

# The Effect of Cold Water Immersion on Cardiac Troponin T and Myoglobin Levels

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## Abstract

**OBJECTIVES:** Skeletal and cardiac damages occurring in the high intensity training, can reduce the efficiency of the athlete in the next training session. Cold water immersion can help for more efficient recovery in athletes. This study was conducted to investigate the effect of cold water immersion applied after exercise on cardiac troponin T and myoglobin levels.

**MATERIAL AND METHODS:** The experimental group comprised of a total of 23 male football players, of which 12 comprised the study group (age 22.4±3.16 years, height 176.9±6.08 cm, BMI 22.61±1.27 kg/m<sup>2</sup>) and 11 comprised the control group (age 21.3±2.21 years, height 175.8±5.52 cm, BMI 21.24±3.15 kg/m<sup>2</sup>). The athletes provided blood samples before exercise (BE) for troponin T (cTnT) and myoglobin (Mb) measurement. Consequently, muscle damage exercise protocol consisting of depth jump, and immediately after this, cold-water immersion at 15°C was applied to experimental group for 10 minutes. The athletes provided blood samples after 2 (2h) and 24 (24h) hours from cold water immersion. Athletes in the control group underwent the same measurements except application of cold-water immersion.

**RESULTS:** While there was a significant difference ( $p<0.05$ ) between the experimental and the control groups regarding troponin values, no significant difference was found for the myoglobin values ( $p>0.05$ ).

**CONCLUSIONS:** In this study, cTnT level, which is regarded as one of the most specific markers of cardiac injury, was found to be lower in the athletes who underwent cold water immersion. These results indicate that the recovery time may be shorter in athletes undergoing cold-water immersion.

## INTRODUCTION

Micro trauma occurring in muscles after intense exercises including eccentric muscle contractions with high frequency and intensity results in remarkable damage in the muscle tissue. Intensive training modes including eccentric contractions lead to damage in muscle fibrils and cause pain, which result in stiffness and damage in the muscles and loss of joint mobility (Proske & Allen, 2005; Banfi *et al.* 2010; Hikida *et al.* 1983; Warhol *et al.* 1985; Lauritzen *et al.* 2009; Warren *et al.* 2001; Corona *et al.* 2009; Schimpchen *et al.* 2017). These physiological changes that occur after exercise are not just confined to the skeletal muscles, but also involve cardiac muscles as well (Burke, 1998). These physiological changes occurring after exercise manifest with abrupt contractions and resemble infarction in skeletal and cardiac muscles after intensive training in branches requiring endurance such as marathon. In addition, the training condition of the athlete plays a determining role on the extent of the damage on the heart and skeletal muscle (Hazar, 2004). The damage occurring in the skeletal and heart muscle after exercise has an adverse impact on the athlete's efficiency in the next training session (Roberts *et al.* 2015).

For more efficient recovery, athletes and trainers frequently use various applications including electrostimulation, supplementation, cold-water immersion, cryotherapy, thermotherapy and massage, in an attempt to overcome these adverse physiological changes occurring after training and competitions (Mor *et al.* 2017; Mor *et al.* 2018; Çolakoğlu *et al.* 2016; Sramek *et al.* 2000; Al Haddad *et al.* 2010). Regarding the effect of the recently popularized cold water immersion on cardiovascular changes, performing this application by exposing a large part of the body to cold water except for the head is known to improve cardiovascular activity as well as to regulate central blood flow and to improve cardiac loading (White & Wells, 2013; Tipton *et al.* 1998). Despite somewhat limited data about the effects of cold water immersion in young athletes, as in adults, cold water immersion after an intensive exercise is thought to be capable of normalizing cardiovascular system and regulating parasympathetic heartbeat and vagal tonus in young adults (Parouty *et al.* 2010; Buchheit *et al.* 2009).

In light of this knowledge, the present study was performed with the aim of investigating the effect of cold-water immersion on cardiac troponin T and myoglobin.

## MATERIALS AND METHODS

### Research Group

This study was conducted with voluntary participation of 23 male football players who performed regular training. These athletes did not perform training during the week before the start of the study, and were assigned to study and control groups as 12 athletes in the experi-

mental group and 11 athletes in the control group. The athletes were informed in detail about the study design.

