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Effects of cold water immersion and protein intake combined recovery after eccentric exercise on exercise performance in elite soccer players

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The purpose of this study is to analyze the effects of the combined recovery method of cold water immersion (CWI) and protein supplement intake after eccentric exercise that causes muscle fatigue in elite soccer players. Eleven semiprofessional soccer players participated in this study. Participants were divided into CWI group, combined protein and CWI group (PCWI), and passive resting group (CON). The participants completed the eccentric exercise for one hour and performed one of three recovery methods. The muscle strength of the quadriceps and hamstring muscles significantly decreased at 48-hr postexercise compared to before exercise in all recovery groups ($P<0.05$), with no significantly different between the recovery groups. The time required to sprint 40 m was significantly longer in all groups at 24 hr and 48 hr after exercise than before exercise ($P<0.05$). The vertical jump height was significantly decreased at 48 hr after exercise compared to before exercise in the CON and CWI groups ($P<0.05$). The muscle soreness values were higher at 6 hr, 24 hr, and 48 hr after exercise than before exercise in all groups ($P<0.001$). The perceived recovery quality was reduced after exercise in the PCWI ($P<0.01$) and CON groups ($P<0.001$) compared to before exercise; it was unchanged in the CWI group. The recovery quality decreased at 6 hr, 24 hr, and 48 hr after exercise in all recovery groups ($P<0.01$). In conclusion, the combined recovery method was less effective than CWI alone for the recovery of exercise performance.

Keywords: Soccer, Eccentric exercise, Recovery, Exercise performance

INTRODUCTION

Soccer is an intermittent exercise in which various types of movements such as jogging and sprinting are performed for more than 90 min. The sport requires intensive powerful activities while performing skills to score a goal (Dolci et al., 2020). Repeatedly performing eccentric contraction movements caused by high-intensity exercises, jumps, momentary decelerations, and direction changes during the game result in muscle fatigue and damage (Mohr et al., 2005; Nédélec et al., 2013). High-level athletes, such as professional soccer players, have to play several matches each season when participating in professional soccer leagues and national team matches. Soccer players endure a high level of physical and psychological stress due to the high number of matches per season, and athletes must recover quickly to improve and/or maintain their performance in subsequent matches.

Unfamiliar high-intensity eccentric exercise leads to exercise-induced muscle damage (Connolly et al., 2003). Soccer players must perform jumping, turning, and shooting movements that consist of eccentric muscle contraction motions; therefore, there is a high risk of muscle injury during a match. High-intensity, repetitive, eccentric contractions result in the destruction of intramuscular structures as muscles lengthen, causing muscle inflammation, pain, and reduced functioning (Byrne et al., 2004). The symptoms of exercise-induced muscle damage include the extravasation of creatine kinase in the blood, increased neutrophilic leukocytosis, decreased joint range of motion, loss of muscle strength, pain, stiffness, swelling, and delayed onset muscle soreness (Hough, 1902).

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Delayed onset muscle soreness increases after 24 hr and peaks from 24 hr to 72 hr, disappears 7 days after exercise, and it affects daily life and reduces exercise performance by causing reduction in muscle functions during sport activities (Cleak and Eston, 1992).

Various recovery methods used to relieve the fatigue accumulated by matches and training in elite soccer players have been reported. Proper nutrition, hydration, and sleep must be provided to recover from high-intensity exercise-induced fatigue. Cold water immersion (CWI) was the most used method by professional soccer players to recover from fatigue after training or matches, followed by static recovery, massage, stretching, compression garments, and electrical stimulation (Nédélec et al., 2013). CWI is an effective recovery method that minimizes muscle damage and fatigue after exercise (Leeder et al., 2012; Sánchez-Ureña et al., 2015). However, the effect of postexercise CWI on exercise performance in subsequent exercise sessions is controversial (Ascensão et al., 2011; Versey et al., 2013). In addition, the use of CWI after exercise can enhance exercise-induced muscle adaptation during rest periods. CWI at 8°C for 10 min after high-intensity exercise increases the expression of peroxisome proliferator activated-receptor γ coactivator-1α and vascular endothelial growth factor mRNA, which play key roles in mitochondrial biogenesis and angiogenesis (Joo et al., 2016).

