# Cold-water immersion for reducing IL-6 and CK in female footballers

*Inmersión en agua fría para reducir IL-6 y CK en futbolistas*

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## Abstract

Introduction: Soccer requires athletes to possess strength and endurance, involving intense physical activities that may lead to fatigue and injury. Such conditions stimulate the release of inflammatory markers, including interleukin-6 (IL-6) and creatine kinase (CK), which can impair recovery and athletic performance. Cold-water immersion (CWI) is a commonly used recovery strategy to mitigate these effects.
Objective: This study aimed to examine the impact of CWI on IL-6 and CK levels, as well as vertical jump performance in female soccer players following a match.
Methodology: A quasi-experimental two-group pretest-posttest design was employed, involving 20 purposively sampled participants. Blood samples were collected at four time points: pre-match, post-match, 24 hours post-match, and 48 hours post-match. Vertical jump performance was used to assess physical recovery.
Results: Results showed no significant difference in vertical jump performance between the groups at 24 hours post-match ($p > 0.05$), but a significant difference was found at 48 hours post-match ($p < 0.05$), favoring the CWI group. Significant differences in IL-6 and CK levels were observed between the groups at both 24 and 48 hours post-match ($p < 0.05$).
Discussion: CWI effectively reduced inflammation and muscle damage, as reflected in IL-6 and CK values, and promoted faster neuromuscular recovery. This finding suggests that CWI supports biological repair and functional recovery within 48 hours post-match.
Conclusions: Cold-water immersion significantly improved physiological recovery in female soccer players, as indicated by lower IL-6 and CK levels and improved vertical jump performance over time compared to the control group.

## Keywords

Cold-water immersion; creatine kinase; interleukin-6; vertical jump; female soccer player.

## Resumen

Introducción: El fútbol requiere que los atletas posean fuerza y resistencia, lo que implica actividades físicas intensas que pueden provocar fatiga y lesiones. Dichas condiciones estimulan la liberación de marcadores inflamatorios, como la interleucina-6 (IL-6) y la creatina quinasa (CK), que pueden perjudicar la recuperación y el rendimiento atlético. La inmersión en agua fría (CWI) es una estrategia de recuperación comúnmente utilizada para mitigar estos efectos.
Objetivo: Este estudio tuvo como objetivo examinar el impacto de la CWI en los niveles de IL-6 y CK, así como en el rendimiento del salto vertical en jugadoras de fútbol después de un partido.
Metodología: Se empleó un diseño pretest-postest cuasiexperimental de dos grupos, con 20 participantes muestreados intencionalmente. Se recogieron muestras de sangre en cuatro puntos temporales: antes del partido, después del partido, 24 horas después del partido y 48 horas después del partido. El rendimiento del salto vertical se utilizó para evaluar la recuperación física.
Resultados: Los resultados no mostraron diferencias significativas en el rendimiento en salto vertical entre los grupos a las 24 horas posteriores al partido ($p > 0{,}05$), pero sí se encontró una diferencia significativa a las 48 horas posteriores al partido ($p < 0{,}05$), a favor del grupo CWI. Se observaron diferencias significativas en los niveles de IL-6 y CK entre los grupos tanto a las 24 como a las 48 horas posteriores al partido ($p < 0{,}05$).
Discusión: La CWI redujo eficazmente la inflamación y el daño muscular, como se refleja en los valores de IL-6 y CK, y promovió una recuperación neuromuscular más rápida. Este hallazgo sugiere que la CWI favorece la reparación biológica y la recuperación funcional en las 48 horas posteriores al partido.
Conclusiones: La inmersión en agua fría mejoró significativamente la recuperación fisiológica en las jugadoras de fútbol, como lo indican los niveles más bajos de IL-6 y CK y un mejor rendimiento del salto vertical a lo largo del tiempo en comparación con el grupo de control.

## Palabras clave

Inmersión en agua fría; creatina quinasa; interleucina-6; salto vertical; jugadora de fútbol.

## Introduction

Soccer is a high-intensity sport that requires a combination of aerobic endurance, strength, agility, and coordination. It is widely played by individuals of all genders and age groups. In Indonesia, female participation in soccer has shown significant growth, marked by increasing numbers of regional and national competitions organized by official institutions (Binat & Kartiko, 2021; Doewes & Nuryadin, 2022). This growing interest highlights the need to focus not only on training strategies but also on post-match recovery interventions tailored to female athletes.

During a typical soccer match, players engage in a range of physical activities such as sprinting, jumping, and abrupt directional changes, covering approximately 10 kilometers per match. These movements, often combined with physical contact, contribute to high musculoskeletal load and increase the risk of fatigue and soft tissue injuries (Almeida et al., 2013). The accumulation of fatigue without adequate recovery has been linked to decreased performance, heightened injury risk, and impaired decision-making on the field (Teoldo et al., 2024)

Recovery is essential to restore physiological and psychological balance after matches. However, with tight match schedules and intensive training loads, optimal recovery time is often limited. Inadequate recovery can result in delayed-onset muscle soreness (DOMS), prolonged fatigue, reduced motivation, and eventually burnout (Marangoni et al., 2023). Therefore, recovery management becomes a crucial aspect of performance maintenance and injury prevention in soccer.

Several recovery strategies have been adopted by athletes, including active recovery, sports massage, compression therapy, and hydrotherapy. Among these, cold water immersion (CWI) has become one of the most widely practiced methods due to its simplicity and effectiveness. CWI typically involves immersing the lower body in water at 10–15°C for 10–20 minutes. This practice is known to promote vasoconstriction, reduce local inflammation, and alleviate muscle soreness after high-intensity exercise (Versey et al., 2013; White et al., 2014)

From a physiological perspective, cold water immersion mitigates inflammation and muscle damage by reducing blood flow and suppressing pro-inflammatory cytokine release. These responses assist in preserving muscle integrity and accelerating the repair process. Additionally, CWI may influence the nervous system by reducing nerve conduction velocity and increasing the release of analgesic neurotransmitters such as norepinephrine, thereby contributing to reduced pain perception (Wang & Ni, 2021; Zhang & Dhalla, 2024).