### Study Design

Body composition measurements of the athletes in experimental and control groups were performed one day before initiation of the exercise protocol. On the day after the body composition measurements, body temperature of the athletes was measured while resting, and this was followed by blood sampling for troponin T (cTnT) and myoglobin (Mb) measurement. Consequently, Muscle Damage Exercise Protocol was applied to the participants in both the experimental and control groups. Immediately after the exercise, athletes in the experimental group was immersed in cold water at 15°C temperature for 10 minutes, with their shoulders, neck and head remaining outside. During this time, athletes in the control group were requested to sit at 25°C ambient temperature. Repeat blood samples for cTnT and Mb measurement were obtained from athletes in both experimental and control groups after passive resting period of 2 (2h) and 24 (24h) hours from the completion of cold water immersion.

### Procedure

*Muscle damage exercise protocol:* Muscle damage exercise protocol consisted of depth jumps from 60 cm height with 20 repeats in 5 sets. The jumps were made with 10 second intervals, with 2 minutes of resting between the sets. The athletes jumped from a height of 60 cm, and were requested to jump as high as possible from 90 degrees flexion as soon as they landed (Goodall & Howatson, 2008; Kirby *et al.* 2012).

*Cold Water Immersion:* Immediately after application of muscle damage exercise protocol, athletes in the experimental group were immersed in cold water at 15°C for 10 minutes while their neck and shoulders remained outside. In order to keep the water temperature constant at 15°C, the water temperature was monitored at regular intervals using a precision thermometer. The water temperature was adjusted by adding chunks of ice to the water (Takeda *et al.* 2014).

*Body composition:* One day before the measurements, weight and height measurements of the athletes were performed with Seca brand height and weight measurement device. Determination of body fat percentage was made via Bioelectrical Impedance (Tanita Body Fat Analyser, Model TBF 300).

*Body temperature measurement:* Body temperatures of the athletes were measured with touch free thermometer (F.BoschFb-Scan).

*Blood Sampling:* For the purpose biochemical evaluation of the athletes, 5 ml of venous blood samples for cTnT and Mb measurements were obtained before muscle damage exercise protocol (BE), and 2 hours (2h) and 24 hours (24h) after, via antecubital veins.

Statistical Analysis

Descriptive statistics of the participants were summarized in table. Since distribution of data was not homogenous, non-parametric comparison methods were employed. In control and experimental groups, the differences between the three measurements (measurement\*group) were analyzed with repeated measures two-way analysis of variance. Variations within the group was analyzed with Friedmann and chi-square tests used for non-parametric more than 2 repeated measurements. Significant differences were compared pairwise via Wilcoxon test. Pairwise comparisons between the groups were made with Mann-Whitney U test.  $p < 0.05$  was accepted as statistically significant.

**RESULTS**

According to the Table 2, mean body surface temperatures in the control group were 36.8°C before exercise, 36.7°C immediately after the exercise, 37.1°C immediately after cold water immersion, 36.6°C 2 hours after cold water immersion, and 36.1°C 24 hours after cold water immersion. The mean body surface temperatures for the experimental group 37°C before exercise, 37.1°C immediately after exercise, 31.2°C immediately after cold-water immersion, 36.5°C 2 hours after cold-

water immersion, and 36.6°C 24 hours after cold-water immersion.

According to the Table 3, mean Mb (Myoglobin) levels in the control group were 38.73±5.77 (ng/ml) before exercise, 135.3±38.52 (ng/ml) at the 2<sup>nd</sup> hour, and 44.31±16.43 (ng/ml) at the 24<sup>th</sup> hour. Mean Mb levels in the experimental group was 39.54±22 (ng/ml) before exercise, 98.33±55.09 (ng/ml) at the 2<sup>nd</sup> hour, and 33.75±16.47 (ng/ml) at the 24<sup>th</sup> hour.

There was no significant difference between control and experimental groups regarding Mb levels ( $p > 0.05$ ). Regarding within group comparison for Mb levels in the control group, there were differences between MB-BE- MB-2h; MB-BE- MB-24h; and MB-2h - MB-24h. In the experimental group, there were differences between MB-BE- MB -2h; MB-BE- MB-24h; and MB-2h - MB-24h. MB-BE values did not show a significant difference between control and experimental groups ( $p > 0.05$ ). MB-2h values did not show a significant difference between control and experimental groups ( $p > 0.05$ ). MB-24h values did not show a significant difference between control and experimental groups ( $p > 0.05$ ).

