

Lower-limb plyometric training improves vertical jump and agility abilities in adult female soccer players

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Abstract:

Plyometric training has been shown to be an effective method for improving jump performance capabilities, strength, agility and sprint ability. However, the results obtained in female soccer players are scarce and controversial. Therefore, the aim of this study was to determine how explosive strength and agility are affected by a 12-week plyometric training program in elite female soccer players. Twenty-eight adult female players were randomly assigned into 2 groups: control group (CG, n = 14, age 26.7 ± 5.3 years) and plyometric group (PG, n = 14; age 26.5 ± 6.9 years). The intervention was performed during the second part of the competitive season. Both groups performed technical and tactical training exercises and matches together. However, the CG followed the regular soccer physical conditioning program, which was replaced by a plyometric program for PG. Neither CG nor PG performed weight training. Plyometric training was carried out 3 days a week for 12 weeks including jumps over hurdles, drop jumps in stands, or horizontal jumps. At baseline and after training all participants were tested on the countermovement jump height (cm) and agility T-test time (s). The PG group showed significantly ($p < 0.001$) improvement than CG in the countermovement jump (3.1 ± 2.4 vs. 0.6 ± 1.2 cm) and T-test (-0.2 ± 0.2 vs. 0.0 ± 0.2 s) following training. Our findings highlight that plyometric training can improve explosive strength in female soccer players, and, most importantly, these improvements concern the jumping ability and sprinting speed with direction changes, power-related components of soccer thought to be necessary for success. We strongly recommend that soccer coaches implement in-season plyometric training to enhance the performance of their players.

Key Words: changes of direction; stretch-shortening cycle; explosive strength.

Introduction

Plyometrics is a well-known form of 'ballistic training', designed to improve jump performance capabilities (Ives, 2013). In addition, plyometric training has been shown to be an effective method for improving strength (Asadi, 2012), agility (Chu, 1998; de Villarreal, González-Badillo, & Izquierdo, 2008) and sprint ability (Chu, 1998; Fischetti, Vilardi, Cataldi, & Greco, 2018). Previous studies have concluded that the effectiveness of plyometric training depends on the volume and frequency of training (de Villarreal, Kellis, Kraemer, & Izquierdo, 2009), and a number of subject characteristics, such as strength training level (Dodd & Alvar, 2007), sex (Bosco & Komi, 1980; Weiss, Relyea, Ashley, & Propst, 1997), age (Bosco & Komi, 1980; Thomas, French, & Hayes, 2009) and sport (De Villarreal, Requena, & Newton, 2010). Plyometric training has been advocated for sports that require explosiveness to increase skills such as vertical jumping ability. Vertical jump height is the primary criterion measure and the most reported variable to assess vertical jump ability. Jump performance ability is governed by an individual's ability to utilise the elastic and neural benefits of the stretch-shortening cycle (Malisoux, Francaux, Nielens, & Theisen, 2006; Weiss et al., 1997). It's scientifically proved that a stretched muscle can produce more power than a non-stretched muscle (Malisoux et al., 2006; de Villarreal et al., 2010).

There are studies that demonstrated increases in this task after a plyometric program in female athletes (Martel, Harmer, Logan, & Parker, 2005; Myer, Ford, Palumbo, & Hewett, 2005) and in male soccer players, both in youth (Diallo, Dore, Duche, & Van Praagh, 2001) and in adult age categories (Polman, Walsh, Bloomfield, & Nesti, 2004). However, the results obtained in female soccer players are scarce and controversial. Chimera, Swanik, Swanik, and Straub (2004) carried out a 6-week plyometric program with female soccer players exercising twice a week. The plyometric group carried out plyometric exercises in addition to the normal soccer training program. The results did not demonstrate significant improvements in jumping ability for the plyometric group. On the other hand, Siegler, Gaskill, and Ruby (2003) developed a 10-week plyometric, intermittent, high-intensity anaerobic program with 34 female soccer players. They registered gains in speed and jumping ability in comparison with players of the control group, who continued with their regular aerobic soccer

conditioning program. Again, a meta-analysis review by Stojanović, Ristić, McMaster, and Milanović (2017) confirmed small increases in squat jump performance and larger improvements in jump performance after at least 10 weeks of plyometric program in female soccer players. Several authors (Fatouros et al., 2000; Markovic, 2007) noted that the discrepancy in the results of previous studies may be caused by different research protocols such as different durations of training methods, different status of the subjects, or different training loads.

