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Short term pre-cooling maneuvers - effect on dehydration and thermoregulation during an exercise test in the warm environment

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Abstract. *Background*. Long period of pre-cooling reduces heat stress and leads to performance improvement in warm environments. This study examined the effectiveness of the shorter period of pre-cooling using cold water immersing or cold shower exposure on hydration status and thermoregulation in the heat condition (temperature=32-34°C, humidity=50%). *Material and Method*. Twenty four volunteer young male soccer players were randomly divided into three groups. The groups completed 45 minutes of treadmill running test either after warm up (non-pre-cooling group, n=8, $VO_{2max,\ ml/kg/min}$=50.69± 5.68), or after warm up+ 10 minutes of cold water immersion (cold water immersion group, n=8, $VO_{2max,\ ml/kg/min}$ =50.62± 6.90), and or after warm up+ 2 minutes of cold shower (cold shower group, n=8, $VO_{2max,\ ml/kg/min}$ = 51.16± 5.92). Oral temperature, plasma lactate, K, and Na were measured at rest (pre test), after (mid test) warm- up or pre-cooling, and after exercise test (posttest). Plasma volume and dehydration were calculated using related formulas. *Results*. Post test oral temperature and plasma lactate were significantly ($P<0.05$) higher, while post test body weight was significantly ($P<0.05$) lower than the pre test in the study groups. No significant differences between the three groups were found for oral temperature, plasma volume, dehydration status, plasma lactate, K and Na at the post test. *Conclusion*. Based on these results, it can be said that there is no evidence for the positive effect of the short time pre-cooling methods on thermoregulation and dehydration during exercise in the heat condition. This trial is registered with Iranian Registry of Clinical Trial (IRCT) number IRCT2015121325503N1.
Key words*: hot temperature, plasma volume, dehydration, body temperature, young soccer players.*

Introduction

Athletes often exposure to the hot environments because of exercise training or competition. Physical activity combined with the ambient heat stress cause body fluids loss and electrolyte imbalance, which can impair cell biochemical reactions and cardiovascular system function (1, 2). The body fluid is usually normal at the beginning of exercise in the heat, but hypohydration or dehydration will be inevitable when exercise continues for a long period of time (3). This exercise induced hypohydration can lead to a mismatch between muscle blood flow and metabolic demands, or can result in a higher body temperature due to skin blood flow restriction (4). There is evidence that reduction in active muscle blood flow is secondary to the skin blood flow restriction and muscle blood flow is maintained in expense of skin blood flow reduction (4). Blood pressure is also found to be at reasonable levels during exercise in the heat (4, 5). Therefore, reduced skin circulation is the most probable consequent of exercise in the hot environment (4). This can result in central temperature elevation, and if left untreated may progress to life threatening heat stroke due to the multi system organ failure (6).

Acclimatization, fluid ingestion and pre-cooling are the most used approaches to prevent the adverse effects of exercise in the heat (7). Cold water immersion (8), ice slurry ingestion (9), cooling garments (10) and cold shower (11) are different pre-cooling maneuvers which have been used to reduce the core body temperature prior to exercise in the heat. Comparison of the of the cooling modalities effectiveness reveals that cold water (preferably cold water immersion) is the superior method for treatment of exertional heat stork and cold showers should also be considered if water immersion is impossible(12).

Several qualitative studies had shown the ergogenic and thermoregulatory effects of cold water immersion and cold shower on aerobic and anaerobic performances (11, 13, 14). It is noteworthy that hundreds of high school students in our country involved in exercise training programs during the summer holidays which lasts for three months. They often do not drink enough fluids when exercising or participating in sports. It is well documented that summer exercise training programs induce more exertional heat–related illness than training during winter and spring (6). Despite this, no study exists in the literature that compares the effects of different pre-cooling methods in Iranian high school students. Studies on the pre-cooling had recommended the longer periods of pre-cooling to attenuate negative physiological effects of exercising in high ambient temperatures (11, 14). From a practical perspective, long period of pre-cooling is impossible when the large numbers of student athletes are involved. We think that if evidence to be provided to demonstrate effectiveness of short term pre-cooling, then it would be more practical in the field settings. This study aimed to examine the effects of two different methods of short term per-cooling on thermoregulation and hydration in young male soccer players during 45 minutes of treadmill running test in the heat condition.

