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Muscle oxygenation and electrical activity changes between genders after cold-water immersion as pre-cooling among amateur young adult mini-marathon runners

KURUSART KONHARN

Abstract

Introduction. No prior research has been conducted to investigate gender differences in physiological responses of muscle oxygenation and electrical activity following pre-cooling at a controlled room temperature. Aim of Study. To determine how cold-water immersion (CWI) affected muscle oxygenation and electrical activity in amateur mini-marathon runners with different genders. Material and Methods. A total of 30 amateur young adult mini-marathon runners were randomly divided equally into two groups. The non-CWI group (n = 15) performed 5 minutes traditional dynamic stretching while the CWI group (n = 15) immersed in ice-cold water for 5 minutes. Main outcome measure(s): muscle oxygenation was measured using Moxy sensor monitor and muscle activity was recorded by electromyography. Results. CWI group showed a 20% significant increase in muscle oxygenation percentage (P = 0.02). Males experienced a 15% greater increase (P = 0.04) than females (P = 0.02). Meanwhile, females in the non-CWI group experienced significant changes in muscle oxygenation percentage, but not males. Muscle electrical activity in the CWI group decreased by 28% at all times (P = 0.02). No statistically significant differences in muscle electrical activity were found between sexes. Conclusions. CWI as pre-cooling had a more beneficial effect in increasing oxygenation and decreasing electrical activity in muscle, with males having a slightly superior result in improving muscle oxygenation after pre-cooling among amateur young adult mini-marathon runners, compared to traditional method.

KEYWORDS: cryotherapy, muscle contraction, long-distance running, muscle oxygenation, active adult.

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Corresponding author: mf_thailand@yahoo.com

[1] *Khon Kaen University,*
[2] *Faculty of Associated Medical Sciences,*
[3] *School of Physical Therapy, Khon Kaen, Thailand*

Introduction

The popularity of long-distance running has grown around the world over the last decade; there were 507,600 total marathon finishers in 2016. The increase in mini-marathon runners indicates the participation of both genders, 46.9% in males and 56.8% in females [25]. A mini-marathon is run over 10.5 km, with an average finishing time of about 1 hour in females and 55 minutes in males [16]. Regardless of a runner's level of ability, a marathon puts a greater demand on their body's physiological systems over a period of several hours.

Long-distance running causes an increase in heat production, which typically exceeds heat loss capacity, and results in an elevation of body's core temperature. Maintaining body's core temperature is a significant element of a marathon runner's performance. Preserving the physical performance can be achieved by using a strategy to prevent fatigue due to the thermoregulatory changes in the ability of the human thermoregulatory system to lower the core temperature [2]. During running, the temperature difference between a body center and skin narrows, demanding an increase in muscles to sustain heat transmission, and in order to obtain adequate cardiac output, a heart rate must be increased, which results in widening margins between the body's core and ambient temperatures [12]. However, an insufficient limitation of the temperature rise while running results in hyperthermia which has a negative effect on running top performance [12, 14]. Moreover, the excessive increase in body temperature is considered detrimental. The cardiorespiratory system's adaptability is critical during strenuous exercises for increasing both convective and diffusive oxygen transport, allowing a body to meet demands for oxygen, substrate delivery, and carbon dioxide removal [7].

In general, dynamic stretching can be used before beginning any exercise routine to warm up a human body or to prepare muscles for exercises. Dynamic stretching can also increase the body temperature, blood flow and circulation [17]. In order to prevent the body's core temperature from rising too quickly during prolonged running, some cooling techniques before the activity have been used with varying degrees of success [14]. Following pre-cooling, it is likely that the reduction in cardiovascular pressure and central blood volume is better maintained, allowing for greater oxygenated blood supply delivery to the working muscles during running. Appropriate application of cold lowers the core body temperature and increases heat storage capacity, allowing athletes to perform at a higher relative level or for a longer period of time [14]. Pre-cooling methods help increase the thermoregulatory system's heat storage capacity before exercising. This enables people to complete more tasks before reaching the critical core temperature threshold during running. However, the use of varying levels of pre-cooling to improve physical performance in runners appears to be controversial. Current evidence shows cold-water immersion (CWI) may be the most effective method of pre-cooling to increase running performance in hot conditions [1].

When it comes to the physical demands of long-distance running, both physiological and environmental factors can have an impact on the running performance. A previous study reported that environmental temperature also has an exceptionally large influence on the effectiveness of a cooling technique. Recent systematic reviews and meta-analysis [2] show that those studies were implemented at environmental temperatures of >30 °C and <12 °C, and cooling degrees of >15 °C. They were conducted at high temperature, which is known to increase thermoregulatory challenge, and thus has an effect on cardiovascular system stress [2, 11]. The ideal environmental condition for a world record is a temperature of 18.6 °C. Therefore, it is interesting to investigate the effects of cold application prior to an exercise in a condition between 20-25 °C. Different genders appear to respond differently to whole-body cryotherapy [9] and environmental temperature [21]. Overall, a rate of cooling and heat loss is proportional to a surface area to volume (mass) ratio and a level of adiposity; whereas the level of adiposity and body size have a significant impact on maintaining core body temperature under cold conditions [9]. Recent studies have discovered that during the maximum exertion phase during an exercise, men have higher muscle oxygen saturation levels than women, due to a greater decline in females, which could be linked to anatomical and functional differences, such as surface area to mass ratio, or higher subcutaneous fat content [8]. To the best of the authors' knowledge, no study has investigated gender differences in physiological responses of muscle oxygenation and electrical activity after pre-cooling, as previous researchers have only studied pre-cooling in relation to thermoregulation with mostly male athletes [8, 11, 20].

In comparison to non-athletes, such as amateur runners [10], more experienced athletes might be more responsive to their physiological limits. Individuals who engage in multiple uncompensated heat stress conditions on a regular basis may develop fatigue symptoms, reducing the amount of time spent at high core temperatures and thus reducing total thermal load experience.