Thus, plyometric training has been applied in numerous studies and shown to increase performance variables. Among these, it was shown that it improves soccer specific skills such as agility (Miller, Herniman, Ricard, Cheatham, & Michael, 2006). However, little scientific information is available to determine if plyometric training actually enhances agility in male soccer, let alone in female soccer. Accordingly, controversial, different and poor results of the researches done on plyometric trainings in women's soccer revealed that more researches should be done. The knowledge about the conditioning of female soccer players should be extended rather than merely implementing extensions of current male training programs. Therefore, the aim of the present study was to determine how explosive strength and agility are affected by a 12-week plyometric training program in elite female soccer players. More specifically, we hypothesized that a plyometric program implemented during the second part of the competitive season, which replaced the standard regular soccer conditioning program, would increase jumping ability and sprinting speed with direction changes in adult experienced female soccer players.

Materials & Methods

Research design

In this randomized controlled trial study design, twenty-eight elite female soccer players participated in the experiment. They were divided into 2 groups according to the training program: control group (CG) and plyometric training group (PG). The independent variable was the treatment effect of the plyometric 12-week training program that was focused on the strength of the lower limbs. The dependent variables were vertical jump height (countermovement jump) and sprint time (T-test). Each variable was measured on 2 occasions: 1 week before the start of the training program and 1 week after the end of the training period.

Participants

Before the start of the intervention, the coach and the women were fully informed about the aims of the study. They provided written informed consent and completed a form giving personal, medical, and training details. All procedures described in this study were in accordance with the ethical standards laid down in the Helsinki Declaration. A total of 28 female soccer players (age 26.6 ± 6.1 years; weight 60.7 ± 5.4 kg; height 160.4 ± 4.1 cm; BMI 23.6 ± 1.6 kg·m⁻²) from two soccer teams playing in the Italian Women's series A League voluntarily participated in the study. Participants were chosen among the players who had at least 5 years of training history and minimum 3 years of plyometric training background, and those who had an orthopaedic injury within the past 8 months were excluded from the study. Goalkeepers were not included in this study. There were no group differences with regard to soccer-related experience. They were randomly allocated either to the CG or to the PG. All players involved in the study attended all the sessions. Following are descriptions of the 2 groups:

- CG: 14 players (age 26.7 ± 5.3 years; weight 60.6 ± 4.6 kg; height 160.7 ± 4.1 cm; BMI 23.5 ± 1.6 kg·m⁻²).

- PG: 14 players (age 26.5 ± 6.9 years; weight 60.8 ± 6.3 kg; height 160.1 ± 4.3 cm; BMI 23.7 ± 1.7 kg·m⁻²).

Both groups trained on average 10 hours a week and played a match weekly.

Training procedures

The specific training program was implemented during the second part of the competitive season (i.e., February, March, and April) after 6 months of training (i.e., from August until January). During the intervention program, the team trained 4 times a week. On Mondays, Wednesdays, and Fridays, the session lasted approximately 120 minutes and on Thursdays only 70 minutes. At each training session, the CG and the PG performed the warm-up and technical and tactical program together. Training games on Thursdays and competitive matches on Sundays were also carried out together. The physical conditioning program was different for the 2 groups. CG women followed the regular standard soccer physical conditioning program. This program was completely replaced by a plyometric program to improve the strength of lower limbs for PG players. After the intervention of 12 weeks, CG and PG continued with their normal soccer training together. During the study, players were not allowed to perform any other training that would impact the results. The training regimen of the team during the study was organized as follows:

- *Monday*. 15 min.: warm up (PG+CG); 40-60 min.: plyometric training for PG (jumps over hurdles) and physical conditioning program for CG (core stability training, reaction speed and stretching); 45-65 min.: technical and tactical training (PG+CG).
- *Wednesday*. 15 min.: warm up (PG+CG); 40-60 min.: plyometric training for PG (drop jumps in stands) and physical conditioning program for CG (speed endurance or general strength training where external resistance was provided using portable rubber bands, ropes, or with the assistance of another soccer player, but it did not include weight training); 45-65 min.: technical and tactical training (PG+CG).

