

## Effects of Pre-Cooling with Cold Water Immersion on Muscle Oxygenation

### among Amateur Young-Adult Mini-Marathon Runners

ผลของการแช่น้ำเย็นก่อนการออกกำลังกายต่อปริมาณออกซิเจนของกล้ามเนื้อในนักวิ่งมินิมาราธอน

สมัครเล่นผู้ใหญ่วัยตอนต้น

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#### ABSTRACT

The purpose of this study was to investigate the effect of cold water immersion (CWI) application before running on muscle oxygenation among amateur young-adult mini-marathon runners. Participants in this study consisted of 10 male young adults (aged  $21.8 \pm 1.9$  years, BMI  $21.88 \pm 2.7$  kg/m<sup>2</sup>), they were randomly allocated into 2 groups (CWI group and control group). The CWI participants were 5-minutes immersed in 8-10°C cold water on plastic tub. The control group completed a dynamic stretching of running-related muscles for 5 minutes. Muscle oxygenation was measured using Moxy sensor in every 1, 8, and 16 minutes that was attached on their dominant side of medial gastrocnemius muscles while performing running on treadmill. Repeated measures ANOVA was used to compare changes within group at different times. After interventions, CWI group showed a slightly decreased of muscle oxygenation percentage on the first minute and 8<sup>th</sup> minute ( $27.00 \pm 9.5\%$  to  $26.00 \pm 9.5\%$ ;  $p > 0.05$ , respectively) then an increase (5.3%) at 8<sup>th</sup> minutes to 16<sup>th</sup> minutes ( $p > 0.05$ ). While after applying dynamic stretching, it can increase muscle oxygenation percentage in every time changes ( $p < 0.05$ ). It can be concluded that CWI resulted in a likely lower muscle oxygen percentage compare to before the intervention.

#### บทคัดย่อ

การศึกษานี้ มีวัตถุประสงค์เพื่อประเมินผลของการแช่น้ำเย็นก่อนการวิ่งต่อปริมาณออกซิเจนของกล้ามเนื้อในการวิ่งของนักวิ่งมินิมาราธอนสมัครเล่นผู้ใหญ่วัยตอนต้น อาสาสมัครในการศึกษานี้เป็นนักวิ่งเพศชายจำนวน 10 คน (อายุ  $21.8 \pm 1.9$  ปี ดัชนีมวลกาย  $21.88 \pm 2.7$  กก./ม.<sup>2</sup>) ถูกสุ่มแบ่งออกเป็น 2 กลุ่ม ได้แก่ กลุ่มที่ได้รับการแช่น้ำเย็น 8-10°C ในอ่างน้ำพลาสติกเป็นระยะเวลา 5 นาที (n=5) กับกลุ่มควบคุม (n=5) ซึ่งได้รับการยืดกล้ามเนื้อที่เกี่ยวข้องกับการวิ่งเป็นระยะเวลา 5 นาที จากนั้นทำการวัดปริมาณออกซิเจนของกล้ามเนื้ออย่างซ้ำกัน (ทั้งก่อนและหลัง) การวิ่งบนลู่วิ่งในช่วงเวลาที่ 1 8 และ 16 โดยใช้เครื่อง Moxy sensor จากนั้นใช้ Repeated measures ANOVA ในการเปรียบเทียบผลลัพธ์ภายในกลุ่มระหว่างช่วงเวลา ผลการวิจัยพบว่า กลุ่มที่ได้รับการแช่น้ำเย็นมีปริมาณออกซิเจนของกล้ามเนื้อลดลงเล็กน้อยระหว่างเวลาที่ 1 และเวลาที่ 8 ( $27.00 \pm 9.5\%$  to  $26.00 \pm 9.5\%$ , ตามลำดับ,  $p > 0.05$ ) และเพิ่มขึ้น 5.3% จากเวลาที่ 8 ไปเวลาที่ 16 ส่วนกลุ่มที่ได้รับการยืดกล้ามเนื้อช่วยเพิ่มปริมาณออกซิเจนตลอดระยะเวลาที่ศึกษา ( $p < 0.05$ ) ดังนั้นจึงสามารถสรุปได้ว่าการแช่น้ำเย็นก่อนวิ่งมีแนวโน้มในการลดการใช้ปริมาณออกซิเจนของกล้ามเนื้อได้