This study therefore aimed to determine the effect of the application of cold before running on muscle oxygenation and electrical activity of muscles in amateur young adult mini-marathon runners at a controlled temperature as the ideal environmental condition. It is hypothesized that the CWI method as pre-cooling at a controlled room temperature would have a beneficial effect on physiological changes such as increasing muscle oxygenation and decreasing electrical activity levels among amateur young adult mini-marathon runners.

Material and Methods

Participants and their characteristics

Thirty amateur young adult mini-marathon runners (16 males and 14 females) were selected and estimated by using the G*Power 3.1.9.7 software with 2-group 2-tailed t-test, using the magnitude of increase in rectal temperature during an exercise from a previous study [6], having an effect size (Cohen's d) of 0.80, an α error probability of 0.05 and a β of 0.2. All participants who were recruited in this study were aged from 19 to 35 years (22.2 ± 3.1 years), with a body mass index (BMI) classified as normal weight (18.5-24.9 kg/m^2). They regularly engaged in moderate-to-vigorous physical activity (30 minutes/day, 3 days/week for 3 months consecutively), and had previously participated in an organized mini-marathon at least twice [6].

Those with severe cold allergies (severe erythema, itchy welts, hives, or irregular breathing) were excluded from the study. Each subject was asked to sign the consent form and then the experimental procedures were verbally explained by the researchers. The recruitment of all subjects was conducted in a month at different times for each subject, and after they passed a health status screening, they were randomly allocated into one of the groups (the CWI or non-CWI group).

The experimental group (the CWI group) consisted of 15 subjects (9 males and 6 females) aged 22.5 ± 4.0 years, with a weight of 60.6 ± 15.0 kg, a height of 168.8 ± 10.7 cm, a BMI of 20.9 ± 2.7 kg/m², a resting heart rate (RHR) of 84.2 ± 15.1 bpm, a resting systolic blood pressure (RSBP) of 118.6 ± 15.2 mmHg, a resting diastolic blood pressure (RDBP) of 75.9 ± 12.6 mmHg, and a resting body temperature (BT) of 37.2 ± 0.5 °C (Table 1). Meanwhile, the control group (the non-CWI group) consisted of another 15 subjects (7 males and 8 females) aged 21.9 ± 2.1 years, with a weight of 53.9 ± 8.2 kg, a height of 164.1 ± 9.3 cm, a BMI of 19.9 ± 1.4 kg/m², a RHR of 82.5 ± 11.7 bpm, a RSBP of 117.1 ± 15.3 mmHg, a RDBP of 71.6 ± 10.8 mmHg, a BT of 37.1 ± 0.5 °C (Table 1). The study protocols have been reviewed and approved by the Center for Ethics in Human Research, Khon Kaen University, Thailand (Ref. No.: HE642206).

Experimental procedures

A quasi-experimental study with two groups (the experimental and control group) was conducted to examine physiological changes between pre- and postinterventions on running performances. A simple random sampling technique (the lottery method) was performed during participants' recruitment; this method of randomization was used to ensure that an equal number of subjects was allocated to each group, and each subject was given an option of choosing a date/session from a testing schedule.

Throughout a health status screening, all participants were instructed to avoid strenuous exercises before the day of the experiment, and to prepare whatever they needed to bring for the data collection (sportwear, running shoes, extra clothes, towels for the CWI subjects, and drinking water were provided). All subjects were ready in their sportswear when they came to a laboratory. Prior to the main study, all participants were asked to complete a health status screening by filling out the Physical Activity Readiness Questionnaire (PAR-Q), and measuring their vital signs; blood pressure was measured using a digital sphygmomanometer (Omron HEM-7361T, Japan) and heart rate measurement was done with a Polar HR monitor and a chest strap (Polar H7 HR Monitor, Finland). Body temperature was

Table 1. Demographic characteristics of participants (mean ± SD)

Characteristics	non-CWI (n = 15)	CWI (n = 15)	p-value	Male (n = 16)	Female (n = 14)	p-value	Total (n = 30)
Male : female (n)	7 : 8	9 : 6					
Age (years)	21.9 ± 2.1	22.5 ± 4.0	0.65	22.9 ± 3.7	21.4 ± 2.2	0.21	22.2 ± 3.1
Weight (kg)	53.9 ± 8.2	60.6 ± 15.0	0.14	65.4 ± 10.8	47.9 ± 5.7	<0.001*	57.2 ± 12.4
Height (cm)	164.1 ± 9.3	168.8 ± 10.7	0.21	173.4 ± 7.2	158.5 ± 6.4	<0.001*	166.5 ± 10.1
BMI (kg/m^2)	19.9 ± 1.4	20.9 ± 2.7	0.20	21.5 ± 2.2	19.1 ± 1.5	0.002*	20.4 ± 2.4
RHR (bpm)	82.5 ± 11.7	84.2 ± 15.1	0.73	81.9 ± 16.3	85.0 ± 9.2	0.53	83.3 ± 13.3
RSBP (mmHg)	117.1 ± 15.3	118.6 ± 15.2	0.79	125.0 ± 15.0	109.6 ± 10.3	0.003*	117.8 ± 15.0
RDBP (mmHg)	71.6 ± 10.8	75.9 ± 12.6	0.33	78.2 ± 13.2	68.6 ± 7.4	0.02*	73.7 ± 11.8
BT (°C)	37.1 ± 0.5	37.2 ± 0.5	0.59	37.1 ± 0.5	37.2 ± 0.4	0.85	37.2 ± 0.5

Note: CWI – cold-water immersion; non-CWI – non-cold-water immersion; BMI – body mass index; RHR – resting heart rate; RSBP – resting systolic blood pressure; RDBP – resting diastolic blood pressure; BT – body temperature

Data are presented as means ± standard deviation. Independent sample t-test was used to analyze the differences between the groups (CWI and non-CWI). Paired sample t-test was used to compare the differences between the genders (male and female).

$^*p < 0.05$

measured using an infrared thermometer (DermaTemp, Exergen, Massachusetts, USA) at rest.