To objectively assess recovery, this study focuses on two key biomarkers: interleukin-6 (IL-6) and creatine kinase (CK). IL-6 is a cytokine rapidly released in response to tissue damage and plays a dual role in inflammation and muscle regeneration (Tanaka et al., 2014). CK, on the other hand, is an enzyme that reflects the degree of muscle membrane disruption and is frequently used as a biomarker of exercise-induced muscle damage (Dewangga et al., 2021). Both IL-6 and CK are sensitive indicators of physical stress and recovery, making them suitable outcome measures in this study (Dewangga et al., 2024).

Although the use of CWI is widespread among athletes, evidence regarding its effectiveness in female soccer players, particularly in relation to IL-6, CK, and performance recovery, remains limited. This study aims to investigate the effect of cold water immersion on post-match recovery in female soccer athletes, by measuring changes in vertical jump performance and serum levels of IL-6 and CK. The findings are expected to inform evidence-based practices for optimizing recovery strategies in women's soccer.

## Method

### *Study Design and Subjects*

This study employed a quasi-experimental two-group pretest-posttest design without random assignment. The design was selected due to logistical and ethical constraints in conducting full randomization, as all participants were members of a single soccer club actively preparing for competition. Group allocation was performed purposively, based on inclusion and exclusion criteria and player characteristics.

A total of 20 female soccer players from the Surakarta Women's Football Academy, all of whom participated in the 2023 Java-Bali Trisula Cup, were recruited. Each group consisted of 10 participants. Due to limited access and resources, no formal power analysis was conducted, and the study was categorized as a pilot study. Findings are intended to inform larger future trials.

### *Inclusion and Exclusion Criteria*

Inclusion criteria: (1) currently competing in the Trisula Cup, (2) physically and mentally healthy, (3) willing to participate in all research procedures, (4) completed one official match within the last 24 hours, (5) consented to blood sampling.

Exclusion criteria: (1) menstruating at the time of blood draw, (2) uncooperative, (3) absent during follow-up evaluations.

### *Intervention Procedure*

Participants were assigned to one of two groups. The active comparator group received thermoneutral water immersion (TWI) at 26°C for 15 minutes post-match. The intervention group received cold water immersion (CWI) at 10°C for 15 minutes. Both interventions were applied immediately after the soccer match, with immersion up to the waist while seated. Participants were instructed to breathe calmly throughout the procedure.

*Vertical Jump Test*

To assess lower-body power, participants performed a vertical jump test at four time points: ~30 minutes before the match (pre), ~15 minutes after the match (post), ~24 hours post-match, and ~48 hours post-match. Participants stood side-on to a wall, marked their standing reach, and then jumped vertically to touch the highest possible point on the wall. Vertical jump height was calculated as the difference between standing reach and jump reach.

*Blood Sampling and Biomarker Analysis*

Blood samples were collected from the brachial vein at four specified time points:

1. Pre-match (~30 minutes before kickoff),
2. Post-match (~15 minutes after match end),
3. 24 hours post-match (~24 hours after kickoff),
4. 48 hours post-match (~48 hours after kickoff).

Samples were stored in vacutainer tubes, kept at -20ºC in an ice box, and centrifuged at 3000 rpm for 30 minutes to isolate serum. Levels of interleukin-6 (IL-6) and creatine kinase (CK) were measured using ELISA at the Clinical Pathology Laboratory of Dr. Sardjito Central General Hospital.

### *Bioethics*

This research was approved by the Health Research Ethics Committee, Faculty of Medicine, Sultan Agung Islamic University, Semarang, Indonesia (Reference Number: 436/X/2023/Bioethics Commission).

### *Statistical Analysis*

All data were analyzed using SPSS version 22. Descriptive statistics were used to summarize participant characteristics and outcome variables, expressed as mean and standard deviation. To assess the effect of the interventions over time and between groups, Repeated Measures ANOVA was employed for the three main outcomes: interleukin-6 (IL-6), creatine kinase (CK), and vertical jump height. Before conducting the ANOVA, key statistical assumptions were verified. Normality of the data distribution was tested using the Kolmogorov–Smirnov test, homogeneity of variance across groups was examined using Levene's test, and sphericity for within-subject factors was evaluated using Mauchly's test. In cases where the assumption of sphericity was violated, the Greenhouse–Geisser correction was applied to adjust degrees of freedom. Bonferroni post-hoc tests were used for multiple comparisons to control for Type I error across time points. Additionally, effect sizes were calculated using partial eta squared ($\eta^2$)

to assess the magnitude of differences, with thresholds of 0.01 considered small, 0.06 moderate, and 0.14 large. Statistical significance was set at $p < 0.05$ for all analyses.

## Results

### *Vertical Jump*

Vertical jump is a test commonly used in sports and physical therapy to measure lower body strength and explosive power (Cormie, Mccaulley, & Mcbride, 2007; Markovic, Dizdar, Jukic, & Cardinale, 2004). To perform this test, first, stand with your feet shoulder-width apart and your arms relaxed at your sides. Then, move by quickly bending your knees and hips in a downward motion, similar to a squat (Bosco, Komi, & Ito, 1981). At the same time, swing your arms back. Without stopping at the bottom, push your body off the ground explosively by straightening your knees, hips, and ankles while swinging your arms forward and upward to gain momentum (Ebben & Petushek, 2010). After jumping, land softly on both feet, bending your knees slightly to absorb the impact (McMahon et al., 2018). It is important to keep your body upright and jump vertically without leaning over (Lees et al., 2004). To track your performance, you can use a device such as a force plate or jumping mat to measure your jump height and power (Hara et al., 2006). Performing multiple jumps with consistent form ensures more accurate results (Moir et al., 2004). Here are the results of this test.