**Keywords:** Pre-cooling, Muscle oxygenation, Mini-marathon

**คำสำคัญ:** การให้ความเย็นก่อนการออกกำลังกาย ปริมาณออกซิเจนของกล้ามเนื้อ มินิมาราธอน

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## Introduction

Running is one of the most common forms of exercise all over the world. Running with long-distance races have increased in popularity in the recent years. Especially the mini-marathon, that has the largest increasing in the number of participants. Mini marathon in Bangkok itself have 10.55 km of distance (Mongkonpattasaruk et al., 2018).

Running can raise core body temperature, which can have an adverse effect on exercise efficiency and contribute to the creation of heat-related illnesses (Bongers et al., 2017). Hyperthermia is caused by an inability to control rising core body temperature. People were advised to acclimate to environmental conditions to counteract the symptoms of hyperthermia, which resulted in fatigue during exercise (James et al., 2015). A strategy to prevent fatigue due to thermoregulation changes is the human thermoregulatory system will try to lower core temperature to preserve physical activity by pre-cooling the body before exercise (Hohenauer et al., 2018).

Pre-cooling is the rapid removal of heat from the body prior to exercise in order to increase heat storage capacity (Ross et al., 2013). Pre-cooling techniques work by increasing the heat storage capacity of the thermoregulatory system to minimize heat stress before exercise. Pre-cooling is a technique for lowering body core temperature before beginning an exercise session. As a result, the margin for metabolic heat output and heat gain is increased (Jones et al., 2012). That enables people to perform more work before the critical limit for core temperature is reached (Arngrimsson et al., 2003).

There is lack of research investigate CWI application before exercise on physical performance, since most of the existing research were conducted in the form of CWI to overcome muscle damage on post exercise (Hohenauer et al., 2020; Paula et al., 2018). Therefore, improper preparation in the middle of repetitive strenuous activity will interfere the body's adaptation, especially the muscles during exercise, which will increase the risk of injury, or interfere with the quality of physical performance.

This study was hypothesized that CWI can decrease oxygenation in the muscle before running to provide a larger heat storage capacity. Following pre-cooling, cardiovascular pressure is reduced and central blood volume is better maintained, allowing for greater skeletal muscle blood supply and oxygen delivery during exercise is performed (Scott et al., 2004).

## Objective of the study

The aim of this study was to determine the different effect of CWI application and non-CWI application (dynamic stretching) on muscle oxygenation in amateur young-adult mini-marathon runners.

## Material and methods

### Study design

This study was a quasi-experimental research design, which used two groups (experimental and control group) with pre-test and post-test design. There was conducted at Physical Therapy research

laboratory, Faculty of Associated Medical Sciences, Khon Kaen University. This study was approved by Ethics Committee of Khon Kaen University, Thailand (Ref. No: HE642206).

### Participants

This study was carried out on 10 male-young adults (19-35 years old). They were amateur runners who are physically active lifestyle (30 minutes/day in moderate-to-vigorous physical activity level, 3 days/week for 3 months consecutively), body mass index classified as normal-weight (18.5-24.9 kg/m<sup>2</sup>), and had experienced as runner in organized mini marathon. The participants with serious allergy of cold exposure (such as serious reddish, itchy welts, hives, abnormal breathing) were excluded. The experimental procedures were verbally explained by researchers. Before data collection, each participant was provided verbally consent and asked to sign the consent form by the researchers.

### Interventions

#### CWI group

The CWI participants immersed at their hip level in cold water immersion by long sitting position in a plastic tub. They were immersed in two different degrees of cold water, the first 3 minutes at 15-20°C for familiarization and 5 minutes at 8-10°C for the main CWI protocol. The CWI was given once, that was before the 2<sup>nd</sup> running session. The temperature of the cold water was continually monitored with a glass thermometer.