Several elite soccer players take supplementations, defined as preparations made to provide nutrients in greater amounts than food-dependent sources, for recovery after exercise. The main component of muscle is protein, and muscle tissue accounts for 60%–70% of the total protein in the human body (Lemon, 1987). Therefore, athletes consume more protein than the general population to achieve muscle hypertrophy and maintain muscle mass. The consumption of protein supplements improved muscle strength and skeletal muscle hypertrophy and prevented muscle mass loss in previous studies (Hoffma et al., 2006; Tipton and Wolfe, 2004). Periodic protein supplementation during long training periods improved exercise performance in athletes (Lemon and Proctor, 1991). Protein supplement intake after eccentric contraction exercises resulted in a faster recovery from exercise-induced muscle fatigue and damage than no supplementation (Etheridge et al., 2008).

Several previous studies have reported that CWI and protein intake are helpful for the recovery of exercise-induced muscle fatigue and damage. However, no study has reported the effects of the combination of protein intake and CWI. This is the first study to examine the effects of the combined recovery method of CWI and protein supplement intake after eccentric exercise that causes muscle fatigue in elite soccer players.

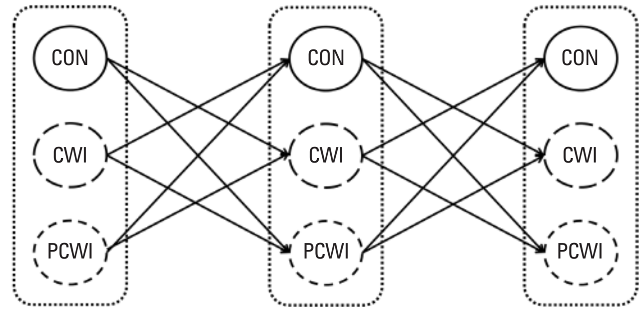

Fig. 1. Schematic illustration of grouping. CON, control; CWI, cold water immersion; PCWI, combined protein and cold water immersion.

MATERIALS AND METHODS

Participants

Eleven semiprofessional (K4 league) soccer players (age, 25.4±5.2 years; height, 182.3±2.0 cm; weight, 76.3±1.5 kg: body mass index, 23.0±0.05 kg/m^2) were included in this study. The players provided written informed consent after the study was explained to them. Players with limited mobility due to serious injuries such as muscle/ligament ruptures, fractures, or dislocations and those who consumed protein supplements during the 3 months prior to the study were excluded. The Ethics Committee of Kangwon National University approved this study (KWNUIRB-2021-09-003-003), which was conducted in accordance with the Declaration of Helsinki.

Study design

The participants were assigned to the recovery method groups using sealed envelopes containing the group assignments. Each participant underwent three experiments; therefore, each participant was assigned to each recovery method throughout the course of the study (Fig. 1). There was a washout period of at least 2 weeks between each treatment. Participants in the control (CON) group sat passively on a chair for 10 min at room temperature (19°C±0.6°C) after one hour of centrifugal exercise. Participants in the CWI group sat with their lower body immersed in 8°C water for 10 min after exercise. Participants in the combined protein and CWI (PCWI) group ingested 1.8 g/kg of powdered protein (Syntha 6 Isolate, BSN, Downers Grove, IL, USA) immediately after exercise (Eliot et al., 2008) and sat with their lower body immersed in 8°C water for 10 min.

Study procedures

The experimental procedures are shown in Fig. 2. Each treatment

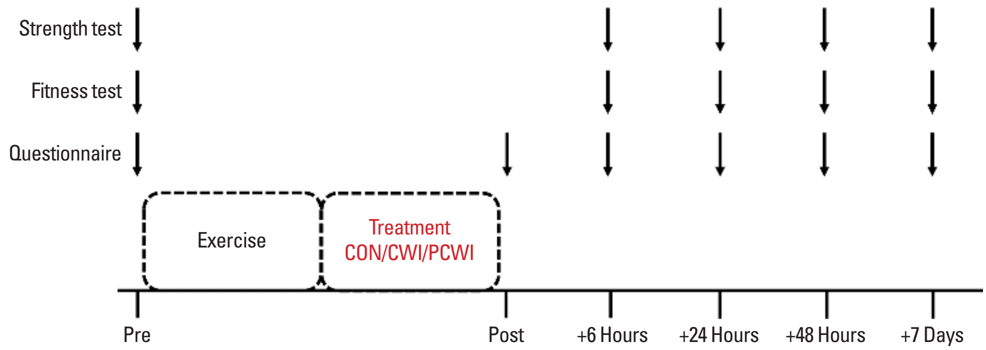

Fig. 2. Schematic illustration of the experimental design. CON, control; CWI, cold water immersion; PCWI, combined protein and cold water immersion.

