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Immediate effects of cold spray application on timing and activation pattern of the knee joint muscles during one-leg landing

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Abstract

Background: Cold sprays (CS) are widely used in sport competitions as an effective, simple, and high available treatment.

Aim: The aim of present study was to investigate immediate and longtime effects of CS application onto the knee joint anterior-medial area of dominant leg on timing and activation pattern of selected muscles during one-leg landing.

Materials and Methods: Thirty (15 control and 15 experimental) able-bodied male participated in this study. Electromyography activity of vastus medialis, rectus femoris, vastus lateralis, biceps femoris, semiendineus and medial gastrocnemius were recorded during one-leg landing tests (with dominant leg) for each of three conditions including before CS application (pre-test), immediately after (post-test 1), and 20 min later (post-test 2). To determine the effect of group and time on each dependent variable, three independent 2×2 multivariate repeated measures analyses of variance were performed ($\alpha < 0.05$).

Results: The results showed following trends rather than significant differences: the muscles in the experimental group had lower activity level and later onset immediately after CS application compared to before application, and they had higher activity level and sooner onset after 20 min from removing CS compared to immediate application. No significant interaction was found for normalized peak activation and time to peak activation ($P > 0.05$). However, decrease in skin temperature after cold spray application was observed.

Conclusion: These results show short-time cold application, which is so practical after many injuries for returning athletes to sport environment, may not predispose individuals to risk of re-injury or failed landing mechanism.

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1. Introduction

Cold sprays (CS) are widely used in sport competitions as a cheap, effective, simple, and high available method to treat acute soft tissue injuries. During sport events, injured athletes often return to competition after appropriate cold treatment application [1, 2]. In many sport fields, acute injuries are the most common type of injuries (89.6%) [3], and it has been recently shown that one of the most common sites of injury is the knee joint [4]. In addition to providing pain relief, local cold application has the potential to produce concomitant effects on many other physiological systems [5].

Controversial evidence surrounds the questions of should athletes return to sport after cold treatment. Numerous studies examined the effect of cold application on proprioception sense [5, 6, 7], muscle activity [2, 8], biomechanics of movement [9], and muscle force [10]. The results of some studies suggest that cold application decreases activity of the neuromuscular system, and although athletes may feel ready to return to competition after treatment, motor performance may be impaired and the individual may be more vulnerable to injury [2, 6, 11, 12]. For example, Fullam et al. (2015) showed that dynamic postural stability was affected immediately after 15 min cold application to the ankle joint [6]. Pritchard et al. (2014) also suggested that athletic performance may be adversely affected when returning to play immediately after cold treatment [11].

In contrast, several authors stated that cold treatment does not increase the risk for re-injury or may have a positive effect on the athlete performance when returning to competition [9, 13, 14]. For example, Stevens et al. (2017) suggested that pre-exercise cold-water immersion and mid-exercise facial water spray involve some physiological changes of central and psychophysiological mechanisms of performance improvement during running [13].

Duñabeitia et al. (2022) also showed that although cold water immersion affects the running movement, but none of the alterations was related to running biomechanical patterns associated with injuries [9]. However, the results of previous studies are controversial with together because of the heterogeneity relating to cooling time/ dosage, body part and outcome measures.

Also, there are some deficits in methodology of the previous studies and their generalizability to real sports environments may not be well approved. First, most previous studies investigated effect of cold application for 10 min and longer [2, 6, 10]; this may exceed duration of treatment in sport environments [1]. In addition, non-practical cooling methods which rarely used during sport competitions (such as water immersion) have been widely studied [8, 9, 14]. Also, there is a little knowledge about the effect of cold application on sport performance tasks (such as landing and jumping). However, cold sprays which are commonly used in sport competitions with short-time application should be more studied to generalize outcomes to sport environments.

Landing from a jump is a common mechanism of lower extremity injuries in the athletic population [15]. Reports illustrate that 58% of all female basketball injuries occurred while landing from a jump [15]. Evaluating muscle activation pattern during these activities may help elucidate the mechanism of injuries. In addition, inter-muscular coordination has a major role in function and prevention of injuries in the knee joint [4]. It is important for athletes so that the influence of various factors, such as applied treatments or interventions, can be better quantified with assessment of timing and muscle activation pattern [16]. Some studies have evaluated the hamstring and quadriceps muscles activation during different activities [4, 17]. Coordinate activation of these antagonist muscle groups are crucial for the knee joint stability and any impairment in this temporal coordination can predispose individuals to injury [4].