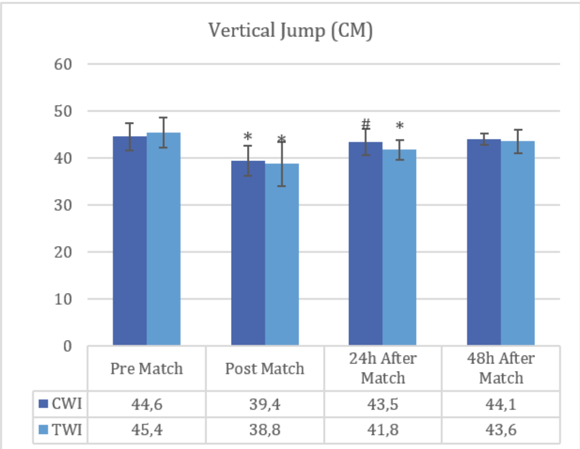
Table 1. Characteristics Sample

| Variable | Mean±SD | Range | 95% CI |
|---|---|---|---|
| Age (years) | 15,9±2,3 | 11,3-20,5 | 14,82 – 16,98 |
| Height (cm) | 162±5,8 | 150,4-173,6 | 159,29 – 164,71 |
| Weight (kg) | 48,9±3,5 | 41,9-55,9 | 47,26 – 50,54 |
| BMI | 21,5±2,35 | 16,8-26,2 | 20,4 – 22,6 |

Table 2. Analysis of vertical Jump between group TWI vs CWI

| | Vertical Jump (cm) | |
|---|---|---|
| | CWI ± SD | TWI ± SD |
| Pre Match | 44,6 ± 2,9 | 45,4 ± 3,2 |
| Post Match | 38,8 ± 3,2* | 38,8 ± 4,7* |
| 24h After Match | 43,5 ± 2,8# | 41,8 ± 2,1* |
| 48h After Match | 44,1 ± 1,2 | 43,6 ± 2,5 |

Figure 1. Result of vertical jump following a one-off soccer match for thermoneutral water immersion and cold water immersion groups. Values are means and standard deviations. *Significant difference versus baseline for both groups (p<0.05). #Significant difference versus TWI group (p<0.05).




Vertical jump performance was assessed at four time points: pre-match, post-match, 24 hours post-match, and 48 hours post-match. A repeated measures ANOVA revealed a significant interaction between time and intervention type ($F(3,54) = 4.86$, $p = 0.005$, partial $\eta^2 = 0.21$), indicating that recovery patterns differed significantly between the cold water immersion (CWI) and thermoneutral water immersion (TWI) groups.

In the TWI group, the mean vertical jump height at baseline was 45.4 ± 3.2 cm. This decreased significantly to 38.8 ± 4.7 cm post-match ($p < 0.001$), reflecting acute neuromuscular fatigue following competition. According to Bonferroni post-hoc analysis, jump height increased to 41.8 ± 2.1 cm after 24 hours, showing a significant improvement from post-match levels ($p = 0.007$) but remaining significantly lower than the pre-match value ($p = 0.043$). After 48 hours, jump height improved further to 43.6 ± 2.5 cm, which was significantly higher than the post-match value ($p = 0.021$), but showed no statistically significant difference compared to the pre-match or 24-hour values ($p > 0.05$).

In the CWI group, the mean pre-match vertical jump was 44.6 ± 2.9 cm, which decreased significantly to 39.4 ± 3.2 cm after the match ($p < 0.001$). No significant difference was observed compared to the TWI group at the same time point ($p = 0.88$). At 24 hours post-match, jump height increased to 43.5 ± 2.8 cm, which was not significantly different from the pre-match value ($p = 0.16$) but was significantly higher than that of the TWI group at the same time ($p = 0.018$; 95% CI: 0.41 to 3.01 cm).

At 48 hours post-match, vertical jump performance in the CWI group further improved to 44.1 ± 1.2 cm, showing no significant difference from the pre-match level ($p = 0.63$), indicating a full return to baseline neuromuscular function. Compared to the TWI group at the same time (43.6 ± 2.5 cm), the difference was also not statistically significant ($p = 0.41$; 95% CI: –0.71 to 1.71 cm).

The recovery observed in the CWI group was more consistent and complete than in the TWI group. This was supported by a moderate between-group effect size at 24 hours (Cohen's $d = 0.54$). Additionally, the within-group improvement from post-match to 48 hours showed a moderate effect size in the CWI group ($d = 0.63$) compared to a small effect size in the TWI group ($d = 0.42$). These results suggest that cold water immersion is a more effective recovery strategy for restoring neuromuscular performance within 24 to 48 hours following match play, compared to thermoneutral water immersion

### *Creatine Kinase*

The creatine kinase (CK) examination procedure begins with explaining the purpose of the examination to the patient and ensuring consent, followed by the preparation of tools such as sterile syringes, blood collection tubes, and medical gloves. After the vein area is cleaned with alcohol cotton, a tourniquet is applied to facilitate blood collection from the vein in the arm. The needle is inserted at a suitable angle until the blood flows into the tube, then the tourniquet is released. After the sample is sufficient, the needle is removed, and the area is pressed with cotton and then covered with a plaster. The blood sample is labeled and sent to the laboratory for analysis using an enzymatic method. The CK results will indicate muscle or organ damage related to the serum CK level. Normal CK values vary by laboratory, but the general range is around 38–174 U/L (for men) and 26–140 U/L (for women) (Lim et al., 2015).