According to the Table 4, mean cTnT values in control group were 6.45±1.24 (pg/ml) before exercise, 8.33±4.49 (pg/ml) 2 hours after exercise, and 4.90±0.99

**Tab. 1.** Anthropometric properties of athletes across experimental and control groups

Group	X	Minimum	Maximum	Mean	Standard Deviation	
Control	Age (years)	11	19	26	21.3	2.21
	Height(cm)	11	168	189	175.8	5.52
	Weight (Kg)	11	51.7	81.2	66.21	10.21
	BMI*	11	17.8	26.3	21.24	3.15
	BFP** (%)	11	5.6	21.2	13.61	5.17
Experimental	Age	12	19	26	22.2	3.16
	Height(cm)	12	167	185	176.9	6.08
	Weight (Kg)	12	62.7	73.4	66.31	2.21
	BMI*	12	18.1	23.4	22.61	1.27
	BFP** (%)	12	6.3	18.3	14.44	2.30

\*BMI: Body Mass Index; \*\*BFP: Body fat percentage

**Tab. 2.** Body surface temperatures of participants

Group	Before exercise (mean)	Immediately after exercise (mean)	Immediately after cold water immersion (mean)	2 hours after cold water immersion (mean)	24 hours after cold water immersion (mean)
Control group (A: 10)	36.8°C	36.7°C	37.1°C	36.6°C	36.1°C
Experimental group (n:10)	37°C	37.1°C	31.2°C	36.5°C	36.6°C

**Tab. 3.** Comparison of myoglobin parameter within and between the groups

Group	n	MB-BE		MB-2h		MB-24h		Within group	Measurement*		
		Mean	SD	Mean	SD	Mean	SD		F	P	
MB (ng/ml)	Control	11	38.73	5.77	135.3	38.52	44.31	16.43	MB-BE--MB-2h, MB-BE--MB-24h, MB-2h--MB-24h	1.583	0.224
	Experimental	12	39.54	22.0	98.33	55.09	33.75	16.47			
<b>Between Groups</b>		<b>TN-BE</b>		<b>TN-2h</b>		<b>TN-24h</b>					
<b>z</b>		-1.361		-1.890		-1.587					
<b>p</b>		0.174		0.059		0.112					

$p < 0.05$ ; MB: Myoglobin; BE: Before exercise; 2h: after 2 hours; 24h: after 24 hours

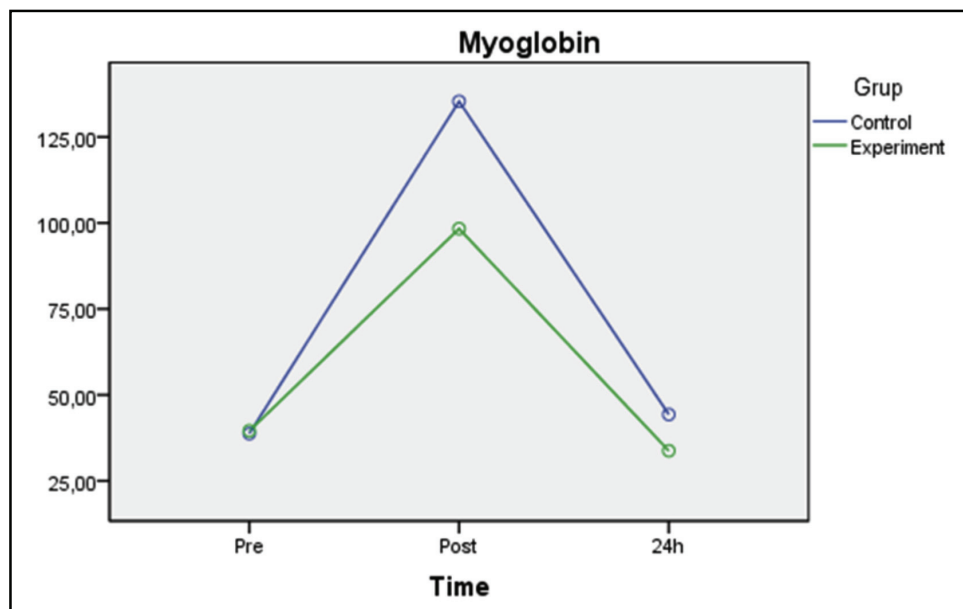
(pg/ml) 24 hours after exercise. Mean cTnT levels in the experimental group were  $5.54 \pm 1.13$  (pg/ml) before exercise,  $5.54 \pm 1.13$  (pg/ml) 2 hours after exercise, and  $4.73 \pm 0.76$  24 hours after exercise (pg/ml).