#### Non-CWI group

The non-CWI participants performed dynamic stretching protocol (without hold stretched) of 5 target muscles; hip extensors and flexors, leg extensors and flexors, and plantar flexors. The subjects performed 3 sets of 10 repetitions of each stretch. Each stretch was performed for one set on both lower extremities, and then on the next target muscle group without a rest. All these dynamic stretching procedures was done in total 5 minutes (Yamaguchi et al., 2015).

### Outcome measurements

#### Moxy sensor

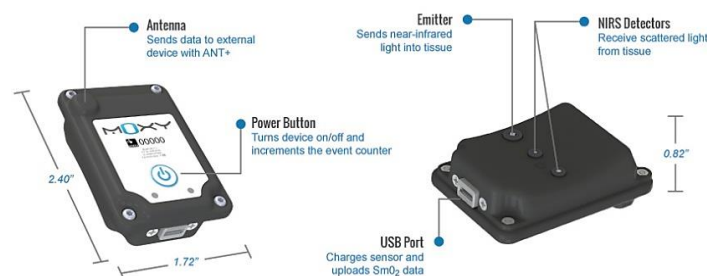


Figure 1 Moxy sensor

Moxy sensor and antenna (ANT) (MOXY-1 sensor bundle by Fortiori Design LLC company, England) was used to measure muscle oxygenation. Each participant was sit on the chair; then the researcher placed MOXY monitor on the proximal of muscle belly (medial gastrocnemius muscles) on

dominant-side lower extremity of the participants (Myers, 2019). The data was recorded in peripedal (software of MOXY) and was exported in Microsoft excel. The validity of this tool is considerably strong (ICC;  $r = 0.77-0.99$ ) (Oatyimprai et al., 2020) in measuring adulthood muscle oxygenation.

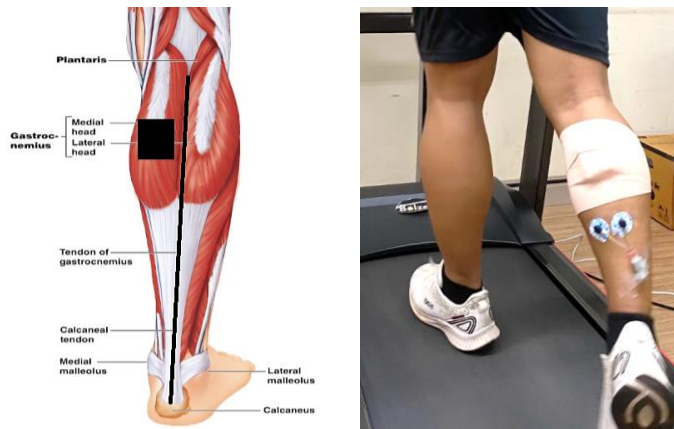


Figure 2 Moxy sensor attachment (1/3 proximal medial gastrocnemius muscle)