Figure 2. Result of CK following a one-off soccer match for thermoneutral waterimmersion and cold water immersion groups. Values are means and standard deviations. *Significant difference versus baseline for both groups (P<0.05). #Significant difference versus TWI group (P<0.05).

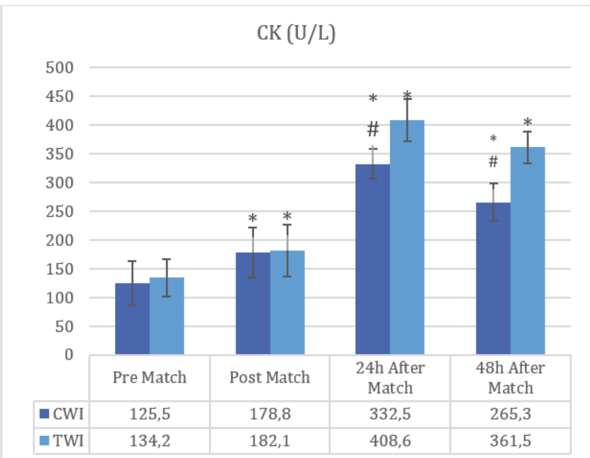


Table 3. Analysis of creatine kinase between group TWI vs CWI

| Creatine Kinase (U/L) | | |
|---|---|---|
| | CWI ± SD | TWI ± SD |
| Pre Match | 125,5 ± 38,5 | 134,2 ± 32,5 |
| Post Match | 178,8 ± 43,5* | 182,1 ± 45,1* |
| 24h After Match | 332,5 ± 25,9#* | 408,6 ± 36,3#* |
| 48h After Match | 265,3 ± 32,4#* | 361,5 ± 27,7* |

Serum creatine kinase (CK) levels were assessed at four time points: pre-match, post-match, 24 hours post-match, and 48 hours post-match. A repeated measures ANOVA revealed a significant interaction between time and intervention group ($F(3,54) = 11.27$, $p < 0.001$, partial $\eta^2 = 0.39$), indicating that the temporal response of CK levels differed significantly between the cold water immersion (CWI) and thermoneutral water immersion (TWI) groups.

In the TWI group, CK levels increased from a baseline of 134.2 ± 32.5 U/L to 182.1 ± 45.1 U/L post-match ($p < 0.001$), reflecting muscle membrane disruption following intense physical exertion. At 24 hours post-match, CK levels rose further to 408.6 ± 36.3 U/L, showing a significant elevation from both pre-match and post-match values ($p < 0.001$). Although a reduction was observed at 48 hours (361.5 ± 27.7 U/L), levels remained significantly higher than at all previous time points ($p < 0.001$), indicating a delayed recovery trajectory.

In contrast, the CWI group had a baseline CK level of 125.5 ± 38.5 U/L, which increased significantly to 178.8 ± 43.5 U/L post-match ($p < 0.001$), with no significant difference from the TWI group at that point ($p = 0.67$). At 24 hours post-match, CK levels peaked at 332.5 ± 25.9 U/L, significantly lower than the TWI group ($p = 0.002$; 95% CI: −123.8 to −36.4 U/L). By 48 hours, CK levels decreased to 265.3 ± 32.4 U/L, remaining elevated compared to baseline ($p < 0.001$), but still significantly lower than the TWI group ($p = 0.008$; 95% CI: −168.2 to −25.1 U/L).

The between-group effect size at 24 hours was large (Cohen's d = 1.10), indicating a substantial impact of CWI in attenuating CK elevation. Within-group analysis also showed a moderate effect size for the CWI group from post-match to 48 hours (d = 0.74), while the TWI group exhibited a greater and more prolonged CK response with a slower reduction in enzyme levels.

These findings suggest that cold water immersion is effective in reducing both the magnitude and duration of muscle damage following match play, as evidenced by significantly lower CK concentrations compared to thermoneutral immersion. The attenuated CK response in the CWI group supports the physiological role of cold exposure in limiting membrane disruption and enhancing muscle recovery after high-intensity physical activity.

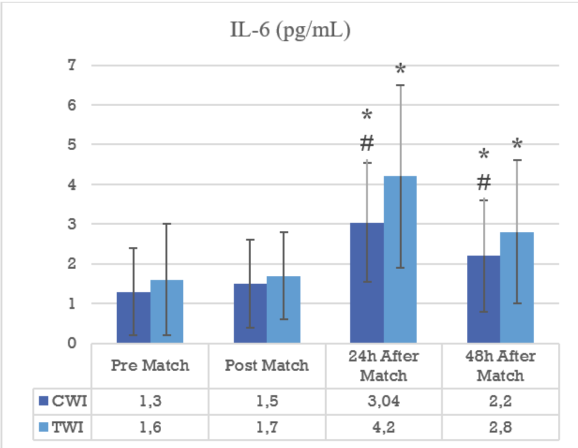
### *Interleukin-6*

The interleukin-6 (IL-6) examination procedure begins with explaining the purpose of the test and obtaining consent from the patient. After that, the vein area is cleaned, a tourniquet is applied, and blood is drawn from the vein using a sterile needle. After the collection, the tourniquet is removed, and the injection site is covered with cotton or plaster. The blood sample is then labeled and sent to a laboratory for analysis using a method such as ELISA to measure IL-6 levels, which functions to detect the level of inflammation in the body.

Table 4. Analysis of creatine kinase between group TWI vs CWI

| Creatine Kinase (pg/mL) | | |
|---|---|---|
| | CWI ± SD | TWI ± SD |
| Pre Match | 1,3 ± 1,1 | 1,6 ± 1,4 |
| Post Match | 1,5 ± 1,1 | 1,7 ± 1,1 |
| 24h After Match | 3,04 ± 1,5#* | 4,2 ± 2,3* |
| 48h After Match | 2,2 ± 1,4#* | 2,8 ± 1,8* |

Figure 3. Result of IL-6 following a one-off soccer match for thermoneutral waterimmersion and cold water immersion groups. Values are means and standard deviations. *Significant difference versus baseline for both groups (p <0.05). #Significant difference versus TWI group (p <0.05).