There were significant differences between control and experimental groups regarding cTnT levels ( $p < 0.05$ ). Within group, comparison for cTnT level in the control group showed significant differences between TN-BE - TN-2h; TN-BE - TN-24h; and TN-2h - TN-24h. In experimental group, there were significant differences between TN-BE - TN-2s; TN-BE - TN-24h, and TN-2h - TN-24h. TN-BE values did not show a significant difference between control and experimental groups ( $p > 0.05$ ). There was a significant difference

between the control and experimental groups regarding TN-2h values ( $p < 0.05$ ). TN-24h values did not show a significant difference between control and experimental groups ( $p > 0.05$ ).

## DISCUSSION

In this study, the effect of cold-water immersion on cardiac damage was investigated. In the study, following administration of a muscle damage exercise protocol consisting of depth jumps, the athletes were immersed in cold water, and troponin T and myoglobin levels were measured as indicators of cardiac damage. Comparison of the two groups regarding cTnT levels showed signifi-



**Fig. 1.** Myoglobin changes between control and experimental groups

**Tab. 4.** Comparison of troponin T parameter within and between the groups

Group	n	TN-BE		TN-2h		TN-24h		Within group	Measurement* Group		
		Mean	SD	Mean	SD	Mean	SD		F	P	
TN (pg/ml)	Control	11	6.45	1.24	8.33	4.49	4.90	0.99	TN-BE--TN-2h, TN-BE--TN-24h, TN-2h--TN-24h	3.490*	0.041
	Experimental	12	5.54	1.13	5.54	1.13	4.73	0.76			
<b>Between Groups</b>		<b>TN-BE</b>		<b>TN-2h</b>		<b>TN-24h</b>					
<b>z</b>		-1.436		<b>-2.268</b>		-0.030					
<b>p</b>		0.151		<b>0.023</b>		0.762					

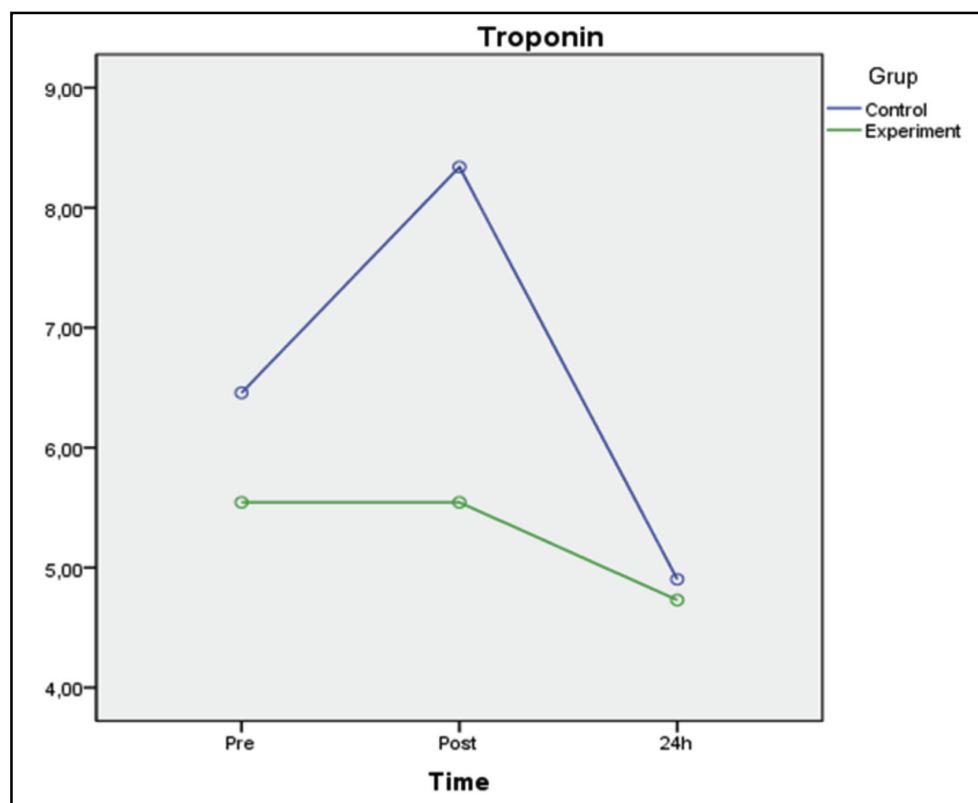
$p < 0.05$ ; TN: Troponin T; BE: Before exercise; 2h: 2 hours after exercise; 24h: 24 hours after exercise