Each subject completed all protocols of the experiment in a single day. Before conducting the main study protocol, both groups performed the same warm-up procedure (dynamic stretching for 5 minutes, moving to full active range of motion at moderate speed and then continuing with light running for 3 minutes). They were asked to

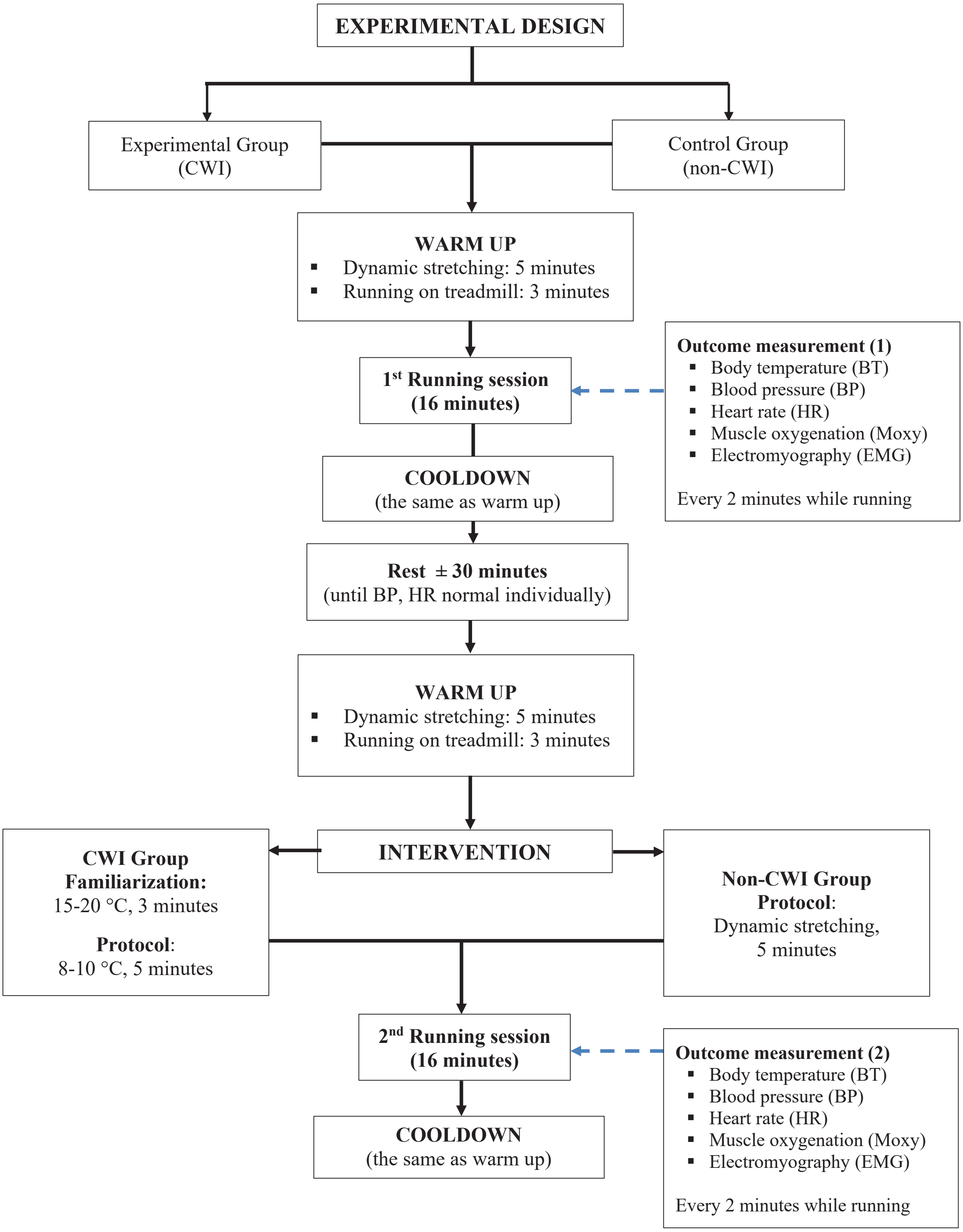

Figure 1. A flowchart of the study

run at their average running speed (pace) recorded within the last 3 months on their usual running devices such as a smartwatch or a smartphone running application.

According to the results of the previous study, in response to body water immersion as the pre-cooling method the muscle and skin temperatures reached a plateau value at the 16th minute from the start [4]. For a baseline measurement, a first running session was performed by running for 16 minutes on a treadmill (Treadmill Bolzen EX, Australia) and each subject ran at their own average running speed at a constant speed with the RPE being monitored [27]. All outcomes were recorded every 2 minutes while they ran. If during the running sessions the subjects had experienced upper-body discomfort, lightheadedness, nausea, faintness, shortness of breath, joint pain or unspecified pain, they could have stopped at any time, and a researcher would have asked them for another additional appointment at a suitable location and time. After each subject finished their first running session, they performed a cooldown procedure which was the same as the warm-up. Then they took a rest for 30 minutes or until their heart rate (HR) and blood pressure (BP) went back to normal [15].

Before the subjects started their second running session, they underwent two different interventions. The experimental group (the CWI group) was asked to immerse their bodies in a plastic tub up to a level of a hip in a long sitting position, with the first 3 minutes as familiarization in 15-20 °C cold water [15], followed by 5-minute immersion in 8-10 °C as the main intervention. Meanwhile, the control group (the non-CWI group) performed dynamic stretching exercises with an average duration of 5 minutes [27]. Next, all subjects in both groups continued with the same warm-up procedure as during the first session, and then they started running on the treadmill as the second running session with the similar outcome measurements. After the run they did a cooldown protocol. All sessions were done at a controlled room temperature of 25 °C (Figure 1).