To the author knowledge, none of previous studies have investigated effects of CS application on muscle timing and activation pattern during landing. Therefore, the aim of present study was to assess effects of 5 sec CS application on muscle activation timing and pattern of select dominant leg muscles, as well as skin temperature, during one-leg landing.

2. Materials and Methods

2. 1. Participation

Thirty healthy males (15 control subjects with age=23.4±2.7 years, height=1.74±0.06 m, and weight=65.2±6.4 kg, and 15 experimental subjects with age=24.1±2.9 years, height=1.77±0.07 m, and weight=67.2±7.8 kg), having no musculoskeletal disorders, not suffering from cold hypersensitivity, health problem or history of injuries in the lower extremities, were recruited to participate in this randomized controlled trial. Proper sample size was calculated with power analysis software (G*power), which showed that at least 12 subjects for each group are necessary (statistical power= 0.80, effect size= 0.40, and alpha level= 0.05). Because cold application outcomes are affected by individual factors such as adiposity, with higher levels acting to limit the magnitude and depth of cooling [1], we tried to choose homogeneous subjects in age, height and weight for control and experimental groups. They were also free of any alcohol, drug, or caffeine intake that might have affected motor performance for at least 24 hours prior to the experiment [2]. They had a normal dorsalis pedis pulse, normal sensation, full range-of-motion, and normal strength in their lower extremities [2]. These criteria were collected by a self-report questionnaire and evaluated and confirmed by a physical education expert. In doubtful cases, the subject could participate in the research only after the approval of an official doctor was taken.

All participants gave their informed consent before participating in the study (ethical code: 2229103). The experimental protocol had been approved by the University Committee on Activities Involving Human Participants and was conducted according to the declaration of Helsinki.

Subjects were randomly assigned to a group using a randomized list and only became aware of their group assignment following the pre-test (once CS was prepared for them).

2. 2. Instrument

Surface electrodes Ag/AgCl with 10 mm diameter were used in bipolar arrangement. Before connecting electrodes, excessive hairs on the Vastus Medialis (VM), Rectus Femoris (RF), Vastus Lateralis (VL), Biceps Femoris (BF), Semitendinosus (ST), and Medial Gastrocnemius (MG) muscles were shaved to reduce resistance between the skin and electrodes. The electrodes were placed on the dominant leg muscles with 20 mm distance between electrodes using SENIAM (surface EMG for a non-invasive assessment of muscles) instructions [18]. Neutral electrodes were also attached to the tibialis bone blade. A Foot-switch force transducer connected to the metatarsal area used to determine impact time of foot and ground during landing. Also, an electrogoniameter (Biometrics Ltd., made in United Kingdom), which has high validity and reliability to assess muscle activity (>0.94 for both) [19] was applied to record knee joint angle. After participants and instruments became ready, data was simultaneously recorded by EMG device (Biomonitor system, ME6000-T16, 2008, made in Finland) with sampling rate 2000 Hertz and common signals rejection 110 decibells, which has high validity and reliability to assess muscle activity (>0.8 for both) [20].

2. 3. Procedure

Data was analyzed using Megawin software version 1.3. The current study was conducted at the Lower Limb Biomechanics Research Laboratory of Bu-Ali Sina University. The room temperature and lighting remained constant for all participants during the test procedure [2, 21]. The dominant leg was determined using three tests namely, ball-kick test, step-up test and balance recovery test [2]. Seven one-leg landing tests (with dominant leg) completed for each of three conditions: before CS application (pre-test), immediately after (post-test 1), and 20 min later (post-test 2). For each condition, five successive landing movements were selected for later analysis. Each participant landed with dominant leg from a wooden box with a height of 30 cm for all conditions. The front edge of the wooden box had about 15 cm distance from where the participants landing spot was marked [22]. The participants did only land (not jump top or front) while their hands put on the hips during landing [22]. Accepted landing included metatarsal impact to the ground at first, balance maintain, ability to land without hopping, knee flexion less than 90°, and keep the trunk direction during landing [22]. All performance tests were carried out using the same shoe (Asics running shoe made in Vietnam).

After the pre-test condition completed, a CS in the experimental group was applied in a supine position to the anterior-medial area of the dominant-leg knee joint for local cooling [2]; while the participants of control group lied in a supine position. The application area did not cover the fibular head and no major peripheral nerves were directly cooled. The distance between cold spray (Pic Solution, Artsana company, made in Italy) to the anterior-medial part of the knee joint (30 cm), the angle of cold (90°) and cold duration (5 sec) were consistent for all participants.