- *Thursday*. 15 min.: warm up (PG+CG); 55 min.: games 7vs7, 5vs5, 10vs10 (PG+CG).
- *Friday*. 15 min.: warm up (PG+CG); 40-60 min.: plyometric training for PG (horizontal jumps) and physical conditioning program for CG (fartlek and stretching); 45-65 min.: technical and tactical training (PG+CG).

Plyometric training

Although all women had previous experience in this type of training, a trained instructor gave specific instructions and showed illustrations of each plyometric exercise before the first session. As mentioned above, the plyometric training took place after the warm-up, 3 days a week for 12 weeks (36 sessions distributed on Monday, Wednesday, and Friday), supervised by the same sport sciences professional. Each plyometric session took 40 to 60 minutes to perform, and the training regimen was based on 3 different exercises, always coming after the procedures described below:

- *Monday*: Series of 5 jumps over hurdles of 60 cm height spaced apart at 45 cm intervals with a rest of 30 seconds after 5 jumps and 4 minutes after 20 jumps;
- *Wednesday*: Series of 5 drop jumps in stands of 50 cm with a rest of 1 minute after 5 jumps and 5 minutes after 20 jumps;
- *Friday*: Series of 5, 8, or 10 horizontal jumps with a rest of 40 seconds after 5, 8, or 10 jumps and 4 minutes after 20 to 30 jumps.

Training sessions were carried out on a hard synthetic floor. Each week, the number of jumps was the same on Monday, Wednesday, and Friday. After every 2 weeks of training, the number of jumps decreased to ensure a proper adaptation to the program, that is was used a 2:1 loading structure. After 12 weeks, the total number of jumps was 3240. Intensity in terms of height/length and speed was always maximal. During the jumps over hurdles and drop jumps, women were instructed to perform a free knee flexion position to ensure an individually and preferred chosen knee flexion angle to achieve the optimal jumping height. In all the exercises, players were asked to minimize ground contact. The plyometric program is described in Table 1.

Table 1. Description of each session of the plyometric 12-week training protocol.

Week (total jumps)	Description		
	Monday (jumps over hurdles)	Wednesday (drop jumps in stands)	Friday (horizontal jumps)
W1 (240 j)	4x(4x5j)/30"/4'	4x(4x5j)/1'/4'	4x(4x5j)/40"/4'
W2 (270 j)	4x(4x5j)/30"/4'+(2x5j)/30"	4x(4x5j)/1'/4'+(2x5j)/1'	3x(4x5j)/40"/4'+(3x10j)/40"
W3 (210 j)	3x(4x5j)/30"/4'+(2x5j)/30"	3x(4x5j)/1'/4'+(2x5j)/1'	3x(4x5j)/1'/4'+(1x10j)
W4 (270 j)	4x(4x5j)/30"/4'+(2x5j)/30"	4x(4x5j)/1'/4'+(2x5j)/1'	2x(4x8j)/40"/4'+(5x5j)/40"
W5 (300 j)	5x(4x5j)/30"/4'	5x(4x5j)/1'/4'	3x(4x5j)/40"/4'+(3x10j)/40"/4'+(2x5j)/40"
W6 (240 j)	4x(4x5j)/30"/4'	4x(4x5j)/1'/4'	4x(4x5j)/40"/4'
W7 (300 j)	5x(4x5j)/30"/4'	5x(4x5j)/1'/4'	3x(4x5j)/40"/4'+(3x10j)/40"/4'+(2x5j)/40"
W8 (330 j)	5x(4x5j)/30"/4'+(2x5j)/30"	5x(4x5j)/1'/4'+(2x5j)/1'	3x(4x5j)/40"/4'+(3x10j)/40"/4'+(4x5j)/40"
W9 (270 j)	4x(4x5j)/30"/4'+(2x5j)/30"	4x(4x5j)/1'/4'+(2x5j)/1'	2x(4x8j)/40"/4'+(5x5j)/40"
W10 (330 j)	5x(4x5j)/30"/4'+(2x5j)/30"	5x(4x5j)/1'/4'+(2x5j)/1'	3x(4x5j)/40"/4'+(3x10j)/40"/4'+(4x5j)/40"
W11 (360 j)	6x(4x5j)/30"/4'	6x(4x5j)/1'/4'	3x(4x8j)/40"/4'+(5x5j)/40"
W12 (300 j)	5x(4x5j)/30"/4'	5x(4x5j)/1'/4'	3x(4x5j)/40"/4'+(3x10j)/40"/4'+(2x5j)/40"