Fig. 3. Measurement of isometric muscle strength: quadriceps (A), hamstring (B).

experiment was conducted for 7 days, and exercise capacity was measured on an artificial turf. The participants rested for 30 min after arriving at the study site before their body composition (Inbody 370, InBody Co. Ltd., Seoul, Korea), muscle strength (Inbody IB-LS, InBody Co. Ltd.), sprinting, and vertical jump were measured. Questionnaires regarding muscle pain and recovery were also completed. The participants then performed eccentric exercises for 1 hr. After the eccentric exercise, the participants performed their assigned recovery method. The participants' exercise abilities were measured at 6 hr, 24 hr, 48 hr, and 7 days postexercise. The questionnaires were also completed at the same time points. The experiment sessions were separated by at least 2 weeks to exclude the influence of the previous recovery method. The participants were permitted to perform general team training during the experiment period.

Eccentric exercise

The patients were instructed regarding the exercise methods and familiarized with the exercise motions prior to the study period. Eccentric exercises performed in this study included single leg bounding, split squat jumps, jump squats, and lateral cone jump movements. Each participant performed 5 sets of 15 repetitions of each exercise. Each set was completed with no breaks, though a 2-min break was permitted between sets. The exercises were performed for 1 hr, and dynamic stretching, running, walking (warm-up), and static stretching (cool-down) were each conducted for 10 min. All exercises were performed under the supervision of the research team.

Assessment of physical fitness

The participants' left and right femoral extension and flexion forces were measured to represent isometric lower extremity muscle strength (Fig. 3). The participants sat on a chair with the under bar adjusted based on the length of their legs. The participants performed voluntary maximal strength tests when signaled by a machine. The researcher verbally encouraged the participants during

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Table 1. Isometric lower extremity muscle strength

Variable	Group	Time				
		Pre	6 Hours	24 Hours	48 Hours	7 Days
QR	PCWI	69.4±9.7	66.6±7.1	61.0±8.6	56.9±8.2**	68.4±10.2
	CWI	71.5±8.6	70.1±7.0	62.9±12.6	61.2±9.4**	70.7±12.3
	CON	68.0±11.9	69.2±13.3	61.5±12.8	59.3±11.8***	72.1±14.1
QL	PCWI	66.3±12.5	63.1±9.3	59.0±8.9	54.7±8.7*	64.5±11.5
	CWI	72.4±9.5	66.4±10.5	64.1±12.0	60.9±9.7***	74.0±10.0
	CON	69.6±11.7	71.0±13.1	65.5±12.4	60.0±12.3*	75.8±13.1
HR	PCWI	39.1±8.6	39.0±7.2	34.6±5.2	30.6±5.1*	40.8±8.8
	CWI	41.4±7.1	38.7±7.0	35.3±7.8*	32.5±7.7***	40.6±7.9
	CON	41.6±8.7	42.7±8.9	37.6±8.0	33.0±7.2***	43.6±9.6
HL	PCWI	35.1±7.0	33.6±6.9	31.0±5.8*	27.8±5.5***	36.0±7.9
	CWI	38.2±4.4	37.5±4.4	33.6±7.2	31.0±4.6**	38.5±6.8
	CON	38.1±6.9	37.4±6.6	33.8±5.9*	29.7±5.9**	41.0±5.1

Values are presented as mean±standard deviation (n=11).
QR, quadriceps right; QL, quadriceps left; HR, hamstring right; HL, hamstring left; PCWI, combined protein and cold water immersion; CWI, cold water immersion; CON, control.
*P<0.05, **P<0.01, ***P<0.001 that significant difference from pre-exercise.

the tests. Each participant also performed a sprint test. Two 40-m sprints separated by a 2-min rest period were performed using the free-start method. Photocell gates (Microgate, Bolzano, Italy) were used to time the sprints, which were run at an outdoor playground with the participants wearing their own soccer shoes. The fastest times were recorded. The participants' vertical jump heights were also measured. Each participant performed two vertical jumps wearing a band-type measuring device on their waists (Sports Imports, Hilliard, OH, USA). The jumps were performed on the soccer field with the participants wearing their own soccer shoes. The vertical jump was performed using only the strength of the lower extremities; no recoil of the arms was permitted. A 2-min rest period was permitted between the jumps. The highest jump was recorded.