After landing tests for the post-test 1 condition was performed, the participants were seated and rested for 20 min without any intervention. The skin superficial temperature of the knee anterior-medial area was measured by infrared electronic thermometer (Manoli company, made in England) for all three conditions. This device measures scope of 0° to 100°C with 0.5°C error, and has high validity and reliability during static situations (>0.9 for both) [23].

A band-pass filter 10-450 Hz was used for initial signal processing and noise removing. Then, EMG row data was converted to a linear envelope through full-wave rectified and low-pass filtered with a second order, phase-corrected Butterworth filter, using a cut-off frequency of 6 Hertz to attenuate movement artifacts [24]. For each muscle, the data was then normalized to the total mean activation of that muscle in the pre-test condition (dynamic normalization). Muscle activation onset time in pre-activation phase during each trial defined as the point when the signal was greater than three times the activity evident during rest and it last at least 50 ms [25]. Rest activity levels were based on EMG data obtained during 5 sec of stationary standing, as recorded prior to landing. The pre-activation and eccentric phases introduced from 100 ms before to foot-ground contact [26, 27] and from this time to 100 ms later [28], respectively.

2. 4. Statistic

The independent variables were groups (control/ experimental) and time conditions (pre-test/ post-test 1/ post-test 2). The dependent variables were normalized mean activation in the pre-activation and eccentric phases, normalized peak activation, time to normalized peak activation, muscle activation onset time in the pre-activation phase, delay in onset for peer agonist/ antagonist knee muscles, and skin temperature. Each of this variable represented the mean of five trials obtained in each group and under each time condition. To determine the effect of group and time on each of the dependent variables, three independent 2×2 (group × time) multivariate repeated measures analyses of variance (MANOVAs) were performed (pre-test vs. post-test 1/ pre-test vs. post-test 2/ post-test 1 vs. post-test 2). In order to prevent a violation of type I error ($\alpha = 0.05$), a Bonferroni correction was applied and significant level was set at $P < 0.0167$. All statistical tests were completed with SPSS software version 22.

3. Results

The results showed the following trends rather than significant differences ($P > 0.0167$): in the pre-activation phase and from the pre-test to the first post-test, all muscles in the experimental group tended to decrease their activity level, while the VMO, RF, BF, ST, and MG in the control group tended to increase their activity level. From the first post-test to the second post-test in this phase, the VMO and MG tended to increase and the BF and ST tended to continue decreasing their activity level in the experimental group in contrast with the control group. In the eccentric phase and from the pre-test to the first post-test, the VMO, RF, ST, and MG in the experimental group tended to decrease their activity level in contrast with the control group. In addition, in this phase and from the first post-test to the second post-test, the VMO and ST in the experimental group tended to increase their activity level in contrast with the control group (Table 1).

Normalized muscle peak activation and time to peak activation. The results showed the following trends rather than significant differences ($P > 0.0167$): from the pre-test to the first post-test, normalized muscle peak activation of the VMO and RF in the experimental group tended to decrease and the BF and MG tended to increase their peak activity level in contrast with the control group. Also, normalized muscle peak activation of the VL and MG from the first post-test to the second post-test tended to decrease and this value for the ST tended to increase in the experimental group in contrast with the control group. In addition, from the pre-test to the first post-test, time to peak activation for the VL and BF in the experimental group tended to reach their peak activity sooner and the VMO, ST and MG later in contrast with the control group. From the first post-test to the second post-test, time to peak activation of the ST and BF tended to reach their peak activity later and the VMO, VL and MG sooner in the experimental group in contrast with the control group (Table 2).

Table 1. Normalized mean activity level, mean (SD)