cant differences between the groups ( $p < 0.05$ ), whereas no significant difference was found regarding Mb levels ( $p > 0.05$ ).

Serum troponin level is one of the most commonly used parameters for detection of cardiac damage. There are many studies showing that increased serum troponin level related to various factors (Nie *et al.* 2011). Myoglobin is a protein found in the muscles. It can be released into circulation following injury to the muscle

or the tissue. Myoglobin, one of the markers for both skeletal and cardiac muscle injury, is an enzyme mostly tested for detection of skeletal muscle damage (Petrofsky *et al.* 2015; Chiu *et al.* 1999).

Although the effects of cold-water immersion on cardiac muscle injury are not fully explained physiologically, it is thought that cold-water immersion may reduce pain via removal of substances causing pain from the body by accelerating blood flow and reducing



**Fig. 2.** Troponin T changes between control and experimental groups



swelling (Park *et al.* 1999; White & Wells, 2013). It has been shown that 10 minutes of cold water immersion at 10°C resulted in the reduction of biochemical (CK, myoglobin), functional and perceptual markers that are indicators of muscle damage, and was effective in accelerating adaptation and regeneration following exercise (Ascensao *et al.* 2011). In one study administering aerobic endurance exercise, while Mb levels measured during exercise did change significantly compared to pre-exercise levels, it peaked after 1 hour from the exercise, and normalized after 12 hours (Suzuki *et al.* 1999).

Ingram *et al.* (2009) found that CRP (C-reactive protein), which is one of the risk factor indicators of myocardial infarction, was lower in the group that underwent cold water immersion after exercise in comparison to both control and contrast water immersion groups. Other studies have shown that cold-water immersion accelerated restoration of parasympathetic functions and repaired cardiac muscle damage (Al Haddad *et al.* 2010; Miyamoto *et al.* 2006; Mourrot *et al.* 2008). Connelly *et al.* (1990) and O'Hare *et al.* (1986) reported reduced plasma norepinephrine (NE) levels after cold-water immersion; and Mono *et al.* (1985) found reduced sympathetic activity in cardiac and skeletal muscles following cold-water immersion. In another study on cold-water immersion, found different to our result, recovery from repeated sprint activity is not improved by either seated or standing cold-water immersion. (Leeder *et al.* 2015). Also, there are a lot of study related to cold water and muscle soreness of athletes. In these studies indicate that, cold water or ice immersion application after concentric and eccentric exercise, has positive effect of recovery. (Lynch & Barry, 2012; Hatzel & Kaminski, 2000; Mattacola & Perin, 1993)

In conclusion, we found that eccentric muscle contractions caused cardiac muscle damage. The circulating levels of Troponin T, which is found in cardiac muscle fibers and is one of the most important indicators of cardiac injury resulting from exercise showed an increase in correlation with the extent of injury, and that this damage was reduced remarkably at the end of 24 hours by cold-water immersion compared to the control group. Development of exercise-induced cardiac damage as determined by myoglobin, which is another indicator of cardiac damage, was not statistically significant. However, it was found that myoglobin levels showed less increase following cold-water immersion in the experimental group. The study results indicate that cold-water immersion is beneficial for reducing the cardiac troponin T and myoglobin levels development that cause cardiac damage in athletes, and shortens the recovery period.

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