Interventions

Cold-water immersion

All subjects in the CWI group were immersed in a long sitting position at the hip level in a plastic tub with a width of 100 × 80 cm, and a depth of 60 cm, one by one, to prevent the spread of infections. Two plastic tubs were provided, water and ice were changed for every new subject. To avoid cold shock and hypothermia caused by a sudden immersion in a low temperature, which may have an adverse effect on normal body functions, the first 3 minutes of CWI were allocated for a familiarization at 15-20 °C, then the primary CWI protocol was conducted at 8-10 °C for the next 5 minutes. A glass thermometer was used to continually monitor the temperature of the cold water.

Dynamic stretching

The subjects in the control group (the non-CWI group) performed dynamic stretching of five target muscles: hips extensors and flexors, legs extensors and flexors, and plantar flexors, as the traditional protocol used by athletes. This protocol was done as follows: (1) hips extensors: the subjects leaned forward and lifted their foot from the floor with their hip and knee joints slightly in flexion. Then, the subjects contracted their hip joint extensors and extended their hip joint so that their leg was extended to the posterior aspect of their body; (2) hips flexors: the subjects contracted their hip joint flexors with their knee joint flexion and then flexed their hip joint so that their thigh could come up to their chest; (3) legs extensors: the subjects contracted their hamstrings and flexed their knee joints so that their heel kicked their gluteus muscle; (4) legs flexors: the subjects contracted their hip joint flexors and flexed their hip joint, raising their thigh parallel to the ground with their knee joint flexed at about 90°. Then, the subjects contracted their quadriceps with the height of their thigh maintained, and then extended their knee joint, so that their leg extended to the anterior aspect of their body; and (5) plantar flexors: the subjects lifted one foot from the floor and fully extended the knee joint. Then, the subjects contracted their dorsiflexors and dorsiflexed their ankle joint, so that their toes were raised. Three sets of each stretch were performed, with 30 seconds per movement; all of these stretching procedures were done in a total of 5 minutes [27].

Outcome measurements

Muscular activity – recorded with a surface electromyography device – and muscle oxygenation – measured with a near infrared spectroscopy (NIRS) device – are considered the main approaches in a monitoring of a targeted muscle [22]. The outcome measurements in this study were muscle oxygenation records from a Moxy monitor and muscle electrical activity records from EMG. When assessing non-standardized muscles, the researchers looked for either muscle belly dominance or an area with minimal adipose tissue, then returned to standardized anatomical landmarks. The Moxy sensor monitor and EMG electrode placement guidelines have a similar goal and are widely used. The standard electrode/sensor should be placed on an area where there is very little chance of a large common-mode disturbance signal. In this study, the medial gastrocnemius muscle on the dominant side of the lower extremity has been measured.

Muscle oxygenation

Muscle oxygenation measurement was conducted with the usage of a Moxy monitor and antenna (Moxy-1 sensor bundle by Fortiori Design LLC company, England). The Moxy monitor is a wireless device that uses four wavelengths of light and assumes that light passes through multiple layers of tissue (epidermis, dermis, fat, muscle) to estimate, the real-time muscle oxygenation percentage (%), from 0 to 100. All subjects were sitting on a comfortable chair; then the researcher placed the Moxy sensor on the proximal muscle belly (medial gastrocnemius muscles). The data were recorded using the muscle oxygen monitor with the PeriPedal program (the software of Moxy) and were exported into Microsoft Excel. The validity of this tool was considered strong (ICC; r = 0.77-0.99) [19].

Electrical activity

The muscle electrical activity was measured using surface EMG electrodes (Biopac EMG100C, Biopac's disposable EL500 series electrodes, USA). The use of surface EMG in sports science mostly concerns determining the physiological mechanism of muscle contraction and relaxation, as well as dealing with muscle condition after interventions. EMG data were collected from the medial gastrocnemius muscles. The EMG electrodes were attached to the distal part of the muscle belly. The skin of those areas was prepared by shaving, and cleaning with an alcohol swab. In this study, the raw EMG signal data were band-pass filtered (cutoff frequencies: 10 Hz low pass filter, 250 Hz high pass filter), and amplitude normalization scaling (mV) was applied.

Statistical analysis

The value was expressed as mean ± standard deviations (SD), and all statistical analyses were performed using the SPSS version 28.0 with the level of significance p-value <0.05 ($p < 0.05$). The Shapiro–Wilk test was used to check the normality of all outcomes, which were normally distributed ($P > 0.05$). The paired sample t-test was used to compare the measured outcomes between before and after interventions within the groups. The independent sample t-test was used to compare the measured outcomes between the experimental groups and between genders in each group. The repeated measures ANOVA was used to compare changes of those measured variables within the groups at different times (2-minute intervals), and the LSD post-hoc test was applied for multiple comparison.

Results

In relation to the participants' characteristics, some significant differences were found in weight, height, BMI, RSBP and RDBP ($p < 0.05$) between genders; however, there were no significant differences in age, RHR, and BT ($p > 0.05$). Also, no significant differences were found in all characteristics between the experiment and control groups ($p > 0.05$) (Table 1).

Muscle oxygenation

Between groups

Figure 2A presents some significant increases in the muscle oxygenation percentage that were shown in the second running session in the CWI group after 5 minutes of cold immersion. Statistical analyses indicated that there was a greater increase by 20% (28.6 ± 10.5% to 35.2 ± 13.5%, $p = 0.021$) in all 16 minutes of the second running session, which presented a significant change ($p < 0.05$), whereas there was no significant difference in the percentage of muscle oxygen in the non-CWI group (46.1 ± 20.0% to 46.1 ± 21.8%, $p > 0.05$) to all measuring time points.