Muscle	Phase	Group	Pre- test	Post- test 1	Post- test 2
VM	Pre-activation	Control	0.435 (0.128)	0.443 (0.154)	0.411 (0.143)
		Experimental	0.433 (0.117)	0.385 (0.092)	0.402 (0.136)
	Eccentric	Control	1.483 (0.304)	1.595 (0.213)	1.550 (0.261)
		Experimental	1.553 (0.125)	1.454 (0.379)	1.575 (0.503)
RF	Pre-activation	Control	0.347 (0.156)	0.383 (0.173)	0.348 (0.140)
		Experimental	0.366 (0.111)	0.330 (0.110)	0.330 (0.131)
	Eccentric	Control	1.633 (0.154)	1.669 (0.280)	1.619 (0.240)
		Experimental	1.633 (0.110)	1.575 (0.316)	1.572 (0.275)
VL	Pre-activation	Control	0.433 (0.174)	0.395 (0.183)	0.399 (0.177)
		Experimental	0.414 (0.151)	0.385 (0.133)	0.374 (0.146)
	Eccentric	Control	1.555 (0.271)	1.564 (0.358)	1.649 (0.363)
		Experimental	1.586 (0.150)	1.625 (0.250)	1.685 (0.352)
BF	Pre-activation	Control	0.462 (0.297)	0.525 (0.234)	0.557 (0.233)
		Experimental	0.532 (0.240)	0.508 (0.249)	0.462 (0.209)
	Eccentric	Control	1.478 (0.401)	1.472 (0.379)	1.460 (0.389)
		Experimental	1.421 (0.278)	1.533 (0.428)	1.479 (0.344)
ST	Pre-activation	Control	0.453 (0.289)	0.499 (0.253)	0.503 (0.230)
		Experimental	0.474 (0.218)	0.415 (0.183)	0.386 (0.217)
	Eccentric	Control	1.507 (0.295)	1.521 (0.366)	1.516 (0.383)
		Experimental	1.534 (0.221)	1.456 (0.502)	1.553 (1.129)
MG	Pre-activation	Control	0.529 (0.284)	0.564 (0.232)	0.540 (0.243)
		Experimental	0.546 (0.219)	0.479 (0.216)	0.483 (0.296)
	Eccentric	Control	1.389 (0.349)	1.461 (0.267)	1.466 (0.360)
		Experimental	1.457 (0.219)	1.401 (0.486)	1.404 (0.412)

Note. The VM indicates to vastus medialis, RF to Rectus Femoris, VL to Vastus Lateralis, BF to Biceps Femoris, ST to Semiendineus, and MG to Medial Gastrocnemius muscles.

Table 2. Normalized muscle peak activation and time to peak activation, mean (SD)

Muscle	Phase	Group	Pre- test	Post- test 1	Post- test 2
VM	Normalized peak	Control	2.044 (0.422)	2.048 (0.495)	2.149 (0.499)
		Experimental	1.992 (0.362)	1.885 (0.553)	2.050 (0.851)
	Time to peak	Control	77.63 (30.08)	81.20 (20.95)	89.03 (21.48)
		Experimental	71.97 (29.83)	88.60 (17.18)	81.70 (23.66)
RF	Normalized peak	Control	2.208 (0.320)	2.308 (0.622)	2.205 (0.451)
		Experimental	2.154 (0.365)	2.122 (0.682)	2.088 (0.569)
	Time to peak	Control	76.67 (24.80)	80.80 (22.08)	71.33 (25.36)
		Experimental	81.27 (22.58)	82.10 (20.40)	72.67 (21.29)
VL	Normalized peak	Control	2.174 (0.652)	2.273 (0.684)	2.487 (0.883)
		Experimental	2.185 (0.604)	2.236 (0.539)	2.165 (0.486)
	Time to peak	Control	70.13 (24.94)	80.67 (26.95)	83.73 (24.72)
		Experimental	81.90 (19.85)	75.90 (20.02)	74.97 (21.90)
BF	Normalized peak	Control	2.107 (0.578)	1.973 (0.637)	2.008 (0.825)
		Experimental	2.017 (0.922)	2.082 (0.803)	2.116 (0.612)
	Time to peak	Control	72.30 (26.23)	81.80 (19.22)	73.33 (18.22)
		Experimental	72.13 (25.04)	69.73 (19.13)	76.57 (14.06)
ST	Normalized peak	Control	2.196 (0.549)	2.149 (0.703)	2.004 (0.609)
		Experimental	2.060 (0.453)	1.984 (0.673)	2.131 (1.541)
	Time to peak	Control	68.57 (25.06)	66.83 (22.87)	59.93 (23.98)
		Experimental	71.23 (24.06)	76.43 (16.21)	80.93 (10.71)
MG	Normalized peak	Control	1.965 (0.620)	1.913 (0.549)	1.996 (0.704)
		Experimental	1.961 (0.549)	1.983 (0.848)	1.966 (0.902)
	Time to peak	Control	66.73 (23.27)	63.37 (19.27)	69.63 (23.32)
		Experimental	75.20 (20.64)	79.97 (19.93)	67.80 (20.39)

Note. The VM indicates to vastus medialis, RF to Rectus Femoris, VL to Vastus Lateralis, BF to Biceps Femoris, ST to Semiendineus, and MG to Medial Gastrocnemius muscles. The unit for time to peak is in miliseconds after foot-ground contact (positive sign).