### Study protocols

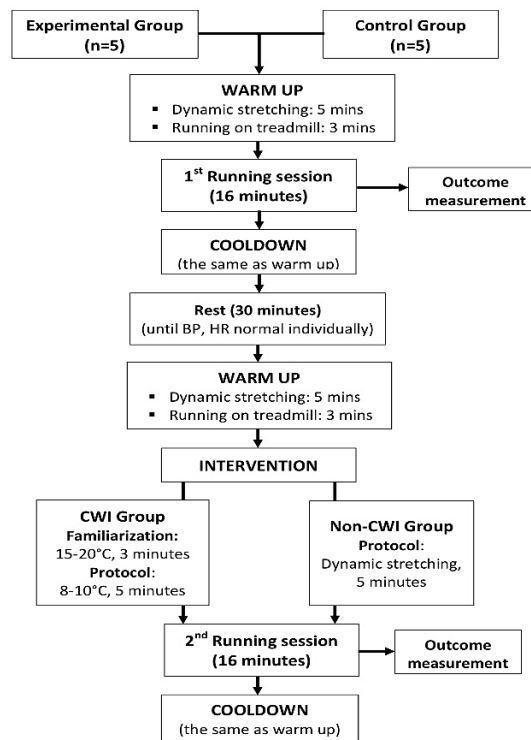


Figure 3 Experimental Design

All participants were allocated into 2 groups; CWI and non-CWI group. Before doing the main study, all participants did the same warm-up protocol (3 sets of 30 seconds each of hip extensor/flexor, adductor/abductor with full extension of legs, trunk circles and passive ankle rotation). They were instructed to do dynamic stretching for 5 minutes and running on a treadmill (Bolzen EX, Australia) for 3 minutes. The next session was the 1<sup>st</sup> running session, all participants ran on treadmill for 16 minutes on their own average running speed (best experienced pace within last 3 months) (Jones et al., 2020). The first outcome measurement was measured. After each subject finished their running, they performed cooling down the same as warm up, then asked to take a rest for 30 minutes (Ryu et al., 2016). The following session was the intervention and the last session was the 2<sup>nd</sup> running session, with the same protocol as the first running session and did the second outcome measurement. All outcome measurements were carried out every 1, 8, and 16 minutes while the participants were running. This study was conducted in controlled temperature (25°C) room.

### Statistical analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc), version 28.0 with licensed by Khon Kaen University with set the level of significance set  $P < 0.05$ . This study aimed to analyze each outcome before and after in both interventions (CWI and dynamic stretching). Independent sample t-test was used to compare the measure outcomes between the groups. Repeated measures ANOVA was used to compare changes within each group at different time. Bonferroni post-hoc test was used for multiple comparisons.

### Results

The sample used in this study consisted of 10 young adult males. Their mean age was  $21.8 \pm 1.9$  years, and BMI value was  $21.88 \pm 2.7 \text{ kg/m}^2$ . Table 1 showed that there was no significant difference between groups on their age, weight, height, and BMI ( $p > 0.05$ ).

**Table 1** Baseline characteristics of participants (expressed as mean $\pm$ SD)

Characteristics of participants	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m <sup>2</sup> )
Groups				
CWI (n=5)	21.6 $\pm$ 2.7	73.8 $\pm$ 12.4	178 $\pm$ 4.3	23.2 $\pm$ 3.1
Non-CWI (n=5)	22 $\pm$ 0.7	60.2 $\pm$ 5.7	171.2 $\pm$ 5.8	20.5 $\pm$ 1.4
Total (n=10)	21.8 $\pm$ 1.8	67 $\pm$ 11.6	174.6 $\pm$ 6	21.8 $\pm$ 2.7
p-value	0.085	0.349	0.175	0.092

Note: Independent sample t-test was performed to analyze variance between characteristics of participants; \* $p < 0.05$ , \*\* $p < 0.01$ .

**Table 2** Effect of pre-cooling on muscle oxygenation between groups in different time points (expressed as mean±SD)

Groups	Before intervention			F	p-value	Partial Eta Squared (Effect size)
	T1 (1 min) (%)	T2 (8 min) (%)	T3 (16 min) (%)			
CWI (n=5)	31.2 ± 13.1	31.8 ± 13.5	33.6 ± 11.9	0.296	0.064	0.069
Non-CWI (n=5)	45.8 ± 16.6	45.4 ± 20	44.8 ± 15.1	0.055	0.629	0.014
After intervention						
CWI (n=5)	27 ± 9.5	26 ± 9.5	27.4 ± 6.1	0.09	0.626	0.022
Non-CWI (n=5)	43.8 ± 18.7 <sup>a</sup>	47.4 ± 17.2 <sup>b</sup>	47.6 ± 17.7 <sup>c</sup>	0.287	0.005**	0.067

Note: One-way repeated measured ANOVA was performed to analyze variance in muscle oxygenation between T1, T2 and T3; Bonferroni post-hoc test was applied for multiple comparison; <sup>a</sup>significant difference between T1 and T2 ( $p < 0.01$ ), <sup>b</sup>significant difference between T1 and T3 ( $p < 0.01$ ), <sup>c</sup>significant difference between T2 and T3 ( $p < 0.01$ ); \*\* $p < 0.01$ .