Material and method

This study was a randomized controlled trail. All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration ethical standards o and the study was conducted following approval from the ethical committee of the University of Zanjan (Islamibc Republic of Iran). Twenty four young male soccer players from provincial teams (Zanjan, Iran) volunteered to participate in this study. Medium effect size was calculated for sample size of 24, α of 0.05 and power of 0.80 by power analysis for repeated measure. The subjects singed an informed consent form. After baseline measurements, they were divided into three equal groups based on the VO_{2max}, age and weight, and were randomly assigned to non-pre-cooling(NP), cold shower pre-cooling (CS) and cold water immersion pre-cooling (CWI) groups (table I).

Table I. Study groups characteristics

	$VO_{2max(mi/kg/min)}$	Weight(kg)	Height(cm)	Age(year)
NP(n=8)	50.69± 5.68	61.90±12.31	170.00±4.78	16.1± 1.9
CWI(n=8)	50.62± 6.90	63.56± 10.28	171.75± 6.40	16.1± 1.1
CS(n=8)	51.16± 5.92	71.03± 12.56	174.08±4.07	16 ± 2

NP; Non-pre-cooling. CWI; Cold water immersion. CS; Cold shower

Baseline measurements. As shown in figure 1, three days before the main trail subjects arrived at exercise physiology laboratory (temperate temperature) for the baseline assessments after overnight fasting. A standard breakfast was consumed at 8 a.m. Height and weight were measured using stadiometer and electronic scale, at 10 a.m.

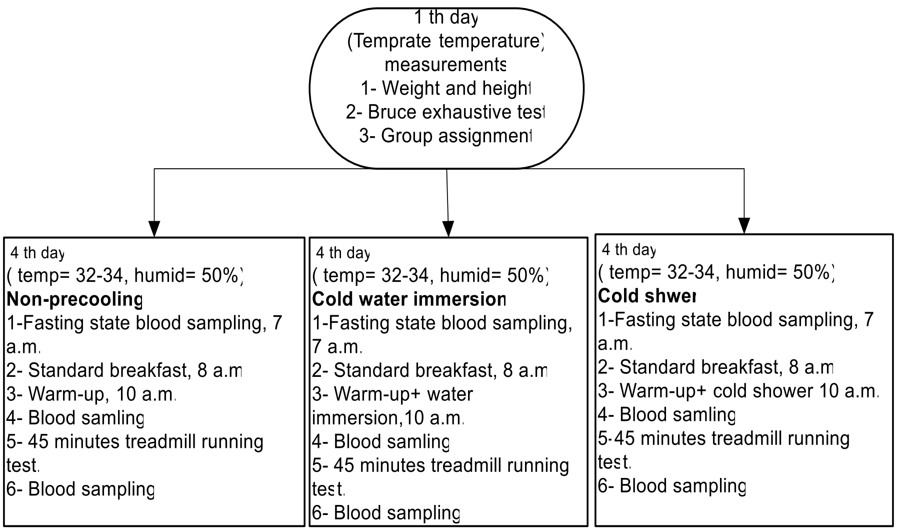

Figure 1. Flow chart

Then, the VO_{2max} was assessed by Bruce Multistage maximal exercise test on a motorized tread mill (Cosmed, Italy) and the following formula was used to calculate the VO_{2max} (T= total time on tread mill in minute)(15):

$VO_{2\,max}$ (ml/kg/min) = 14.8 - (1.379 × T) + (0.451 × T2) - (0.012× T3)

Three days after baseline assessments, subjects reported to the heated laboratory following a 10 hour overnight fasting. The temperature in the laboratory was maintained at 32-34°C throughout the experiments.

Blood samples collection and blood variables assessment. Blood samples were collected from the left antecubital vein as follows. Pretest Fasting blood sample at 7 a.m. Mid test sample: all the groups consumed standard breakfast at 8 a.m. Blood samples were drowned either after 10 minutes of warm up (NP), or after 10 minutes warm up+ 10 minutes of cold water immersion (CWI), and or 10 minutes warm up+ 2 minutes of cold shower (CS) at 10 a.m. Post test sample: in the all study groups immediately after treadmill running test.

The system K-4500 automated hematology analyzer was used to determine Hemoglobin (mg/dl) and hematocrit (%) concentrations in the blood samples. Dill and Costill method was used to calculate the pretest, mid test and post test plasma volume from the values of hematocrit (Ht,%) hemoglobin (Hb, mg/dl) (16)

Plasma lactate concentrations were measured using Kobas Auto-analyzer RA 1000 and kits manufactured by Pars Azmoon Company of Iran. Zist Chem kits (Zist Chem. Company, Tehran, Iran) and Electrolyte Analyzer were used to assess the plasma Na and k concentrations.