Serum interleukin-6 (IL-6) levels were measured at four time points: pre-match, post-match, 24 hours post-match, and 48 hours post-match. The analysis using Repeated Measures ANOVA revealed a significant interaction between time and intervention group ($F(3,54) = 9.63$, $p < 0.001$, partial $\eta^2 = 0.35$), indicating that the inflammatory responses differed significantly between the cold water immersion (CWI) and thermoneutral water immersion (TWI) groups over time.

In the TWI group, IL-6 levels increased slightly from 1.6 ± 1.4 pg/mL at baseline to 1.7 ± 1.1 pg/mL post-match, with no statistically significant difference ($p = 0.78$). At 24 hours post-match, IL-6 rose sharply to 4.2 ± 2.3 pg/mL, a significant increase compared to both pre- and post-match values ($p < 0.001$). Although levels decreased at 48 hours to 2.8 ± 1.8 pg/mL, they remained significantly elevated compared to baseline ($p < 0.001$).

In the CWI group, IL-6 increased from 1.3 ± 1.1 pg/mL pre-match to 1.5 ± 1.1 pg/mL post-match, but this change was not statistically significant ($p = 0.67$), nor was it different from the TWI group ($p = 0.73$). At 24 hours post-match, IL-6 reached 3.04 ± 1.5 pg/mL, which was significantly lower than the TWI group at the same time point ($p = 0.021$; 95% CI: –2.19 to –0.18 pg/mL). At 48 hours, IL-6 levels decreased to 2.2 ± 1.4 pg/mL, remaining significantly lower than the TWI group ($p = 0.034$; 95% CI: –1.21 to –0.09 pg/mL).

The between-group effect size at 24 hours was moderate (Cohen's $d = 0.68$), suggesting a clinically relevant anti-inflammatory effect of CWI. Moreover, the relative IL-6 reduction from 24 to 48 hours was greater in the CWI group ($d = 0.52$) than in the TWI group ($d = 0.37$), indicating a faster inflammatory resolution.

These findings support the anti-inflammatory potential of cold water immersion in female athletes, with consistently lower IL-6 levels at both 24 and 48 hours post-match compared to thermoneutral immersion. This is in line with previous literature showing that cold exposure can suppress pro-inflammatory cytokine activity and promote recovery by modulating systemic inflammation.

## Discussion

Recovery is a fundamental aspect of training programs and competitive sports. It is not a passive phase but an active process designed to restore physiological, biochemical, and psychological balance following intense physical activity. Without adequate recovery, accumulated fatigue may impair performance, delay muscle regeneration, and increase the risk of injury. Therefore, implementing structured and evidence-based recovery strategies is essential to maintain both physical and mental readiness for subsequent training or competition sessions (Darani et al., 2018).

Numerous recovery methods are widely used in sports settings, including stretching, sports massage, nutritional therapy, hydrotherapy, and electrotherapy. One of the simplest, most affordable, and widely applied methods is cold water immersion (CWI). This involves submerging the lower or full body in water at 10–15°C for 10–20 minutes and is believed to accelerate muscle recovery and reduce systemic inflammation (Moore et al., 2022; Orunbayev, 2023).

This study evaluated the effectiveness of CWI compared to thermoneutral water immersion (TWI) in promoting physiological recovery in female soccer athletes. Two primary biomarkers were used: creatine kinase (CK) as an indicator of muscle damage and interleukin-6 (IL-6) as a marker of systemic inflammation. The findings demonstrated that the CWI group experienced faster recovery than the TWI group, as shown by lower CK and IL-6 levels and

***Vertical Jump Performance and Neuromuscular Recovery***

Vertical jump performance serves as a reliable and practical indicator of neuromuscular fatigue and lower-limb power output in both laboratory and field settings. In the context of soccer, this metric is particularly relevant due to the sport's reliance on repeated bouts of explosive movements such as sprinting, jumping, and sudden changes in direction (Sedano & Maroto-Izquierdo, 2025)). These actions, when performed under competitive pressure, lead to substantial mechanical loading and microtrauma of muscle fibers, which in turn result in delayed neuromuscular function and performance deficits.

In this study, both the CWI and TWI groups demonstrated an immediate post-match decline in vertical jump height, which reflects acute fatigue and exercise-induced muscle damage (EIMD). This is consistent with previous research showing that intense match-play leads to impaired excitation-contraction coupling, reduced motor unit recruitment, and disrupted calcium homeostasis in muscle fibers (Machado et al., 2016; Rosell et al., 2017). However, the CWI group exhibited a significantly faster recovery, returning to near-baseline values within 48 hours, whereas the TWI group remained substantially below pre-match levels.

This accelerated recovery in the CWI group is likely due to multifactorial physiological mechanisms, including reduced inflammation, preservation of neuromuscular function, and decreased soreness. CWI has been shown to attenuate neuromuscular impairment by stabilizing the sarcolemma and reducing the buildup of metabolic byproducts that impair muscle excitability (Ascensão et al., 2011; Ihsan et al., 2016). Furthermore, CWI may also alleviate delayed onset muscle soreness (DOMS), which is known to negatively affect jump performance by altering proprioceptive input and limiting voluntary muscle activation.