Muscle activation onset time. The results also showed the following trends rather than significant differences (*P*> 0.0167): from the pre-test to the first post-test, all the muscles in the experimental group tended to be active later, while the VMO, RF, ST, and MG in the control group tended to be active sooner. Also, from the first post-test to the second post-test, all the muscles in the experimental group tended to be active sooner in contrast with the control group (Figure 1).

Muscle agonist-antagonist activation onset time. The results showed that from the pre-test to the first post-test, activation onset time VMO-VL (*P*= 0.021), VMO-ST, VMO-BF (*P*= 0.02), and RF-BF tended to decrease and VL-ST to increase in the experimental group in contrast with the control group. From the first post-test to the second post-test, in the experimental group the VMO-ST, RF-ST, RF-BF, and VL-ST tended to decrease.

Also, from the pre-test to the second post-test, in the experimental group the VMO-VL (*P*= 0.023), VMO-ST (*P*= 0.071), VMO-BF (*P*= 0.094), RF-ST, and RF-BF tended to decrease in contrast with the control group. However, no significant interactions between time and group were found (*P*> 0.0167; Figure 2).

Skin temperature. The results showed statistically significant interactions between time (pre-test vs. post-test 1) and group (*P*= 0.001) with decreasing temperature in the experimental group and no distinct change in the control group, as well as between time (post-test 1 vs. post-test 2) and group (*P*= 0.001) with increasing temperature in the experimental group and no distinct change in the control group (Figure 3).

Normalized muscle activation pattern. The normalized level of muscle activation was dragged during 100 ms before to 100 ms after foot-ground contact and introduced as a muscle activation pattern. It seems that for the two groups the muscle activation patterns are very similar among three conditions (Figure 4).

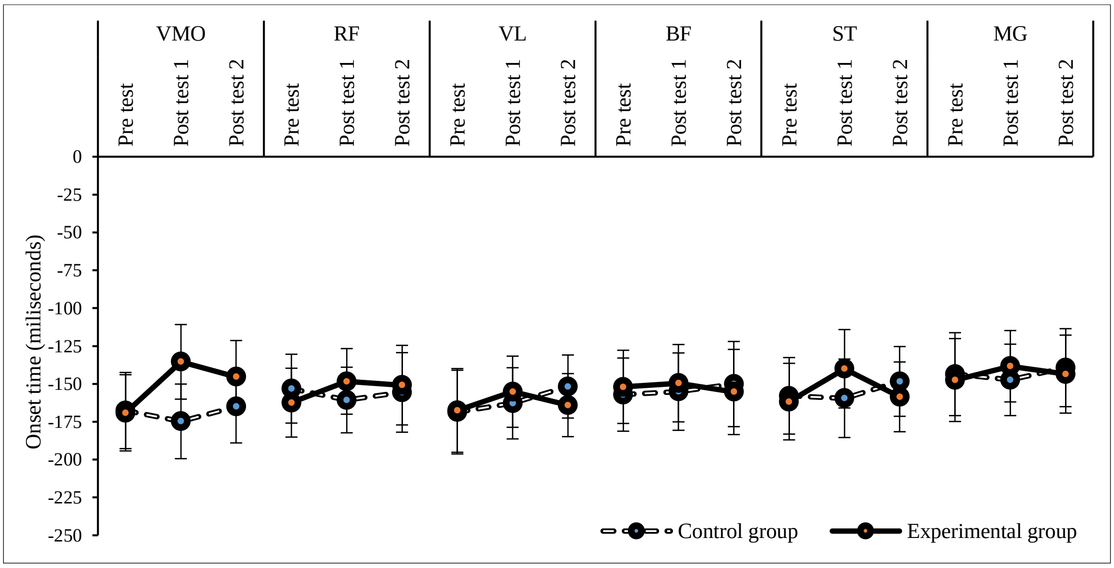

Figure 1. Muscle activation onset time (mean ± standard deviation) for the control and experimental groups at three conditions: pre-test, post-test 1, and post-test 2. Negative sign indicates to the time before foot-ground contact