The results in Table 2 were analyzed using one-way repeated measured ANOVA between three different times and using Bonferroni post-hoc test. In CWI group, before applied with immersion from the first minute to 16<sup>th</sup> minutes while at the 1<sup>st</sup> running session, there was shown that it was likely to be increase in muscle oxygenation ( $31.20 \pm 13.1\%$  to  $33.60 \pm 11.9\%$ ) but it was not significant ( $p > 0.05$ ). Moreover, muscle oxygenation in non-CWI group showed a decrease throughout the time periods although it was neither nor significant. While at the 2<sup>nd</sup> running session, after immersion in CWI group between the first minute to 8<sup>th</sup> minutes of running, it showed a slightly decrease of muscle oxygenation percentage ( $27.00 \pm 9.5\%$  to  $26.00 \pm 9.5\%$ ) then an increase (5.3%) at 8<sup>th</sup> minutes to 16<sup>th</sup> minutes, but a significant increase all time showed in non-CWI group which performed dynamic stretching ( $p < 0.01$ ).

## Discussion

This study examined the effect of cold water immersion (8-10°C) before running on muscle oxygenation results in amateur young adult mini marathon runners. Based on the obtained results of 1<sup>st</sup> running session or before the intervention was applied, this study showed the increasing of muscle oxygenation percentage occurred in CWI group all the time but contrary there was a decrease throughout the minutes in the control group. In runner's body, it uses oxygen to create energy or adenosine triphosphate (ATP). When running intensity increases, so does oxygen utilization. The harder the muscles work during exercise, the demand for oxygen increases. As a result of running, the heart rate is elevated to supply more oxygen to the working muscles. While different effects can also occur in the same activity condition, due to the effects of muscular contraction on blood flow during long distance running. At roughly 30-50% of maximal muscular contraction, blood flow begins to become occluded. At 70% of maximal muscular contraction, blood flow is completely occluded. This blood flow reduction caused by exercise intensity which will affect a significant drop in oxygen percentage in the muscle (Myers, 2020).

After interventions were performed, there was various changes from each group whereas mostly some increasing results in muscle oxygenation obtained. As the results showed, in CWI group after immersed in cold water 8-10°C, there was a slight decrease of percentage in early minutes (1 to 8 minutes), then an increase occurred in another half time (8 to 16 minutes) which indicate a possible increase in muscle oxygen availability and enhancement of oxidative capacity after immersion (Yeung et al., 2016)

The results of this study are consistent with previous results of Yeung et.al. (2016) who used 12-15°C cold water immersion method for 10 minutes duration. A previous study reported a reduction in tissue oxygenation in the second fatiguing protocol by 4% during subsequent exercise. It has been suggested that cold water immersion leads to cutaneous vasoconstriction, resulting in a decrease in peripheral blood flow which may reflect microvascular adaptation to the cold. This redistribution of blood from the periphery to the core augments venous return and increases stroke volume. The net effect of these changes is to enhance blood and oxygen delivery to working muscles, and possibly to enhance exercise performance (Roberts, 2015; Yeung, 2016).

There are several limitations in this study. Moxy sensor only monitors oxygenation changes in a small area of the muscle, and we only have limited unit to use, whereas there are many important running muscles to be measured. The present study examines the effect of cold water immersion on muscle oxygenation while running on treadmill, future studies using cold water immersion in actual practice or competition should be considered.

## Conclusion

The CWI application before running seems to produce a lower muscle oxygen percentage, but dynamic stretching resulted an increase muscle oxygenation percentage. Therefore, CWI and dynamic stretching can be conversely effect among amateur young-adult mini-marathon runners.

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