The percentage of dehydration was calculated using the next formula (17): % Dehydration= (Pretest weight – Posttest weight)/Pretest weight x 100

Oral temperature. Oral temperatures were recorded in sub lingual pocket at pre (Baseline), mid (NP group, at the end of warm-up; CWI group, at the end of warm up+ water immersion; CS group, at the end of warm up+ cold shower) and post test (immediately after treadmill running test) by Beurer FT 09 Digital Clinical Thermometer (Germany)**.**

NP group conducted 45 minutes of the treadmill running test after a 10 minutes of warm (10 minutes tread mill run[7km/h]), while the same test was done by CWI and CS groups after 10 minutes warm up+ 10 minutes cold water immersion and 10 minutes warm up+ 10 minutes cold shower, respectively.

Pre-cooling maneuvers:

- *Cold water immersion.* A small pool filled with water of 24°C and the water temperature was maintained between22- 24 °C throughout immersion test. Participants in the CWI group immersed up to the neck for 10 minutes.
- *Cold shower*. We conducted a small pilot study involving 5 subjects**.** Shivering response was observed during 10 and 5 but not in 2 minutes of cold shower exposure. Therefore cold shower exposure time was reduced to 2 minutes to eliminate the shivering response. The subjects of the CS group stood under showers for 2 minutes. Water temperature was gradually reduced from 30- 32°C to18- 20°C (4°C / 30 seconds). All the experiments were conducted at the temperature and humidity of 32-34 °C and 50%, respectively.

Treadmill running test. During soccer match, the mean speed of running has approximately been reported 10 km/h in young soccer players (18). With respect to this, a 45 minutes treadmill running test was designed to simulate this running speed. During the exercise test, treadmill started at 7 km/h, the speed was increased by 1 km/hr every 2 minutes up to the 10 km/h, after then the constant speed was maintained throughout the test.

Statistical analysis. The Shapiro-Wilk test verified normal distributions of the data for VO_{2max}, weight, height and age. If assumptions for normality were not met (plasma volume, oral temperature, plasma lactate, Na and K), data were log transformed prior to analysis. The untransformed values are shown in the text and tables. NP, CWI and CS groups were as between subjects' factor, and time (pre test, mid test and post test) was regarded as within subjects' factor. A mixed repeated measures analysis of variance was used to assess the main effects and interaction effect of these factors on the dependent variables. If the significant time effects were observed, then one way repeated measure with Boferroni as post-hoc were used for further analysis.

Within group weight change was assessed by independent t test and one way analysis of variance was used to compare the subjects' characteristics, dehydration status and changes in weight. Research data were analyzed by SPSS-19. Significant level was set $p \leq 0.05$.

Results

Body weight and dehydration: all the experimental groups showed significant body weight reduction after the treadmill test in the heat (NP: P=0.00; CWI: P=0.00; CS: P=0.001). No significant between groups differences were found on body weight reduction measures [F (2, 23) = 2.26, p= 0.12] (table II). Dehydration was calculated 1.61±0.37 %, 1.69±0.51 % and 1.85±0.47% for the CWI, CS and NP groups, respectively, with no significant differences between the study groups [F (2, 23) = 0.60, p=0.55] (table II).
Plasma volume: all the three groups did not show significant change in the plasma volume during experimental period [F (2, 42) = 1.15, P= 0.2]. There were no significant between groups differences on plasma volumes at pre, mid and post test [F (2, 21) =0.64, p=0.53] (table II).

Table II. Comparison of body weight, dehydration status and plasma volume between study groups in the heat environment (temp=32-34 °C, hum= 50%)

	NP(n=8)		**CWI(n=8)**		**CS(n=8)**	
	Pretest (rest)	Post test (b)	Pretest (rest)	Post test (b)	Pretest (rest)	Post test (b)
Weight (kg)	61.90± 12.31	60.71± 11.87*	63.56± 10.04	62.51± 1 0.04*	71.03±1 2.56	70.53±12.68*
Plasma volume (ml/100ml)	54.04± 4	56.06± 2	56.2± 2.4	56.5± 2	55.4± 1.0	56± 1.7
Weight loss (kg) (pretest-post test)		1.18± 0.5		1.17±0.3		1.17±0.30
Dehydration% (post test)		1.85±0. 4		1.61±0.3		1.69±0.5

*NP; Non-pre-cooling. CWI; Cold water immersion. CS; Cold shower. (a) after warm-up; (b) after exercise test ;(c) after warm-up+ water immersion ;(d) after warm up +cold shower. *significantly different from pretest. P≤ 0.05.*

Oral temperature: all the study groups showed significant change of oral temperature during the experiment [F (2, 42) = 61.4, P= 0.002, Partial Eta Squared=0.74]. Using One way repeated measure and Boneferroni tests, post test oral temperature was found higher than the pre and mid test in the NP (p=0.001, p= 0.04), CWI (p=0.007, p=0.002) and CS (p=0.001, p= 0.001) groups. There were no significant between group differences in pre, mid and post test oral temperatures [F (2, 21) =2.48, p= 0.10]. A reflection of the similar thermal response to treadmill test in the three study groups (table III).