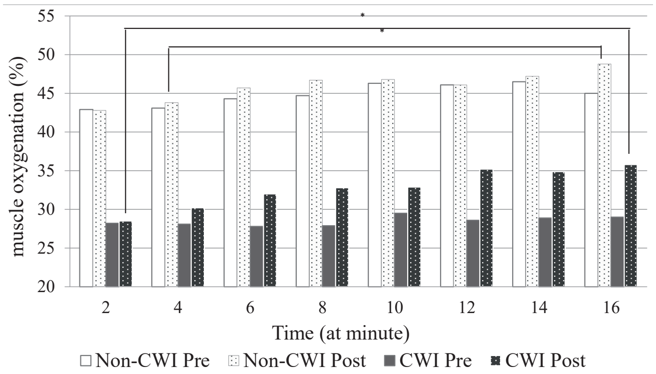

Figure 2A. Muscle oxygenation percentage changes on pre- and postinterventions during 2nd to 16th minute among the non-CWI and CWI groups (significant difference $p < 0.05$)

Between genders

Figure 2B shows the non-CWI and CWI subjects' muscle oxygenation changes between genders. After dynamic stretching, females showed significantly different muscle oxygenation percentage (41.0 ± 21.3% to 51.4 ± 21.3%, $p = 0.015$), but males did not (44.9 ± 23.4% to 45.9 ± 21.7%, $p > 0.05$). Meanwhile, in the

CWI group after cold immersion, although it started with the similar values during the first 2 minutes in both genders, the results showed that the muscle oxygenation percentage during the second running session in males increased more than in females from the 4th minute. The muscle oxygenation percentage in males increased by 23% in all 16 minutes of running (28.9 ± 9.2% to 37.9 ± 15.2%, p = 0.06) compared to the 14% of increase in females (27.8 ± 12.0% to 32.7 ± 14.2%, p = 0.20). However, no significant differences between genders were found (p > 0.05).

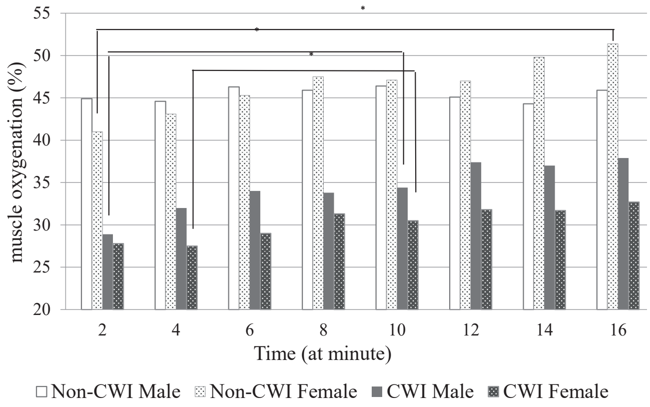

Figure 2B. Muscle oxygenation percentage changes between the male and female subjects among the non-CWI and CWI groups postinterventions (significant difference p < 0.05)

Electrical activity

Between groups

Figure 2C shows the muscle electrical activity level changes in both groups before and after the participants were subjected to the interventions. In the non-CWI group, there was an increase of the electrical activity level in all time points of the second running sessions after the dynamic stretching exercises; however, it was not statistically significant (1249.6 ± 480.8 mV to 1694.1 ± 1667.4 mV, p > 0.05). Meanwhile, an overall significant decrease in the electrical activity level (1402.9 ± 831.6 mV to 1009.8 ± 537.9 mV, p = 0.028) was found in the CWI group after cold immersion, compared to the first running session before cold immersion.

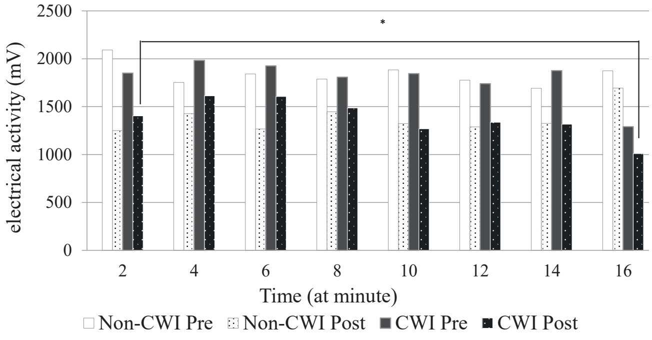

Figure 2C. Muscle electrical activity level changes on pre- and postinterventions during 2nd to 16th minute among the non-CWI and CWI groups (significant difference p < 0.05)

Between genders

Figure 2D shows the non-CWI and CWI groups' electrical activity changes between male and female runners. In both groups of the experiment, the male runners were more likely to present the higher electrical activity levels than the female runners (p > 0.05). However, after the dynamic stretching exercises, a slight decrease was found in females during the first 6 minutes but there were the stable changes of the muscle electrical activity level during the 6th to 14th minutes (p > 0.05). The participants of the CWI group demonstrated a larger decline in the electrical activity level (2092.6 ± 1459.3 mV to 1522.8 ± 926.9 mV) in males compared to females, whose measurements showed only a small decrease in the electrical activity level (1336.5 ± 435.9 mV to 1165.1 ± 392.9 mV). However, there were no statistically significant changes in both groups among genders (p > 0.05).

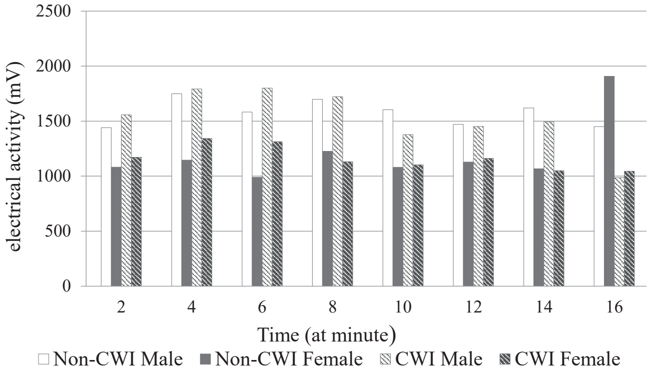

Figure 2D. Muscle electrical activity (mV) changes between the male and female subjects between the non-CWI and CWI groups postinterventions

Discussion

The main finding of this study was the fact that the 8-10 °C CWI application for 5 minutes as the pre-cooling method was significantly effective in increasing the percentage of oxygenation in muscles and decreasing the muscle electrical activity compared to the traditional procedure of the dynamic stretching in amateur young adult mini-marathon runners, while the dynamic stretching group showed no significant results in both outcomes.