Note. j= jumps; example: 4x(4x5j)/30"/4' = 4 sets x (4 series x 5 jumps); 30 seconds rest after 5 jumps and 4 minutes rest after 20 jumps.

Testing procedures

Before the initial testing, each player was familiarized with the testing protocol. Participants were informed that they should stop physical activities 24 hours before and they should stop drinking and eating 3 hours before the test. Tests were done on a hard synthetic floor. To standardize testing procedures, the same trained test leaders carried out the entire test procedure using identical order and protocol. All players were tested on 2 separate occasions: PRE, 1 week before the start of the training program; POST, 1 week after the end of the intervention program. During the pre-testing visit, the participants filled out an informed consent. After confirming that they did not meet any exclusion criteria, height and body mass were measured using a standard stadiometer (SECA[®]) and an electronic scale (Tanita[®]). Next, the first session of testing was completed in the following order.

Vertical Jumping Ability. Before the start of the test session of the explosive strength, each woman carried out a standardized 15-minute warm-up period. The jumping ability of the players was evaluated with the *Countermovement jump test* which assesses the subject's ability to use a stretch-shortening cycle with leg muscles, and it involves many complex and interacting neural and mechanical processes (Bosco, Tarkka, & Komi, 1982). This test began with the subject standing in an upright position. A fast downward movement to approximately a 90° knee flexion was immediately followed by a quick upward vertical movement, as high as possible for the subject, all in one sequence. The test was performed with hands on hips. The subjects were

required to land at the same point of takeoff and rebound with straight legs when landing to avoid knee bending and alteration of measurements. The CMJ was conducted on a resistive and capacitive platform connected to a digital timer (accuracy ± 0.001 seconds) (Ergojump, Psion XP, MA.GI.CA., Rome, Italy) that recorded the flight and contact times of each individual jump. The height of the jump was calculated from the flight time (in seconds) by applying ballistic laws. Each participant was given five trials, which were separated by 40 seconds intervals, to complete their highest jump. The maximal height (cm) reached in the trials was taken as the dependent variable. The test-retest reliability reported a high reliability for the CMJ (ICC = 0.98).

Agility and sprinting ability. After a short recovery of 3 minutes after stretching exercises, players practiced the T-test for 10 minutes to familiarize themselves with the test procedure. T-test is a reliable and valid measure of sprinting speed, lower-body power, and agility and predictor of sports performance in nondisabled men and women (Pauole, Madole, Garhammer, Lacourse, & Rozenek, 2000). It consisted by direction changes such as forward sprints, left and right shuffles, and backpedalling (Figure 1). The subjects were instructed to sprint from a standing starting position to a cone 10 m away, followed by a side-shuffle left to a cone 5 m away. After touching the cone, the subjects side-shuffled to the cone 10 m away and then side-shuffled back to the middle cone. The test was concluded by back-peddalling to the starting line (Miller et al., 2006). Participants performed three trials with five minutes recovery between trials. The best time of the trials, to the nearest 0.01 sec., was recorded for statistical analysis. Times to complete the T-test were measured every time by the same two assisting people using a hand-held stopwatch. The average of the times measured by the two assistants was used for statistics. The test-retest reliability reported a high reliability for T-test (ICC = 0.94). Until the end of the experiment the experimental status of the participants (PG or CG) was unknown for both assistants.