Table III. Oral temperature at rest [mean (±SD)], after warm up or pre-cooling and after exercise test in the heat environment (temp=32-34 °C, hum= 50%)

	NP(n=8)			**CWI(n=8)**			**CS(n=8)**		
	Pretest (rest)	Mid test (a)	Post test (b)	Pretest(rest)	Mid test (c)	Post test (b)	pretest(rest)	Mid test(d)	Post test (b)
Oral temp°	36.52± 0.23	36.71± 0.72	37.42± 0.52*†	36.16± 0.50	36.17± 0.54	37.61± 0.51*†	36.26±0 .37	36.37± 0.39	37.42± 0.73*†

*NP; Non-pre-cooling. CWI; Cold water immersion. CS; Cold shower. (a) after warm-up; (b) after exercise test ;(c) after warm-up+ water immersion;(d) after warm up +cold shower. *, significantly different from pretest; †, significantly different from mid test. P≤ 0.05.*

Plasma sodium and potassium: In the study groups, no significant changes were found in plasma Na and K concentrations from the pre to post test [Na: F(2,42)=2.66, p= 0.82. K: F (2, 42) = 1.56, p= 0.2]. These variables were not significantly different between the NP, CWI and CS groups at pre, mid and post test assessments [Na: F (2, 21) =0.34, p= 0.71. K: F (2, 21) =.24, p=0 .78] (table IV).
Plasma lactate: there was significant change in plasma lactate from pre to post test in the three study group [F (2, 42) =39, P=0.001, Partial Eta Squared=0.65)]. Post test plasma lactate was significantly higher than that of the pre [NP (p=0.01), CWI (p= 0.02) and CS (p= 0.004)] and mid test [NP (p=0.001), CWI (p=0.03) and CS (p= 0.003)] in the study groups. Between groups comparisons revealed that there were no statistical differences in the plasma lactate among the NP, CWI and CS at pre, mid and post test [F (2, 21) =0.19, p=0.82] (table IV).

Table IV. Plasma electrolytes and lactate [mean (±SD)] at rest, after warm up or pre-cooling and after exercise test in the heat environment (temp=32-34 °C, hum= 50%).

	NP(n=8)			**CWI(n=8)**			**CS(n=8)**		
	Pretest (rest)	Mid test (a)	Post test (b)	Pretest (rest)	Mid test (c)	Post test (b)	Pretest (rest)	Mid test (d)	Post test (b)
Na^+ (mEq/L)	140.32± 1.18	140.83± 1.47	141.76± 0.89	141.33± 1.58	142.48±1. 88	141.86±1. 00	141± 0.67	141.71± 2.06	142.32± 1.71
K^+ (mEq/L)	4.04± 0.38	3.75± 0.19	3.79± 0.20	3.92± 0.25	3.94± 0.42	3.72± 0.15	4.12± 0.45	3.90± o.21	3.76± 0.15
Lactate (mg/dl)	15.01± 0.77	14.92± 1.30	26.61± 2.88*†	14.31± 0.73	16.56± 0.70	28.05± 4.40*†	15.70±0. 77	15.18± 2.33	24.25± 2.18*†

*NP; Non-pre-cooling. CWI; Cold water immersion. CS; Cold shower. (a) after warm-up; (b) after exercise test ;(c) after warm-up+ water immersion;(d) after warm up +cold shower. * significantly different from pretest; †, significantly different from mid test. P≤ 0.05.*

Discussion and Conclusion

This study was designed to examine the effects of two different maneuvers of pre-cooling (cold water immersion or cold shower) on oral temperature, plasma volume, plasma electrolytes (Na and K) and lactate in the heat environment**.** In this study no significant changes were found in Oral temperature either after warm up + cold water immersion or warm up+ cold shower , compared with warm-up alone (table III). Oral temperature Significant increase was observed in the NP, CWI and CS groups from the mid to the post test, without any significant differences between the study groups at post test (table III). Increasing the margin for heat production and increasing the time to reach exhaustion during physical activity at heat ambient is the basis of the precooking (19). Marino and Booth (14) reported a significant core temperature after drop with water immersion. Drust et al (11) also showed reduction in core temperature by having subjects stand under a cold shower. In contrast to our result, the lower core temperature in the pre-cooling condition has been maintained throughout exercise test and resulted in less thermoregulatory strain than the non-pre-cooling condition (9).