Running economy is the key factor of endurance running performance, since it is a useful predictor of performance in endurance racing. The concept of the energy cost is another important physiological parameter used to explain performance in running. It has been speculated in this research that the CWI method causes an increase in oxygen delivery and reduces muscle activity, which could contribute to the maintenance of muscle health and the reduction of delayed onset muscle soreness. Since a long-distance running exercise, such as a mini-marathon, is one of the most strenuous activities, the procedure to promote muscle endurance and to beat muscle fatigue earlier than expected is essential for athletes. In other words, the ability of an athlete to minimize their energy expenditure at their own pace/exercise intensity in a race would be identified as one of the determining factors of optimal performance.

Unfortunately, only a few studies have examined the effect of CWI on the muscle oxygenation percentage in a strenuous activity. A study by Yeung et al. [28] observed that CWI at 12-15 °C resulted in an increase in muscle oxygenation after maximal dynamic knee extension and flexion contractions, both concentrically. Cold water caused a greater reperfusion and an increase in oxygen delivery to target muscles. CWI caused cutaneous vasoconstriction, which reduced peripheral blood flow and had an impact on muscle metabolism [29]. During the running protocols following the cold exposure in the current study, the muscle oxygenation percentage showed some increases that indicated a higher oxygen utilization and oxygen demands in the runners' muscles. The obtained results showed that there was a possible increase in muscle oxygen availability and enhancement of oxidative capacity after 5 minutes of CWI. Skeletal muscle oxygen consumption can increase 50-fold during an exercise, with unexpected increases in oxygen delivery of up to tenfold, because skeletal muscles are very dependent on oxidative metabolism. Improvements in the body's oxidative system lead to a better athletic performance. In addition, the change in high-energy phosphorus metabolites is affected by both oxygen deliveries in muscles and oxidative capacity. The current findings of a significant increase in muscle oxygen percentage after 5-minute cold immersion with low temperature suggest that cold exposure can help amateur runners fulfill their muscle oxygen consumption needs in long distance running.

Moreover, this study was conducted at a controlled room temperature, where the results of muscle oxygenation and electrical activity were not affected by the effects of heat from the environment in which the athletes performed their strenuous activity during measurement. Whereas a previous study on the effects of pre-cooling on intermittent-sprint performance in the heat found only minor benefits [5] the current study, which was conducted at a controlled room temperature of 25 °C, a significant effect was seen as a result of the application of 8-10 °C CWI for 5 minutes prior to the running activity, with the heat strain obtained from the increased activity. A comparison of the results of the increase in oxygenation percentage in the non-CWI group showed that it increased at a slower rate and was not as significant as in the CWI group. It demonstrated that in the pre-cooled muscle condition, oxygen consumption increased, and this was not affected by the heat stress since the activities were performed at a controlled temperature. This result was supported by the Seebacher et al. [24] study showing that at low temperatures, oxygen consumption in a human's body increases. Muscle oxygen consumption was significantly higher at 15 °C than at higher temperatures. Muscles consumed significantly more oxygen at lower temperatures for the same work output. As the temperature dropped, the metabolic cost of muscle performance and activity increased. To maintain activity across a temperature range, the human body must increase the ATP production.

The current study showed that the male runners had a significantly higher increase in the muscle oxygenation percentage in the cold immersion group than the female runners. It has been demonstrated that the amount or total mass of an oxygen-carrying protein called hemoglobin in blood influences performance and appears to differ between males and females. Increased hemoglobin levels improve a person's capacity to carry oxygen throughout the body, as well as their performance during endurance activities. Even when taking age and race into consideration, females have 12% lower hemoglobin levels than males. Testosterone in males may enable them to reach a desirable hemoglobin level more easily because of the synergistic effect of androgens on bone marrow, which might be associated with the differences in physiological changes in the current study. For the same amount of blood flow, females have a lower overall oxygen delivery. This is caused by the fact that females have lower blood volume relative to a body size (65.4 kg vs 47.9 kg), limiting how much blood flow can be redistributed to exercising muscles without adversely affecting delivery to other tissues during an exercise. This demonstrates a gender difference in endurance-related biological needs.

In the present study, the electrical response of muscle contraction has been assessed through EMG, and is detected by two electrodes placed on the surface of the target muscle. The amplitude of the EMG signal has the potential to provide a measure of the magnitude of muscle contraction force. This signal is produced by the active muscles under skin. Meanwhile, another outcome in this study showed that electrical activity was significantly decreased by 28% in the CWI group. Cold water exposure lowered the conductibility. It is supported by Hurr [13], who employed 12 subjects performing a cooling technique as a recovery method, which resulted in a steadily decreasing vertical jump performance over 30 consecutive vertical jumps in all three sets. Between genders, females have a lower electrical activity level of muscles than males. Since males have a higher proportion of type II fibers than females, they can produce more muscle force. A previous study discovered that testosterone may increase a number of type II fibers, and males have a high proportion of these fibers. Males are stronger than females because the male hormone (testosterone) enhances muscle growth. However, neither fat mass percentage nor adipose tissue thickness differences between males and females were measured in this study. The decrease of the electrical activity level differences after cold immersion between genders has been attributed to the differences in muscle mass, muscle morphology, contracting muscle metabolism, and neuromuscular activation. It was highlighted that the muscle mass differences between males and females have a direct impact on absolute muscle force development during exercises at relative levels, resulting in higher intramuscular pressure in males due to their higher strength, which ultimately affects oxygen delivery and consumption in the contracting muscle through greater compression of active tissue vasculature [18]. Oxygen availability plays an important role in modulating motor unit recruitment and discharge patterns of activated motor units during contraction. In a prior study, it was discovered that venous blood oxygenation declines as muscle contraction force increases, implying that oxygen extraction increases with prolonged muscle contraction. The greater the changes in muscle oxygenation, the lower the amount of muscle electrical activity, which indicates muscle fatigue [26]. However, it is important to note that the recorded muscle activity from EMG could be contaminated by active neighboring muscles which also could have been affected by cold immersion. Furthermore, the interpretation of muscle activity measured by EMG is challenged since the data can be influenced by some factors, such as muscle length, muscle fiber type, and muscle contraction velocity. Cold-induced muscle vasoconstriction slows ATP utilization, calcium release and uptake from the sarcoplasmic reticulum, as well as creates differences in oxygen transportation to a working muscle, while one of the most important aspects of sustaining efficient muscle activity is the delivery of oxygen to the muscle [26]. Mantooth et al. [18] stated that oxygenation plateauing was also associated with the recruitment of all type I muscle fibers, as well as with the additional recruitment of type II muscle fibers at higher intensities. Nonetheless, studies that tested lower intensities revealed that oxygenation at exhaustion was higher than during the midpoint phase of endurance testing, which may be partly attributed to a potential increase in total hemoglobin levels in muscle tissue during prolonged contractions [18, 23].