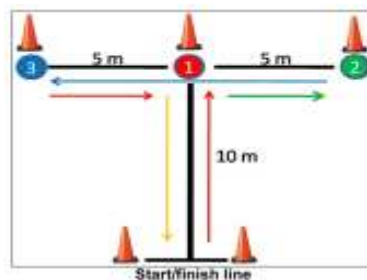


Figure 1. T-test procedure

Statistical Analysis

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. Percentage changes were calculated as [(posttraining value – pretraining value)/pretraining value] x 100.

The reliabilities of the countermovement jump and T-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). 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 (η^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 ($\eta^2_p \geq 0.14$). We accepted $p < 0.05$ 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 attended all training sessions (100% compliance) and there were no injuries resulting from either training program. The PG and CG groups did not differ significantly at baseline in anthropometric characteristics and in both performance measures ($p > 0.05$). Pre- and post-intervention results for all outcome variables are presented in Table 2.

Countermovement Jump. A significant 'Time x Group' interaction ($F_{1,26} = 11.74, p = 0.0020, \eta^2_p = 0.31$) and main effect of 'Time' ($F_{1,26} = 26.91, p < 0.0001, \eta^2_p = 0.51$) were found, but no significant main effects of

'Group' was detected. The post-hoc analysis revealed a significant increase in maximal height reached from pre- to post-test in the PG ($p = 0.0003$, $d = 0.55$).

T-Test. The statistical analysis revealed a significant 'Time x Group' interaction ($F_{1,26} = 4.94$, $p = 0.0352$, $\eta^2_p = 0.83$) and main effect of 'Time' ($F_{1,26} = 11.51$, $p = 0.0022$, $\eta^2_p = 0.92$) but not of 'Group'. The post hoc analysis revealed a significant decrease in the run time from pre- to post-test in the PG ($p = 0.0005$, $d = 0.73$).

Table 2. Changes in performance variables after 8-week for the PG and CG. Data are mean (\pm SD).

Variables	PG (n = 14)				CG (n = 14)			
	Pre-test	Post-test	Difference		Pre-test	Post-test	Difference	
			Absolute	%			Absolute	%
CMJ (cm)	33.6(5.5)	36.8(5.8)*†	3.1(2.4)	9.7(8.0)	32.1(6.4)	32.7(5.7)	0.6(1.2)	2.6(4.3)
T - Test (sec)	8.8(0.3)	8.5(0.3)*†	-0.2(0.2)	-2.6(2.1)	8.9(0.3)	8.8(0.4)	0.0(0.2)	-0.5(2.7)

PG = Plyometric training group; CG = Control group; CMJ = countermovement jump. *Significantly different from baseline ($p < 0.05$). †Significant 'Time x Group' interaction = significant effect of the training program.

Discussion & Conclusions

Plyometric programs are often implemented during the preseason to bring players to an appropriate initial level of fitness. Such a preseason regimen may serve to improve the athletic performance of footballers by enhancing muscular strength, endurance, and power. Furthermore, previous authors (Reilly, Bangsbo, & Franks, 2000; Markovic & Mikulic, 2010; Meylan & Malatesta, 2009; Miller et al., 2006) have recommended continuation of a plyometric training program into the soccer season to maintain and increase explosive ability. However, little is known about the possible influence of plyometric training on tasks such as jumping ability and agility in female soccer players and what is known is controversial. Therefore, our aim in this study was to determine how explosive strength and agility are affected by a 12-week plyometric training program. As we expected, the main findings of the current study indicated that a 12-week plyometric training program focusing on the lower limbs, in addition to the regular soccer training, increased explosive strength of the lower limbs as well as sprinting speed with direction changes in adult experienced female soccer players.