Studies have also shown that vertical jump assessments are highly sensitive to muscle recovery status, especially within the first 24–72 hours post-exercise (Machado et al., 2016). In practical settings, the recovery of vertical jump height can serve as a non-invasive, objective tool to monitor recovery status in athletes and to guide return-to-play decisions. The fact that the CWI group in this study returned to baseline performance levels within 48 hours suggests that this intervention not only facilitated biological repair but also restored functional performance, which is critical in tournament environments where athletes must compete again within short timeframes.

Given that explosive muscle power is a decisive factor in technical actions such as shooting, aerial duels, and acceleration in soccer, restoring vertical jump capacity can have a direct impact on in-game performance and tactical effectiveness (Rosell et al., 2017). Thus, CWI emerges not just as a recovery modality, but as a performance-preserving intervention with tangible implications for team success, especially in female soccer populations where recovery physiology may differ due to hormonal and structural factors (Taneja, 2018).

***CK, IL-6 Responses, and the Context of Female Soccer***

CK levels significantly increased in both groups following the match, indicating sarcolemma disruption due to mechanical stress. However, the CWI group exhibited a smaller increase and a faster decline in CK levels, suggesting better maintenance of membrane integrity and reduced leakage of enzymes into the bloodstream. Ascensão et al. (2011) explained that CWI may reduce protein leakage by stabilizing the muscle cell membrane and lowering its permeability. CWI also minimizes metabolic stress and mitigates damage from eccentric contractions, as noted by Stožer et al. (2020).

IL-6, a pro-inflammatory cytokine rapidly released in response to tissue injury, also increased in both groups post-match. Yet, the CWI group displayed a significantly lower and quicker reduction in IL-6 levels. These findings support those of Ihsan et al. (2016), who demonstrated that CWI attenuates IL-6 release by suppressing local immune activity and cytokine expression. Lower IL-6 levels are crucial for faster tissue regeneration, pain reduction, and readiness for subsequent training.

Importantly, female soccer players may respond differently to exercise-induced muscle damage and inflammation compared to their male counterparts due to distinct hormonal and physiological profiles. Female athletes generally have lower muscle mass, different estrogen levels, and are more prone to delayed onset muscle soreness (DOMS) (Dannecker et al., 2012). Estrogen, while protective against muscle damage, may prolong inflammation by modulating immune cell activity (Taneja, 2018).

This study contributes valuable data by confirming that CWI is not only effective in reducing CK and IL-6 levels but also promotes faster functional recovery in female soccer players. Given that most prior studies have focused on male athletes, this highlights the relevance of CWI for gender-specific recovery protocols and underscores the importance of tailoring interventions to the unique physiological demands of female athletes.

### *Mechanistic Insights and Synergistic Pathways*

The recovery-enhancing effects of CWI involve complex interactions between molecular, vascular, and neuroendocrine systems. Vascularly, cold exposure triggers peripheral vasoconstriction via sympathetic activation and α-adrenergic receptors. This reduces local blood flow, limits the delivery of pro-inflammatory cytokines (e.g., IL-6, TNF-α), and minimizes immune cell infiltration into damaged muscle tissue (Montgomery et al., 2008).

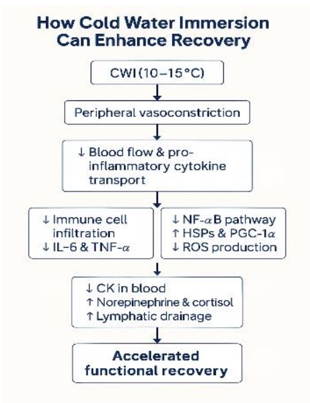
At the molecular level, CWI inhibits the activation of the NF-κB pathway, a key regulator of pro-inflammatory gene expression. Suppression of this pathway leads to decreased IL-6 production and reduced expression of oxidative enzymes that can harm tissue (Ihsan et al., 2016). Additionally, CWI may upregulate heat shock proteins (HSPs) and PGC-1α, which facilitate mitochondrial biogenesis and muscle regeneration (Aguiar et al., 2016).

CWI also lowers tissue metabolic rates, reducing oxygen demand and reactive oxygen species (ROS) production. ROS accumulation causes lipid peroxidation and membrane damage, contributing to CK release. By mitigating ROS, CWI helps preserve cell membrane stability and limits secondary muscle damage (Ascensão et al., 2011; Ramos et al., 2021).

From a neuroendocrine perspective, cold exposure stimulates the HPA axis, increasing norepinephrine and cortisol secretion within physiological limits. Norepinephrine provides analgesic effects, while cortisol suppresses excessive immune activation. CWI also enhances lymphatic drainage, facilitating the removal of inflammatory byproducts and promoting tissue resolution (Wang & Ni, 2021).

Together, these mechanisms explain why the CWI group in this study demonstrated significantly lower IL-6 and CK levels and faster recovery of neuromuscular performance compared to the control group.

Figure 4. Proposed mechanism by which cold water immersion (CWI) facilitates post-exercise recovery, reducing inflammation and muscle damage markers (IL-6, TNF-α, CK) and promoting neuromuscular restoratio



### *Practical Applications*

These findings have direct implications for coaches, physiotherapists, and sports practitioners. CWI can be easily implemented across various levels of competition due to its low cost, simplicity, and efficacy. A 15-minute immersion at 10°C post-match significantly enhances recovery of muscle performance within 24–48 hours, making it especially valuable in congested match schedules—particularly in women's soccer tournaments.

### *Study Limitations and Future Directions*

Despite promising results, this study has several limitations. First, the small sample size drawn from a single team limits the generalizability of the findings. Second, subjective variables such as perceived fatigue or muscle soreness were not assessed. Third, the study focused solely on CK and IL-6; additional biomarkers such as IL-10, TNF-α, and cortisol would offer a more comprehensive view. Future research should include larger and more diverse samples, alternative recovery modalities, and integrated interventions involving nutrition, compression, or active recovery protocols.