A study by Brodeur et al. [3] determined that the duration of the muscle oxygen levels remained elevated after stretching. The muscle oxygen levels were reported to drop as soon as a resting phase began. Oxygen levels remained elevated following dynamic stretching until the 2-minute mark [25]. It can be concluded that the effect of the increasing oxygen level in the dynamic stretching group was only valid for a short period of time, which was supported by our study's findings that both, the increase in oxygen level and the electrical activity level were not significant. Performance improvements of the dynamic stretching subjects appeared to last only a few minutes after stretching was completed.

The majority of previously conducted studies were predominantly focused on males, particularly because certain anthropometric and hormonal differences, such as a stage of a menstrual cycle, may have confounded results for female participants, and they did not focus on body composition, body fat, and body/extremity circumference [1, 11, 14, 25]. As verified by the results of the present study, it was reported that the male subjects showed a higher and more significant increase in the muscle oxygen percentage than the female subjects. Many physiological parameters, such as skeletal muscle ratio and subcutaneous fat thickness, differ depending on a gender. An earlier study identified estrogen as the most consistently identified source of these differences [20].

According to Peiffer et al. [21], cold exposure has an effect on lowering skin or body temperature. The 5-minute CWI intervention in this study was demonstrated to be the most practical in terms of lowering core temperature, while having the least impact on muscle temperature. Therefore, it may not be possible to conclude that 5-minute CWI is better at maintaining muscular function than longer interventions. As long as it does not lower muscle temperature, acute cooling has some potential to improve exercise performance.

There are some notable limitations of this study. Firstly, the results of such laboratory-based experiments are likely to lack ecological validity and mundane realism. This means that because of the controlled environment in laboratory experiments, the outcome may not yield an accurate reflection of what would be seen in the real world. However, it appears to be more beneficial for physiological changes in a controlled environment, and it would be suitable for a real situation of a well-organized sporting event, such as a mini-marathon competition, which typically starts in the early morning hours when environmental temperatures are at their lowest and constant. Secondly, although the city of Khon Kaen has organized international mini-marathons 18 times, participants were limited to amateur runners from the Khon Kaen province, Thailand. Thirdly, some cooling techniques may have different effects depending on a part of a human body. The last limitation resulted from the equipment limitations; only one muscle could be measured during the experiment. A further investigation of the other core muscles, which are used during the running gait, would be recommended.

The application of CWI as a pre-cooling intervention could help improve a runner's physical performance, which allows them to use and apply the method for their daily preparation before a running activity. Further studies must be conducted to investigate the effects of CWI as a pre-cooling intervention on physical performance outcomes and comparisons between athletes and non-athletes; the differences between the dominant and non-dominant side of an extremity might also be worth monitoring.

Conclusions

The purpose of this study was to determine the effect of the pre-running CWI application at 25 °C controlled temperature on electrical activity and muscle oxygenation in amateur young adult mini-marathon runners. According to the obtained findings, 5-minute CWI at 8-10 °C appears to have a greater effect than the dynamic stretching exercises as a warm-up/cool-down routine, and gives a positive impact which can be associated with running performance in both males and females, particularly in male runners. Therefore, our findings are of clinical relevance to sports medicine specialists and it is highly recommended for athletes to immerse their legs in cold water before a prolonged exercise (or as pre-cooling), which can have a more beneficial effect on increasing oxygen flow and decreasing electrical activity in muscles to maximize competition performance.

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Conflict of Interest

The author has no conflicts of interest to report.

References

1. Baláš J, Kodejška J, Krupková D, Giles D. Males benefit more from cold water immersion during repeated handgrip contractions than females despite similar oxygen kinetics. J Physiol Sci. 2020;70(1):13. http://doi.org/10.1186/s12576-020-00742-5
2. Bongers CC, Hopman MT, Eijsvogels TM. Cooling interventions for athletes: an overview of effectiveness, physiological mechanisms, and practical considerations. Temperature (Austin). 2017;4(1):60-78. http://doi.org/10.1080/23328940.2016.1277003S
3. Brodeur ZR, Paustian MJ, Monteleone-Haught DA, Lamm RA, Pagano AG, Ellis CE. The effects of static and dynamic stretching on muscle oxygen saturation in the rectus femoris. J Exerc Sci. 2022;15(3):702-708.
4. Castle PC, Macdonald AL, Philp A, Webborn A, Watt PW, Maxwell NS. Precooling leg muscle improves intermittent sprint exercise performance in hot, humid conditions. J Appl Physiol (1985). 2006;100(4):1377-1384. http://doi.org/10.1152/japplphysiol.00822.2005
5. Cheung S, Robinson A. The influences of upper-body pre-cooling on repeated sprint performance in moderate ambient temperatures. J Sports Sci. 2004;22(7):605-612. http://doi.org/10.1080/02640410310001655813
6. Choo HC, Peiffer JJ, Lopes-Silva JP, Mesquita RNO, Amano T, Kondo N, et al. Effect of ice slushy ingestion and cold-water immersion on thermoregulatory behavior. PLoS One. 2019;14(2):e0212966. http://doi.org/10.1371/journal.pone.0212966
7. Coelho Rodrigues Jr JF, Prado DM, Sena AF, Veneroso CE, Cabido CE, Sevílio Jr MN. Physiological responses during the long-distance race in the warm environment in runners: a pilot-study. J Sports Med Phys Fitness. 2021;61(6):779-787. http://doi.org/10.23736/S0022-4707.21.11426-4