The players who incorporated the plyometric exercise were able to significantly increase their vertical jump height by 3.1 ± 2.4 cm, that is by 9.7 ± 8.0 % compared to the control group. These changes were interpreted as a moderate effect size showing the effectiveness of the plyometric training program. From previous researches, plyometric training was suggested as an alternative to strength training oriented at lower extremity for female athletes (Myer et al., 2005). Campo et al. (2009) investigated the effects of plyometric training on elite female soccer players that consisted of hurdle jumps, drop jumps, and horizontal jumps for 12 weeks, 3 times a week, as experienced in the present research. Vertical jump height significantly increased after the plyometric training as compared with a control group. Furthermore, several authors have reported significant improvements in vertical jump using plyometric training in male and female athletes (Diallo et al., 2001; Fischetti et al., 2018; Matavulj, Kukulj, Ugarkovic, Tihanyi, & Jaric, 2001; Polman et al., 2004; Martel et al., 2005; Myer et al., 2005; Ramirez-Campillo et al., 2014). Results of the present study are in agreement with this statement because it was shown that the plyometric training program caused significant difference in jump ability between pre-training and post-training values for PG as compared with a control group. These results are also in agreement with those of Siegler et al. (2003), who reported gains in jumping ability after a 10-week plyometric program in female players. Conversely, Chimera et al. (2004) did not find significant increases in jumping ability after a 6-week plyometric program. The difference in frequency of training could be the reason of the discrepancy in results (Fatouros et al., 2000; Markovic, 2007).

Many authors suggested that muscular performance gains after plyometric training are attributed to a neural adaptation located in the nervous system (Carter, Kaminski, Doux Jr, Knight, & Richards, 2007; Maffiuletti, Dugnani, Folz, Di Pierno, & Mauro, 2002; Potteiger et al., 1999; Toumi, Best, Martin, Guyer, & Pourmarat, 2004). According to these authors, the neuromuscular factors such as increasing the degree of muscular coordination and maximizing the ability to use the muscles' stretch-shortening cycle are very important. Moreover, the improvement of muscular coordination after the training period would be partly related to the specificity of movements used during the training program (Diallo et al., 2001). This is especially the case for the velocity because training at a specific velocity improves explosive strength around that particular velocity (Manolopoulos, Papadopoulos, Salonikidis, Katartzi, & Poluha, 2004; Toumi et al., 2004). The specificity of exercises and the knee flexion angle during strength training could also be considered a possible factor influencing the gains in jumping ability (Carter et al., 2007; Maffiuletti et al., 2002).

In our study, subjects who underwent plyometric training were able to decrease significantly their times (-0.2 ± 0.2 s; i.e. $-2.6 \pm 2.1\%$) improving the T-test agility performance, in agree with previous studies (Meylan & Malatesta, 2009; Miller et al., 2006; Thomas et al., 2009). Therefore, we found a positive relationship between plyometric training and improvements in vertical jump height and agility performance. Previous research demonstrated increased proprioception after plyometric training (Myer et al., 2005). Also, leg muscle power has been shown to be moderately related to agility (Mayhew, Piper, Schwegler, & Ball, 1989). In this case the performance gains could be attributed to neuromuscular adaptations and specificity principle as were already described above for the jumping ability. These speculated adaptations could have improved the ability to rapidly and forcefully switch from decelerating to accelerating movements.

Thus, the present research provides novel findings in the field of advanced training methodologies in the female soccer field. Furthermore, we want to highlight that tried to provide useful advice such as not using mobile devices (e.g., smartphones or tablets) before testing and training sessions to avoid a decline in fitness performance (Greco, Tambolini, Ambruosi, & Fischetti, 2017). This strategy may have helped to improve performances after intervention program, of course, this is just our hypothesis, future studies are needed to test procedures to control this effect.

Finally, it could be concluded that a 12-week plyometric training can improve explosive strength in female soccer players, and, most importantly, these improvements concern the jumping ability and sprinting speed with direction changes. The results of this study highlight the potential of using plyometric training techniques to improve the power-related components of soccer thought to be necessary for success. We strongly recommend that soccer coaches implement in-season plyometric training to enhance the performance of their players.

Conflict of interest - The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contribution

Francesco Fischetti contributed to the research design and conception, data interpretation, critical review of draft manuscripts, and written the manuscript. Stefania Cataldi contributed to the writing of the manuscript and data collection and interpretation. Gianpiero Greco contributed to the research design and conception, statistical analysis, data interpretation, critical review of draft manuscripts, and written the manuscript. All authors contributed intellectually to the manuscript, and all authors have read the manuscript and approved the submission.

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