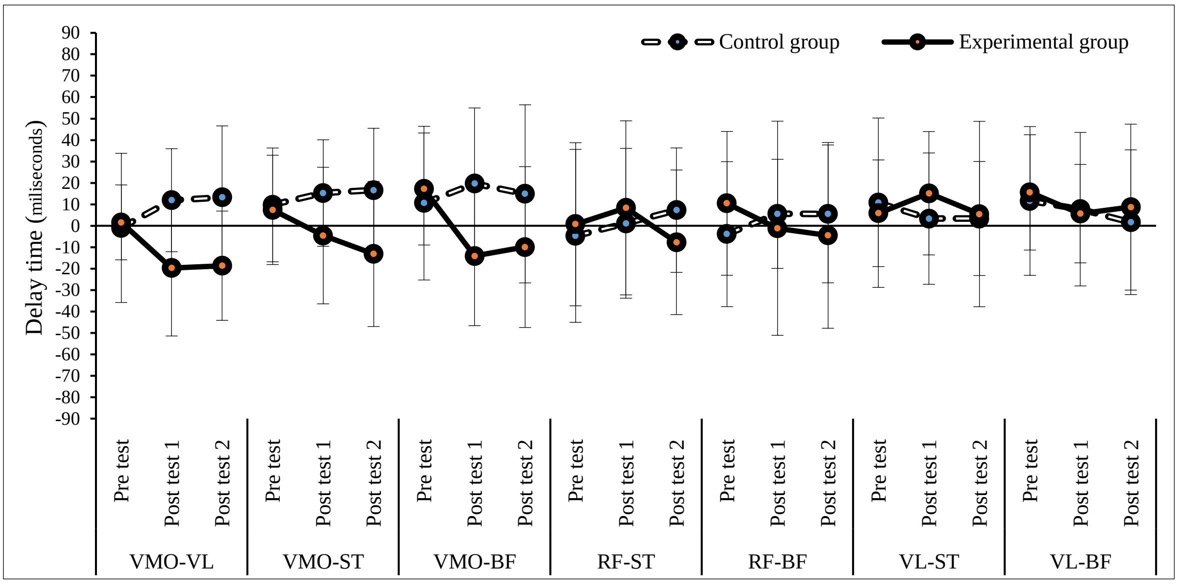

Figure 2. Peer agonist-antagonist activation onset time (mean ± standard deviation) for the control and experimental groups at three conditions (pre-test, post-test 1, and post-test 2)

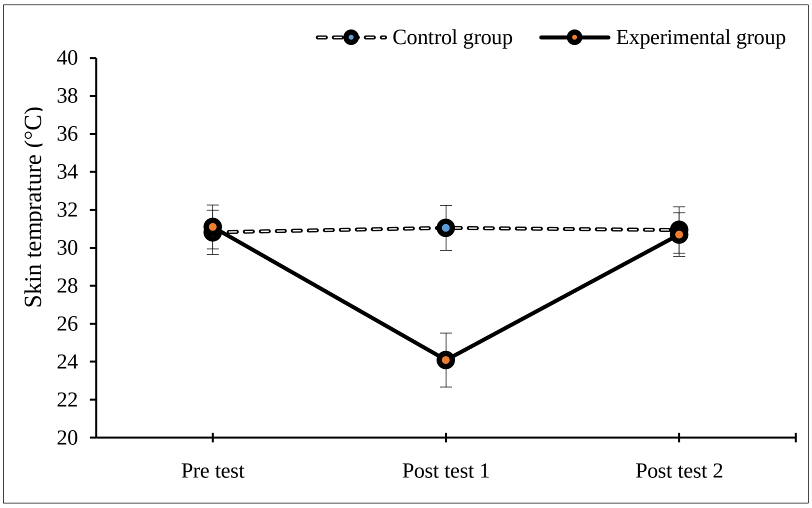

Figure 3. Skin temperature (mean ± standard deviation) for the control and experimental groups at three conditions: pre-test, post-test 1, and post-test 2

4. Discussion

The aim of present study was to investigate the effect of short-time CS application on timing and activation pattern of the knee dominant leg muscles during one-leg landing. The EMG signal is a common measure reflecting the neuromuscular control strategies during dynamic tasks. The results showed that in the pre-activation phase, all muscles in the experimental group tended to decrease their activity level immediately after the CS application. These findings are in accordance with Bleakley et al. (2012) who showed that 20 min cold-pack on the knee joint tended to decrease the average EMG activity of muscles in the pre-activation phase immediately and 20 min after treatment during a plyometric exercise [2]. However, our results showed that in this phase and from the first post-test to the second post-test, the VMO and MG tended to increase and the BF and ST tended to continue decreasing their activity level in the experimental group in contrast with the control group, which is in contrast with the study of Bleakley et al. (2012).