8. Espinosa-Ramírez M, Moya-Gallardo E, Araya-Román F, Riquelme-Sánchez S, Rodriguez-García G, Reid WD, et al. Sex-differences in the oxygenation levels of intercostal and vastus lateralis muscles during incremental exercise. Front Physiol. 2021;12:738063. http://doi.org/10.3389/fphys.2021.738063
9. Hammond LE, Cuttell S, Nunley P, Meyler J. Anthropometric characteristics and sex influence magnitude of skin cooling following exposure to whole body cryotherapy. Biomed Res Int. 2014;2014:628724. http://doi.org/10.1155/2014/628724
10. Hodgson JR, Chapman L, Pope FD. Amateur runners more influenced than elite runners by temperature and air pollution during the UK's Great North Run half marathon. Sci Total Environ. 2022;842:156825. http://doi.org/10.1016/j.scitotenv.2022.156825
11. Hohenauer E, Costello JT, Deliens T, Clarys P, Stoop R, Clijsen R. Partial-body cryotherapy (–135°C) and cold-water immersion (10°C) after muscle damage in females. Scand J Med Sci Sports. 2020;30(3):485-495. http://doi.org/10.1111/sms.13593
12. Hohenauer E, Stoop R, Clarys P, Clijsen R, Deliens T, Taeymans J. The effect of pre-exercise cooling on performances characteristics: a systematic review and meta-analysis. Int J Clin Med. 2018;9:117-141. http://doi.org/10.4236/ijcm.2018.93012
13. Hurr C. Acute local cooling to the lower body during recovery does not improve repeated vertical jump performance. Int J Environ Res. 2021;18(9):5026. http://doi.org/10.3390/ijerph18095026
14. Jones PR, Barton C, Morrissey D, Maffulli N, Hemmings S. Pre-cooling for endurance exercise performance in the heat: a systematic review. BMC Med. 2012;10:166. http://doi: 10.1186/1741-7015-10-166
15. Knight KL, Draper DO. Therapeutic modalities: The art and science. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2013.
16. Legaz Arrese A, Munguía Izquierdo D, Serveto Galindo JR. Physiological measures associated with marathon running performances in high-level male and female homogeneous groups. Int J Sports Med. 2006;27(4):289-295. http://doi.org/10.1055/s-2005-865628
17. Lin WC, Lee CL, Chang NJ. Acute effects of dynamic stretching followed by vibration foam rolling on sports performance of badminton athletes. J Sports Sci Med. 2020;19(2):420-428.
18. Mantooth WP, Mehta RK, Rhee J, Cavuoto LA. Task and sex differences in muscle oxygenation during handgrip fatigue development. Ergonomics. 2018;61 (12): 1646-1656. http://doi.org/10.1080/00140139.2018.1504991
19. Oatyimprai K, Eungpinichpong W, Buranruk O, Konharn K, Tudpor K. Effect of traditional Thai massage on muscle oxygen saturation in low back pain patients: a preliminary study. Int J GEOMATE. 2020;19(72): 54-61. http://doi.org/10.21660/2020.72.5636
20. Paredes-Ruiz MJ, Jodar-Reverte M, Gonzalez-Moro IG, Ferrer-Lopez V. Effects of gender on oxygen saturation of thigh muscles during maximal treadmill exercise testing. Sport Mont. 2021;19(1):7-11. http://doi.org/10.26773/smj. 210203
21. Peiffer JJ, Abbiss CR, Watson G, Nosaka K, Laursen PB. Effect of cold-water immersion duration on body temperature and muscle function. J Sports Sci. 2009; 27 (10): 987-993. http://doi.org/10.1080/0264041090 3207424
22. Perrey S. Muscle oxygenation unlocks the secrets of physiological responses to exercise: time to exploit it in the training monitoring. Front Sports Act Living. 2022;4:864825. http://doi.org/10.3389/fspor.2022.864825
23. Philippe M, Wegst D, Müller T, Raschner C, Burtscher M. Climbing-specific finger flexor performance and forearm muscle oxygenation in elite male and female sport climbers. Eur J Appl Physiol. 2012;112(8):2839-2847. http://doi.org/10.1007/s00421-011-2260-1
24. Seebacher F, Tallis JA, James RS. The cost of muscle power production: muscle oxygen consumption per unit work increases at low temperature in Xenopus laevis Duadin. J Exp Biol. 2014;217:1940-1945. http://doi.org/10.1242/jeb.101147
25. Tenaglia SA, McLellan TM, Klentrou PP. Influence of menstrual cycle and oral contraceptives on tolerance to uncompensable heat stress. Eur J Appl Physiol Occup Physiol. 1999;80(2):76-83. http:/doi.org/10.1007/s00421 0050561
26. Yamada E, Kusaka T, Arima N, Isobe K, Yamamoto T, Itoh S. Relationship between muscle oxygenation and electromyography activity during sustained isometric contraction. Clin Physiol Funct Imaging. 2008;28(4):216-221. http://doi.org/10.1111/j.1475-097X.2008.00798.x
27. Yamaguchi T, Takizawa K, Shibata K. Acute effect of dynamic stretching on endurance running performance in well-trained male runners. J Strength Cond Res. 2015;29(11):3045-3052.http://doi.org/10.1519/JSC.0000000000000969
28. Yeung SS, Ting KH, Hon M, Fung NY, Choi MM, Cheng JC, et al. Effects of cold-water immersion on muscle oxygenation during repeated bouts of fatiguing exercise: a randomized controlled study. Medicine. 2016;95(1): e2455. http://doi.org/10.1097/MD.0000000000002455
29. Zinner C, Sperlich B, editors. Marathon running: physiology, psychology, nutrition and training aspects. Cham: Springer; 2016.