## Conclusions

This quasi-experimental study evaluated the effects of cold water immersion (CWI) on post-match recovery among 20 female soccer players from a single regional academy. The results showed that the CWI group experienced significantly lower increases in creatine kinase (CK) and interleukin-6 (IL-6) levels at 24 and 48 hours post-match compared to the thermoneutral water immersion (TWI) group. Furthermore, vertical jump performance in the CWI group returned to near-baseline levels within 48 hours, indicating a more effective neuromuscular recovery.

Although the findings suggest that CWI is a simple, cost-effective, and physiologically beneficial strategy to support recovery in female soccer players, generalization must be made with caution due to the small and homogeneous sample. Future research should include larger and more diverse populations, explore sex-specific physiological factors, and integrate additional biomarkers and performance indicators to develop more comprehensive and individualized recovery protocols for female athletes.

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## References

Aguiar, P. F., Magalhães, S. M., Fonseca, I. A. T., da Costa Santos, V. B., de Matos, M. A., Peixoto, M. F. D., Nakamura, F. Y., Crandall, C., Araújo, H. N., Silveira, L. R., Rocha-Vieira, E., de Castro Magalhães, F., & Amorim, F. T. (2016). Post-exercise cold water immersion does not alter high intensity interval training-induced exercise performance and Hsp72 responses, but enhances mitochondrial markers. Cell Stress & Chaperones, 21(5), 793–804. https://doi.org/10.1007/s12192-016-0704-6

Almeida, P. S. M. de, Scotta, Â. P., Pimentel, B. de M., Júnior, S. B., & Sampaio, Y. R. (2013). Incidência de lesão musculoesquelética em jogadores de futebol. Revista Brasileira de Medicina Do Esporte, 19(2), 112–115. https://doi.org/10.1590/s1517-86922013000200008

Ascensão, A., Leite, M., Rebelo, A. N., Magalhäes, S., & Magalhäes, J. (2011). Effects of cold water immersion on the recovery of physical performance and muscle damage following a one-off soccer match. Journal of Sports Sciences, 29(3), 217–225. https://doi.org/10.1080/02640414.2010.526132

Binat, M., & Kartiko, D. (2021). Minat Siswi SMA Kemala Bayangkari 1 Surabaya Dalam Permainan Sepak Bola. Jurnal Pendidikan Olahraga Dan Kesehatan, 09(03), 19–24. https://ejournal.unesa.ac.id/index.php/jurnal-pendidikan-jasmani/issue/archivehttps://ejournal.unesa.ac.id/index.php/jurnal-pendidikan-jasmani

BOSCO, C., KOMI, P. V., & ITO, A. (1981). Prestretch potentiation of human skeletal muscle during ballistic movement. Acta Physiologica Scandinavica, 111(2), 135–140. https://doi.org/10.1111/j.1748-1716.1981.tb06716.x

Cormie, P., Mccaulley, G. O., & Mcbride, J. M. (2007). Power Versus Strength-Power Jump Squat Training. Medicine & Science in Sports & Exercise, 39(6), 996–1003. https://doi.org/10.1097/mss.0b013e3180408e0c

Dannecker, E. A., Liu, Y., Rector, R. S., Thomas, T. R., Fillingim, R. B., & Robinson, M. E. (2012). Sex Differences in Exercise-Induced Muscle Pain and Muscle Damage. The Journal of Pain, 13(12), 1242–1249. https://doi.org/10.1016/j.jpain.2012.09.014

Darani, M. M., Abedi, B., & Fatolahi, H. (2018). The effect of active and passive recovery on creatine kinase and C-reactive protein after an exercise session in football players. International Archives of Health Sciences, 5(1), 1. https://doi.org/10.4103/iahs.iahs_31_17

Dewangga, M. W., Irianto, D. P., Dimyati, Sumaryanto, Nasihun, T., Febrianta, Y., Wahyuni, Wijianto, & Agustiyawan. (2021). Different Effects of Acute and Chronic Strenuous Physical Exercise on Superoxide Dismutase (SOD), Malondialdehyde (MDA) Levels, and Sperm Quality of the Wistar Rats. Journal of Kerman University of Medical Sciences, 28(6), 539–547. https://doi.org/10.22062/jkmu.2021.91825

Dewangga, M. W., Susilo, T. E., Cahyadi, M. M., Saputra, H., Furqony, I. Y., Putra, Z. Y., Wilger, R. V., Candrika, A. A., & Rosyid, F. N. (2024). Physical performance and markers of muscle damage response after single soccer matches in amateur women's soccer players. Fizjoterapia Polska, 24(2), 136–143. https://doi.org/10.56984/8ZG5608X8S

Doewes, R. I., & Nuryadin, I. (2022). Analysis of the participation of women in football in Indonesia. Sport TK, 11, 1–13. https://doi.org/10.6018/sportk.522901

Ebben, W. P., & Petushek, E. J. (2010). Using the Reactive Strength Index Modified to Evaluate Plyometric Performance. Journal of Strength and Conditioning Research, 24(8), 1983–1987. https://doi.org/10.1519/JSC.0b013e3181e72466

Hara, M., Shibayama, A., Takeshita, D., & Fukashiro, S. (2006). The effect of arm swing on lower extremities in vertical jumping. Journal of Biomechanics, 39(13), 2503–2511. https://doi.org/10.1016/j.jbiomech.2005.07.030

Ihsan, M., Watson, G., & Abbiss, C. R. (2016). What are the Physiological Mechanisms for Post-Exercise Cold Water Immersion in the Recovery from Prolonged Endurance and Intermittent Exercise? Sports Medicine, 46(8), 1095–1109. https://doi.org/10.1007/s40279-016-0483-3

Lees, A., Vanrenterghem, J., & Clercq, D. De. (2004). Understanding how an arm swing enhances performance in the vertical jump. Journal of Biomechanics, 37(12), 1929–1940. https://doi.org/10.1016/j.jbiomech.2004.02.021

Lim, E., Miyamura, J., & Chen, J. J. (2015). Racial/Ethnic-Specific Reference Intervals for Common Laboratory Tests: A Comparison among Asians, Blacks, Hispanics, and White. Hawai'i Journal of Medicine & Public Health : A Journal of Asia Pacific Medicine & Public Health, 74(9), 302–310.