During jumping or falling, the lower extremity muscles are activated before landing to absorb the impact shock and control stability of the knee joint dynamically [29], which the intensity and timing of this pre-landing activity are scaled to the expected impact [23].

de Britto et al. (2014) stated that muscle pre-activation may be more effective in preventing excessive forces within the ACL than muscle recruitment after landing initiates [20].

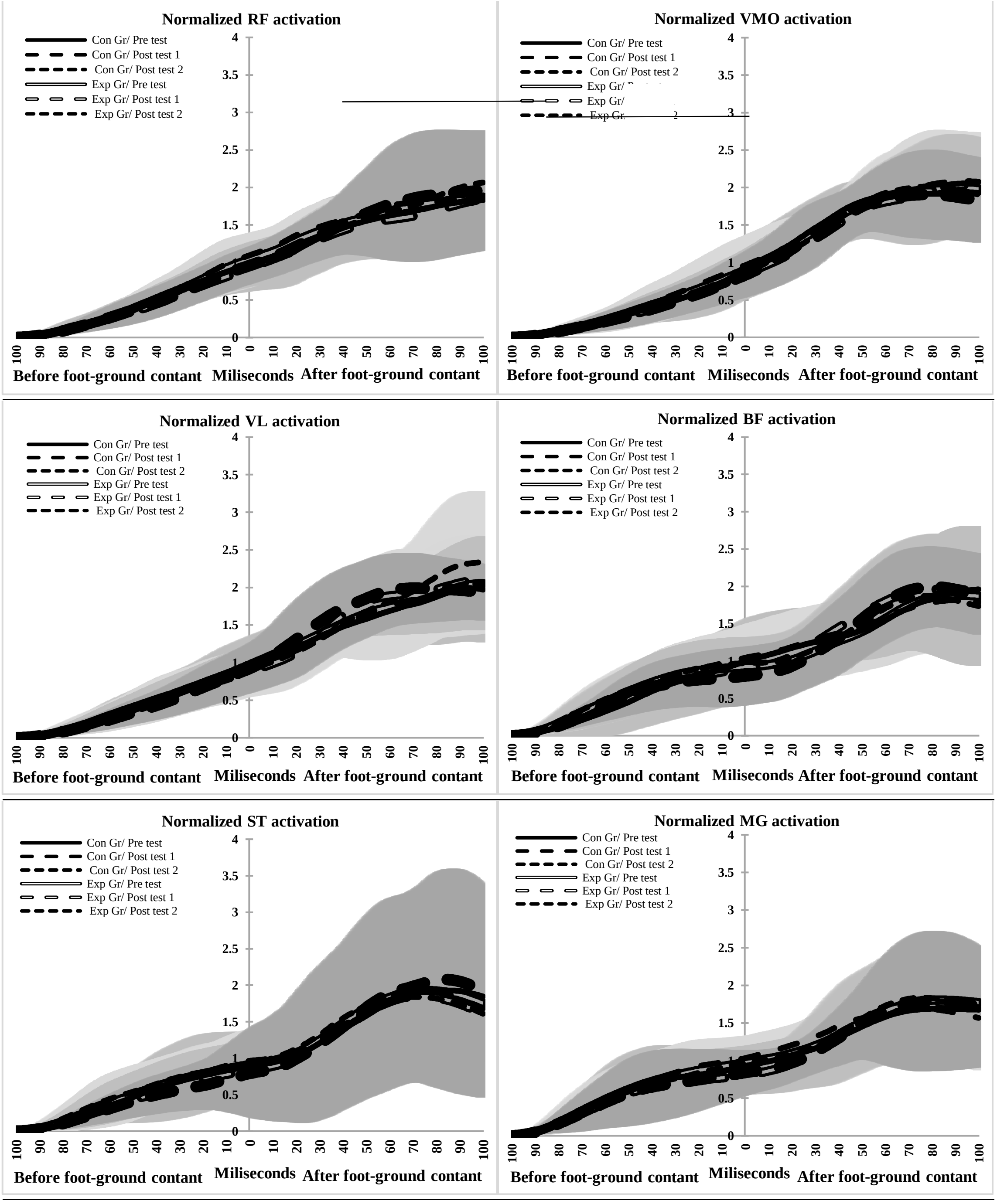

Figure 4. Normalized VMO, RF, VL, ST, BF, and MG muscle activation pattern during landing (between ± 100ms of foot-ground contact) for the control and experimental groups at three conditions: pre-test, post-test 1, and post-test 2 (Con stands for control group and Exp stand for experimental group)

The activation of the muscles prior to the landing is pre-programmed (learned through previous experience) and dispatched from higher centers in the nervous system [2, 24]. To rationalize our finding, the mechanism of decrease in muscle activity during this phase and following the CS application may be related to an altered joint proprioception [2, 24]. Proprioceptive input has been suggested to contribute to the pre-landing muscle responses associated with drop-landing [24].