Questionnaires

Subjective muscle soreness was assessed using a visual analog scale that included "no pain" at one end of a 100-mm line and "extremely sore" at the other end (Zhang et al., 2000). The recovery level was measured using the total quality recovery questionnaire, as described in a previous study (Kenttä and Hassmén 1998). The total quality recovery consists of scores ranging from 6 to 20 points, with higher scores indicating better recovery.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics ver. 26.0 (IBM Co., Armonk, NY, USA). All values are expressed as mean±standard deviation. A two-way repeated-measures analysis of variance (ANOVA) was used to analyze the differences between the recovery groups and time periods. A one-way repeated-measures ANOVA was used to identify differences between the time periods within the recovery groups. Statistical significance was set at $P<0.05$.

RESULTS

Muscle strength

The muscle strength of the quadriceps and hamstring muscles were not significantly different 6 hr after exercise compared to before exercise (Table 1). However, the muscle strength significantly decreased at 48-hr postexercise in all recovery groups ($P<0.05$). The muscle strength returned to pre-exercise levels by 7 days postexercise. The muscle strength was not significantly different between the recovery groups at any time point (Table 1).

Sprint

The time required to sprint 40 m increased in the PCWI group 6 hr after exercise. The time was significantly longer in all groups at 24 hr and 48 hr after exercise than before exercise ($P<0.05$). The 7 days postexercise sprint times were not significantly different than those recorded prior to exercise in any recovery group (Fig. 4).

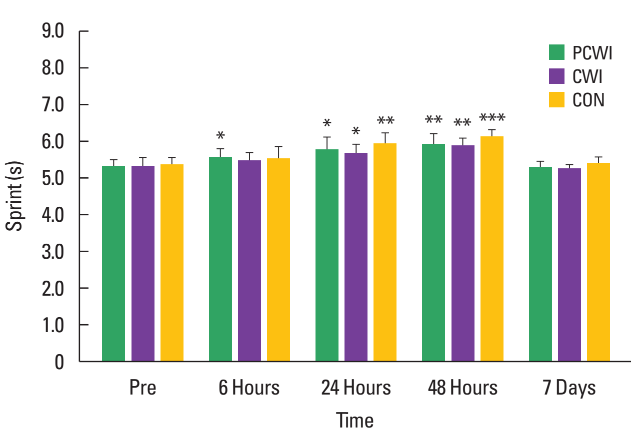

Fig. 4. The time required to sprint 40 m. PCWI, combined protein and cold water immersion; CWI, cold water immersion; CON, control. *$P<0.05$, **$P<0.01$, ***$P<0.001$ that significant difference from pre-exercise.

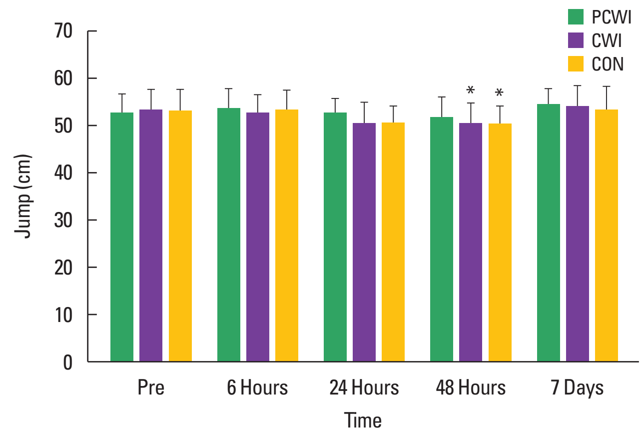

Fig. 5. The vertical jump height. PCWI, combined protein and cold water immersion; CWI, cold water immersion; CON, control. *$P<0.05$ that significant difference from pre-exercise.