Machado, A. F., Ferreira, P. H., Micheletti, J. K., de Almeida, A. C., Lemes, Í. R., Vanderlei, F. M., Netto Junior, J., & Pastre, C. M. (2016). Can Water Temperature and Immersion Time Influence the Effect of Cold Water Immersion on Muscle Soreness? A Systematic Review and Meta-Analysis. Sports Medicine, 46(4), 503–514. https://doi.org/10.1007/s40279-015-0431-7

Marangoni, L. S., Pottratz, S., & Boiangin, N. (2023). The Impacts of Burnout on Athletic Identity and Attitude towards Sport. Youth, 3(4), 1121–1136. https://doi.org/10.3390/youth3040071

Markovic, G., Dizdar, D., Jukic, I., & Cardinale, M. (2004). Reliability and Factorial Validity of Squat and Countermovement Jump Tests. The Journal of Strength and Conditioning Research, 18(3), 551. https://doi.org/10.1519/1533-4287(2004)18<551:RAFVOS>2.0.CO;2

Moir, G., Button, C., Glaister, M., & Stone, M. H. (2004). Influence of Familiarization on the Reliability of Vertical Jump and Acceleration Sprinting Performance in Physically Active Men. The Journal of Strength and Conditioning Research, 18(2), 276. https://doi.org/10.1519/R-13093.1

Montgomery, P. G., Pyne, D. B., Hopkins, W. G., Dorman, J. C., Cook, K. K., & Minahan, C. L. (2008). The effect of recovery strategies on physical performance and cumulative fatigue in competitive basketball. Journal of Sports Sciences, 26(11), 1135–1145. https://doi.org/10.1080/02640410802104912
Moore, E., Fuller, J. T., Buckley, J. D., Saunders, S., Halson, S. L., Broatch, J. R., & Bellenger, C. R. (2022). Impact of Cold-Water Immersion Compared with Passive Recovery Following a Single Bout of Strenuous Exercise on Athletic Performance in Physically Active Participants: A Systematic Review with Meta-analysis and Meta-regression. Sports Medicine, 52(7), 1667–1688. https://doi.org/10.1007/s40279-022-01644-9
Orunbayev, A. (2023). Recovery Strategy In Sports. American Journal Of Social Sciences And Humanity Research, 03(12), 135–147. https://doi.org/10.37547/ajsshr/Volume03Issue12-18
Ramos, Z. N. A., Colmenero, B. R. R., Dávila, M. Z. G., Maldonado, G. E. M., Flores, J. R. H., & Cruz, G. H. (2021). Influencia del masaje ZNAR y la inmersión en agua fría en el proceso inflamatorio, Creatin Kinasa y percepción al dolor muscular en jugadores de voleibol (Influence of ZNAR massage and cold water immersion on the inflammatory process, Creatine Kinase and. *Retos, 44,* 95–102. https://doi.org/10.47197/retos.v44i0.89399
Rosell, D. R., Custodio, R. M., Márquez, F. F., García, J. M. Y., & Badillo, J. J. G. (2017). Traditional vs. Sport-Specific Vertical Jump Tests: Reliability, Validity, and Relationship With the Legs Strength and Sprint Performance in Adult and Teen Soccer and Basketball Players. Journal of Strength and Conditioning Research, 31(1), 196–206. https://doi.org/10.1519/JSC.0000000000001476
Sedano, S., & Maroto-Izquierdo, S. (2025). Effectiveness of Different Neuromuscular Recovery Strategies in Elite Youth Female Football Players. Sports, 13(2), 36. https://doi.org/10.3390/sports13020036
Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harbor Perspectives in Biology, 6(10), a016295–a016295. https://doi.org/10.1101/cshperspect.a016295
Taneja, V. (2018). Sex Hormones Determine Immune Response. Frontiers in Immunology, 9(August), 1–5. https://doi.org/10.3389/fimmu.2018.01931
Teoldo, I., Dambroz, F., & Brito, J. (2024). Performance Of Soccer Players Under Acute Physical Fatigue: An Approach Based On Cognitive, Tactical And Physical Aspects. Heliyon, 10(9), e30516. https://doi.org/10.1016/j.heliyon.2024.e30516
Versey, N. G., Halson, S. L., & Dawson, B. T. (2013). Water immersion recovery for athletes: Effect on exercise performance and practical recommendations. Sports Medicine, 43(11), 1101–1130. https://doi.org/10.1007/s40279-013-0063-8
Wang, Z. R., & Ni, G. X. (2021). Is it Time to put Traditional Cold Therapy in Rehabilitation of Soft-tissue Injuries out to Pasture? World Journal of Clinical Cases, 9(17), 4116–4122. https://doi.org/10.12998/wjcc.v9.i17.4116
White, G. E., Rhind, S. G., & Wells, G. D. (2014). The effect of various cold-water immersion protocols on exercise-induced inflammatory response and functional recovery from high-intensity sprint exercise. European Journal of Applied Physiology, 114(11), 2353–2367. https://doi.org/10.1007/s00421-014-2954-2
Zhang, H., & Dhalla, N. S. (2024). The Role of Pro-Inflammatory Cytokines in the Pathogenesis of Cardiovascular Disease. International Journal of Molecular Sciences, 25(2). https://doi.org/10.3390/ijms25021082

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