Furthermore, Surenkok et al. (2008) showed the negative effects of local cold application on the knee joint position sense [25]. Hence, it seems that decrease in proprioception sense, induced by joint cooling or other interventions, could modify the central program and thus a decrease of the pre-landing muscle activity. In this area, the temperature change resulted from cold application is crucial [25]. Although our results showed statistically significant decrease temperature in the experimental group after the CS application, this decrease (to about 22°C immediately after and no regardful change in temperature after 20 min of removing the CS application compared with before the CS application) could not sufficient to significantly change the level of muscle activity.

In addition, the result of present study showed no significant difference in the activation level of the muscles during the eccentric phase. Although some previous studies showed no decrease in muscle activation following cold application [10, 27], some of them showed controversial results [2, 8].

Shimose et al. (2014) showed that EMG activity of the VM, RF and VL increased significantly or tended to increase with skin cooling [27]. Also, Pietrosimone and Ingersoll (2009) investigated the effect of 20 min focal knee joint cooling during a knee extension maximal voluntary isometric contraction (MVIC) and found an increase in the VL muscle activity [10].

In contrast, Bleakley et al. (2012) showed that cryotherapy decreases activity of the lower extremity muscles [2]. These controversial results may be due to the heterogeneity relating to cooling time/ dosage, body part and outcome measures. In addition, because EMG profiles can be influenced by the low-pass filter or other EMG signal processing steps [16], caution must be taken when comparing results from studies that used different cut-off frequencies and data processing methods. In addition, it is noted that cooling protocol used in the current study was different from their protocol in time and cooling method.

Furthermore, the results of the current study showed no significant difference in normalized peak activation, time to peak activation and activation pattern of the muscles among three conditions. Also, a non-significant delay was observed in activation onset of the VMO, VL, VMO-BF, and VL-BF muscles following the CS application. In this area, the neuromuscular characteristics may explain the discrepancy of some injuries (like ACL rupture) [26].

Wikstrom et al. (2008) showed that successful jump landing trials had earlier activation times and higher preparatory and reactive EMG amplitudes; thus, the neuromuscular control differences between successful and failed trials because of earlier muscle onset and greater amplitude [15]. According to their suggestion, delay in onset activation of muscles prior to foot-ground contact and decrease in the pre-activation of muscles may predispose individuals to failed landing. However, it has been accepted that the clinical relevance of the effect of cold application on the muscle activity strongly depends on type and intensity of the movement task; the largest efficacy is for endurance exercise, whereas the effect on sprint and intermittent sprint performance is considerably smaller [2, 25, 27]. Generally, all previous studies involving reflexive and/or high intensity tasks (like plyometric exercises) revealed decreases in motor performance, and none of them found no or even positive effects of cold application on a plyometric task [2], which is because of an accurately neuromuscular control of task, where the faster task is completed, the more accurate control needs to be implemented.

However, all of these studies investigated long-time cold application (more than 5 min), which is not practical in sports field and may have different outcomes (like more decrease in temperature). During sport, very brief time of cooling (<1 min) are sometimes used during a break in play, where the rationale is to provide a counterirritant for pain, rather than to induce large/ deep temperature reductions [1]. However, future studies are needed to assess other outcomes of short-time CS application on human activity as well as EMG activation level and its variables.

5. Conclusions

It seems that short-time CS application, which is so practical after many injuries for returning athletes to sport environment, may not predispose individuals to risk of re-injury or failed landing mechanism. This finding is in contrast with numerous studies showed negative effects of cold application in motor performance during a plyometric task. However, all of these studies investigated long-time cold application (more than 5 min), which is not practical in sports field and may have different outcomes. Future studies need to assess other effects of the CS application on sport performance tasks and risk of re-injury for real sport environments.

Limitations

Although SENIAM guidelines were used, some movements of the muscle under the skin are expected. However, it is unlikely that electrode placement would reach any variability due to movement, which occurs in a random manner across all participants and therefore hardly affect the results.

Conflict of interest

The authors declared no conflicts of interest.

Authors' contributions

All authors contributed to the original idea, study design.

Ethical considerations

The authors have completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc. The *study* was conducted in accordance with the Declaration of Helsinki and all subjects provided the written consent form before participation.

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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