Table 2. Perceived muscle soreness and recovery quality

Variable	Group	Time					
		Pre	Post	6 Hours	24 Hours	48 Hours	7 Days
Muscle soreness	PCWI	0.9±.02	4.0±1.3***	5.1±0.9***	6.2±1.4***	8.7±1.2***	1.1±0.4
	CWI	0.9±0.4	3.2±1.1***	4.5±0.7***	5.9±0.9***	8.4±1.0***	0.9±0.3
	CON	0.9±0.3	3.3±1.3***	4.0±1.2***	6.4±0.9***	8.4±1.0***	1.1±0.7
Recovery quality	PCWI	17.6±1.5	13.6±1.9**	11.2±2.2***	10.3±2.4***	9.5±1.2***	18.2±1.4
	CWI	17.9±2.2	15.0±1.8	12.6±1.8**	10.3±2.1***	9.5±1.7***	17.6±1.4
	CON	18.7±0.9	13.1±1.6***	10.9±1.9***	9.9±1.1***	8.7±1.3***	18.4±1.4

Values are presented as mean±standard deviation (n=11).
PCWI, combined protein and cold water immersion; CWI, cold water immersion; CON, control.
**$P<0.01$, ***$P<0.001$ that significant difference from pre-exercise.

Vertical jump

The vertical jump height of the participants in the PCWI group was not changed during the study period. The vertical jump height was significantly decreased at 48 hr after exercise compared to before exercise in the CON and CWI groups ($P<0.05$), though the vertical jump height had recovered by 7 days after exercise in both groups (Fig. 5).

Muscle soreness and recovery quality

The perceived muscle soreness was not significantly different between the groups throughout the study period. However, the muscle soreness values were higher at immediately after exercise, 6 hr, 24 hr, and 48 hr after exercise than before exercise in all groups ($P<0.001$). The perceived recovery quality was reduced after exercise in the PCWI ($P<0.01$) and CON ($P<0.001$) compared to before exercise; it was unchanged in the CWI group. The recovery quality decreased at 6 hr, 24 hr, and 48 hr after exercise in all recovery groups ($P<0.01$) (Table 2).

DISCUSSION

This study analyzes the effects of the combined recovery method of protein intake and CWI on recovery and exercise performance after eccentric exercises in elite soccer players. This is the first study to apply the complex recovery methods after eccentric exercises to elite soccer players. The combined recovery method was less effective than CWI alone for the recovery of exercise performance in this study.

Measuring muscle strength after exercise is one of the most accurate ways to measure muscle damage or fatigue (Morton et al., 2005). In this study, the muscle strength at 6 hr after exercise was not significantly less than that before exercise. As muscle strength loss due to exercise-induced muscle damage typically occurs immediately or several hours after exercise (Peake et al., 2017), the eccentric exercises used in this study did not cause muscle damage in the elite soccer players, even when performed for 60 min. It is possible that muscle damage did not occur because the participants

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in this study were elite soccer players who were familiar with eccentric exercise. In a previous study, muscle damage was observed in the general population after less intense eccentric exercises were performed (Abaïdia et al., 2017). In addition, as elite athletes consistently perform eccentric exercises during training, they may have developed resistance to eccentric exercise-induced muscle damage via the "repeated bout effect" (McHugh, 2003). In contrast, muscle strength was measured by voluntary maximal contraction without electrical stimulation in this study. Voluntary maximal contraction should be performed with electrical stimulation to accurately measure muscle strength (Morton et al., 2005).

In this study, muscle strength was not different between the recovery groups throughout the study period. However, muscle strength decreased in all of the groups 48 hr after exercise. As the muscle strength was not significantly decreased 6 hr or 24 hr after exercise, the decrease in muscle strength at 48 hr observed in all three groups may have been due to factors other than the eccentric exercise. Muscle fatigue is measured as a decrease in motor function or muscle strength, a change in electromyogram activity, or an abnormality in contractile function. The maximum muscle force gradually decreases after continuous physical activity (Enoka and Duchateau, 2008). The decrease in muscle strength at 48 hr after exercise observed in this study may be due to the accumulation of muscle fatigue during team training sessions. The eccentric exercises were often performed on a Monday, after the participants had a weekend break from team training. In general, elite soccer teams tend to complete high-intensity training once a week to maintain the athletes' physical fitness during the season, according to the periodization theory (Thorpe et al., 2015). The decrease in muscle strength 48 hr after exercise may be due to the fact that the elite soccer players who participated in this study also participated in general team training. Although the team training intensity was low during the experiment period, the training intensity could not be completely controlled due to the nature of the semiprofessional soccer team pursuing success in their matches.

