)				Pyramid Training group (n = 10)			
	Pre-test	Post-test	Difference		Pre-test	Post-test	Difference	
			Absolute (ES)	%			Absolute (ES)	%
<b>Muscular strength</b>								
Barbell Bench Press (kg)	99.8 (17.5)	112.9 (17.6)*†	13.1 (0.74)	13.1	95.3 (16.4)	99.0 (17.2)*	3.7 (0.22)	3.9
Barbell deadlift (kg)	118.4 (37.8)	137.7 (37.4)*†	19.3 (0.51)	16.3	110.0 (27.8)	115.3 (28.8)*	5.3 (0.19)	4.8
Lat pull-down (kg)	87.0 (18.6)	104.2 (20.0)*†	17.2 (0.89)	19.8	75.5 (13.8)	78.3 (12.6)*	2.8 (0.21)	3.7
Standing barbell military press (kg)	54.6 (8.4)	67.7 (10.0)*†	13.1 (1.41)	24.0	54.5 (10.4)	56.4 (10.2)*	1.9 (0.18)	3.5

Experimental group: combined high-load (2 sessions per week) and Pyramid training (1 interspersed session per week). ES = Cohen’s *d* effect size. \*Significantly different from pre-test ( $p < 0.05$ ). †Significant ‘Time x Group’ interaction = significant effect of the training program.

## Discussion & Conclusions

Through a randomized controlled trial study design with experimenter blinding, we tested the hypothesis that high-load RT would result in greater increases in muscular strength compared with Pyramid training. The rationale of hypothesis was based on the principle of specificity since the high-load condition should have elicited a greater increase in 1-RM strength. To the authors’ knowledge, this is the first study to evaluate muscular strength in high-load versus pyramid training in well-trained young men. The study produced several important results as it sought to clarify the effectiveness of high-load training and the pyramidal method to increase maximum strength in trained individuals. The main finding of this study was that the high-load RT is effective to promote adaptations in muscular strength in well-trained young men. They have achieved significant gains in muscle strength with a percentage ranging from 13.1% to 24% , and changes observed were interpreted qualitatively as large effect size. Instead, subjects who trained at high-intensity without drastic volume reductions, that is by Pyramid method, achieved significant but minimal improvements in muscular strength with a ranging from 3.5% to 4.8%.

Our results confirm previous hypotheses, that is that the high-load condition causes a greater increase in 1-RM strength (Buckner et al., 2017; Kraemer & Ratamess, 2004; Mattocks et al., 2016; Mitchell et al., 2012; Ratamess et al., 2009; Schoenfeld et al., 2015). These changes obtained after eight weeks can be attributed to the principle of specificity. Experimental group trained high-load twice a week performing same multi joint exercises of the maximal strength testing session, then gains in terms of muscle strength were specific to the type of exercise, training intensity and muscle groups involved. Maximal strength is optimized by a combination of increased muscle CSA and enhanced neural efficiencies (Cormie, McGuigan, & Newton, 2011; Duchateau, Semmler, & Enoka, 2006). Studies comparing muscular adaptations between low- versus high-load exercise have generally shown greater increases in 1-RM for those training with heavier loads (Campos et al., 2002; Mitchell et al., 2012; Ogasawara, Loenneke, Thiebaud, & Abe, 2013). In effect, it has been hypothesized that the superiority of high-load exercise is related to neural improvements; as such training allows the lifter to get practice at the performance of heavy lifts (Mitchell et al., 2012).

However, also the Pyramid training group carried out the same exercises performed during the assessment tests but the increase in muscle strength was less. This supports effectiveness of the Pyramid method in increase muscular strength although in a limited way compared to high-load training. The Pyramid system theoretically allows training with higher loads, at least during the final sets of an exercise, without reducing the training volume from a loading zone standpoint, thus maintaining a favourable anabolic environment for increased muscle hypertrophy and thus strength gains. This confirm that training volume is an important contributing factor for increasing muscular strength (Marshall et al., 2011; Radaelli et al., 2015; Rhea et al., 2002; Rønnestad et al., 2007) even if it is not effective at increasing maximal strength such as using high loads and low repetitions (Campos et al., 2002).

Thus, our findings confirm previous studies. In addition, we have combined two training methods (high-loads + pyramid method) that have provided results of high importance in increasing the maximal strength in the trained subjects. The novelty brought by our work lies in the effectiveness of the mixed method that has produced very large gains in maximal muscle strength. It is known in the scientific literature the effectiveness of combined training methods in increase fitness performance (Fischetti & Greco, 2017; Fischetti, Vilardi, Cataldi, & Greco, 2018). Furthermore, unlike what was done in the literature, the experiment was performed on well-trained young men eliciting positive results despite the risk of the “ceiling effect”. This means that the training protocol we have implemented respects the scientific principles that underlie the training methodology and has provided a new advanced training method as it has been effective and valid to produce the pre-established results. The present study had some limitations that must be considered when extrapolating conclusions based on the results. The study period lasted only 8 weeks. While this duration was sufficient to produce significant increases in muscular strength, it is not clear whether results between groups would have diverged over a longer time. Moreover, our subject population consisted exclusively of young resistance-trained men. Findings therefore cannot necessarily be generalized to other populations including adolescents, women and elderly. It is possible that differences in hormonal influences, anabolic sensitivity of muscle, recuperative abilities, and other factors could alter muscular adaptations to high-load protocols in these individuals.

We conclude that high-load RT is an effective method to promote positive short-term adaptations of muscular strength in well-trained young men. However, even the Pyramid method has increased muscle strength even though it has not been as effective as high-load RT. Finally, practitioners also have the option of using a combination of different RT systems over time, as this may help to maintain interest in and motivation to perform RT by allowing a varied RT program.

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#### **Authors' contribution**

Francesco Fischetti designed the study, was involved in the interpretation of data, wrote and revised the manuscript. Francesco Camporeale designed the study, collected data and was involved in the interpretation of data. Gianpiero Greco designed the study, carried out the statistical analysis, interpreted the data, wrote and revised the manuscript. All authors contributed intellectually to the manuscript, and all authors have read the manuscript and approved the submission.

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