The true invasive core temperature measurement was impossible in this study; therefore the oral temperature was used as the index of core temperature. There is debate about the oral temperature accuracy in measuring core body temperature. It has been stated that Sublingual pocket is close to the sublingual artery and reflects changes in core body temperature; therefore oral cavity temperature is considered as a reliable index of core temperature when the thermometer is placed into the sublingual pocket (20). In the current study, oral temperatures were recorded in the sublingual pocket, therefore it can probably be said that our pre-cooling maneuvers did not reduce core body temperature in the pre-cooled groups, which was probably due to the brief exposure time. We conducted10 and 2 minutes of cold water immersion and cold shower exposure ,respectively, compared with 60 minutes of immersion (14) or cold shower exposure (11) in the other studies. Thus, it can be said that the lack of positive thermoregulatory effect of pre-cooling during exercise test was due to the fact that the pre-cooling maneuvers did not create significant gradient for heat dissipation.

Pretest body weights were similar in the NP, CWI and CS groups, indication of similar hydration before exercise at heat condition (table I). Significant weight loss occurred from pretest to the end of the exercise test in the three groups, without any significant differences between the groups, indication of a similar dehydration status in the study groups (table II). It is well documented that body weight reduction immediately after exercise is an indication of body water deficit and dehydration that can adversely affect performance and thermoregulation (21). Dehydration levels were calculated 1.61±0.37 %, 1.69±0.51% and 1.85±0.47% for the CWI, CS and NP groups, respectively (table II), which caused no significant increase in the plasma volume at the end of the treadmill test (table II). The underlying mechanism of plasma volume increment seems to be the intracellular fluids contribution which behaving as temporary reservoirs for plasma volume recovery (22). All the three study groups showed no significant changes in the plasma Na and K concentrations from pre to the post test (table IV). The concentration of Na in sweat has been reported to increase with an increasing sweating, which may lead to low plasma sodium concentration (2). Our subjects in the three groups were dehydrated after exercise test, the most probably because of excessive sweating. We expected plasma Na decline after exercise test, due to the sweating. The concentration of sodium in sweat was not measured in this study, but mathematical models indicate that the sweat Na loss is insufficient to produce plasma Na reduction (23).

Another explanation is that about one fourth of total body sodium exists in bones and cartilages and is recruitable to compensate reduction in plasma sodium (24). In line with our finding, plasma potassium concentration has been reported to remain stable after prolonged exercise and dehydration (25). Since, loss of potassium in sweat and urine is so low that it cannot affect the potassium content of plasma (26). These results indicated that dehydration status was similar in the study groups. In contrast with our results, a previous study had documented that pre-cooling delays or decreases the sweat response and postpone dehydration during physical activity in the heat (27). This is achieved by creating a greater body heat storage capacity, delaying the onset of heat dissipation mechanisms, and lengthening the time required to reach sweat threshold (28). The short durations of the pre-cooling maneuvers in our study may explain the discrepancy in results between our study and previous studies.

We found similar plasma lactate response to exercise test in the study groups (table IV). No significant change from pre to the mid test and significant increase from mid test to the post exercise test (table IV). It is established that high ambient temperature induces several metabolic adjustments which might also result in the increased plasma lactate during exercise at heat (29). Because, reduced skeletal muscle blood flow due to the dehydration in the heat environment could decrease aerobic metabolism efficiency and increase reliance on anaerobic glycolysis, which results in a raise in plasma lactate during exercise at heat (30). It has been shown that Pre-cooling attenuates dehydration and reliance on anaerobic metabolism during exercise at high ambient temperature. In the current study, despite the pre-cooling, either through water immersing or cold shower, plasma lactate response and dehydration status was not different between the per-cooled groups and control condition (table II and IV). To our knowledge, the main reason for the similar plasma lactate response in the pre-cooled and control group was the lack of the effectiveness of the pre-cooling methods to attenuate dehydration.

The results of this study showed that there are no thermoregulatory, metabolic and body fluid balancing advantages to the short term pre-cooling during exercise at high ambient temperature. Additional studies using long periods of pre-cooling and soccer specific tests are needed to examine the effects of pre-cooling maneuvers.

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