Most of the movements performed by soccer players are aerobic, though movements at critical moments, such as counterattacks or scoring chances in front of the goal, are anaerobic (Dolci et al., 2020). Therefore, it is important for soccer players to perform anaerobic exercises that allow them to move explosively over short distances (Nédélec et al., 2013). Sprinting and jumping are necessary skills in soccer, and several studies have measured the anaerobic capacity of athletes via sprinting and jumping tests (Castagna and Castellini, 2013; Haugen et al., 2014). In this study, the sprint time was not significantly different between the recovery groups at any time point. The sprint time decreased at 24 hr and 48 hr after exercise in all recovery groups. These results are consistent with those of previous study that reported that there was no difference in soccer players' springing ability between baseline and 48 hr of high-intensity exercise in CWI (Bouzid et al., 2018). Therefore, no difference in sprint ability was observed based on the recovery treatments, as sprinting is an exercise that is familiar to soccer players as it is frequently performed for a short period of time during matches or training. A previous study regarding rugby players reported similar results. After 40 min of rugby-specific exercises, the explosive movements of athletes who recovered via CWI were not different than the explosive movements of those in the CON group (Garcia et al., 2016).

The vertical jump height was not significantly different between the recovery groups in this study, which is consistent with the result of previous studies. Jump performance was unaffected after acute high-intensity postexercise CWI compared to CON, although the type of exercise was different between the studies (Bailey et al., 2007; White et al., 2014). CWI applied after each four matches for 4 days showed no differences with thermoneutral water immersion (Rowsell et al., 2009). A previous study that evaluated jump performance based on the consumption of protein supplementation reported contradicting results regarding postexercise CWI. Protein supplement intake before sleep after an evening professional soccer match was found to be effective in maintaining the jumping performance 36 hr and 60 hr after the match (Abbott et al., 2019). The inconsistent results between the studies may be due to differences in protein intake methods (presleep ingestion vs. postexercise ingestion) and the levels of the participating soccer players (professional vs. semiprofessional). Exercise performance according to various consumption methods of protein supplements must be investigated in elite soccer players in future studies.

Questionnaires are often used to evaluate the degree of perceived muscle injury and recovery after soccer matches and training (Osiecki et al., 2015; Rey et al., 2012). The perceived muscle soreness was higher in all recovery groups immediately after exercise to 48 hr after exercise when compared to perceived muscle soreness prior to exercise, and the perceived muscle soreness was not significantly different between the recovery groups. These results are consistent with those of previous studies regarding the effects of CWI and protein intake (Abbott et al., 2019; Burnley et al., 2010). The perceived recovery significantly decreased immediately after exercise in the PCWI and CON groups, though no significant change was noted in the CWI group, indicating that postexercise CWI is helpful for the recovery from fatigue. The perceived recovery decreased in all groups at 6 hr, 24 hr, and 48 hr after exercise with no differences between the recovery groups. However, the exercise intensity of team training sessions negatively affected the perceived recovery.

This study is not without limitations. First, the intensity of team training sessions could not be controlled during the study period. Due to the nature of the study, the players should be prohibited from participating in team training during the study period. However, this could not be controlled as the participants in this study play soccer for a living. Second, the effects of the acute recovery methods after exercise were analyzed in this study. To effectively investigate the effects of the postexercise recovery methods, a longer training period is necessary. Therefore, future studies should include longer training periods.

In conclusion, the effects of acute recovery methods after eccentric exercise on exercise performance were similar between the recovery groups. However, the perceived recovery was higher in CWI compared to the other recovery methods at immediately postexercise. Based on these results, CWI is recommended as recovery method to enhance the rate of recovery following training sessions in elite soccer players. The future studies are needed to analyze the effects of the combined recovery method of protein intake and CWI during prolong period of training.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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