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Tissue viability imaging of skin microcirculation following exposure to whole body cryotherapy (-110°C) and cold water immersion (8°C)

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Cryotherapy is currently used in various clinical, rehabilitative, and sporting settings. However, very little is known regarding the impact of cooling on the microcirculatory response.

Objectives: The present study sought to examine the influence of two commonly employed modalities of cryotherapy, whole body cryotherapy (WBC; -110°C) and cold water immersion (CWI; 8±1°C), on skin microcirculation in the mid- thigh region. **Methods:** The skin area examined was a 3 × 3 cm located between the most anterior aspect of the inguinal fold and the patella. Following 10 minutes of rest, 5 healthy, active males were exposed to either WBC for 3 minutes or CWI for 5 minutes in a randomised order. Volunteers lay supine for five minutes after treatment, in order to monitor the variation of red blood cell (RBC) concentration in the region of interest for a duration of 40 minutes. Microcirculation response was assessed using a non-invasive, portable instrument known as a Tissue Viability imaging system. After a minimum of seven days, the protocol was repeated. Subjective assessment of the volunteer's thermal comfort and thermal sensation was also recorded. **Results:** RBC was altered following exposure to both WBC and CWI but appeared to stabilise approximately 35 minutes after treatments. Both WBC and CWI affected thermal sensation ($p < 0.05$); however no between-group differences in thermal comfort or sensation were recorded ($p > 0.05$). **Conclusions:** As both WBC and CWI altered RBC, further study is necessary to examine the mechanism for this alteration during whole body cooling.

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Key Words: Cryotherapy; Microcirculation; Tissue Viability Imaging; TiVi; Cooling; Cold Water; Whole Body; Occupational Cooling

INTRODUCTION

Various modalities of cooling, including whole body cryotherapy (WBC) and cold water immersion (CWI), have gained widespread acceptance in clinical (28), rehabilitative (3), and sporting settings (17). While the removal of heat from the body in the form of CWI has been used for centuries, WBC is a relatively new technique consisting of exposure to extremely cold temperatures (typically between -100 to -130°C) in a climatic chamber for a short duration of time in

minimal clothing (1). Cryotherapy can be used as a basic treatment of acute tissue injury and to treat symptoms associated with local inflammation (38). It has also been shown to reduce pain, muscle spasm, blood flow, nerve conduction velocity, and skin, muscle, and core temperature (21). As a result both WBC and CWI are used by patients suffering from rheumatic diseases, fibromyalgia, and ankylosing spondylitis and by athletes before, during, and after sporting activity.

However, cooling the skin can also produce a

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powerful localized vasoconstriction that can decrease skin blood flow essentially to zero (4) thus diverting blood flow from the skin to the central organs (14). It is believed that cooling, via the sympathetic nervous system, stimulates localized neurotransmission from noradrenergic nerves to cause peripheral vasoconstriction in order to minimise heat loss to the environment (4, 15). Previous clinical studies have shown that decreasing tissue temperatures reduces the demand of oxygen and attenuates the liberation of vasodilatation mediators, which results in a decrease of the skin microcirculation due to the reduction in the periphery of blood volume (34). As cryotherapy has been postulated to generate this powerful local vaso-motor response with initial vasoconstriction followed by secondary vasodilation (40) it is likely that, in conjunction with an alteration in the permeability of the capillary vessels, the skin microcirculation in the upper human dermis may be altered.

Skin microvasculature is extremely complex in structure and the role of blood supply in providing the entire body with oxygen has been studied extensively in recent decades. It is at the microcirculatory level that the exchange of material between the intravascular and interstitial space occurs. As a result, skin microcirculation is very important in a clinical sense (32, 39). Tissue Viability (TiVi) has recently emerged as a promising, portable, and low-cost polarization technology for dermal microvascular viability and red blood cell concentration assessment (27). The method has previously produced high-resolution images of the red blood cell concentration in the upper human dermis and is easier to deploy in a clinical setting in comparison to other commonly employed imaging techniques (30).

Despite the wealth of existing physiological literature on thermogenesis (35), thermoregulation (12), cold stress (45), heart rate variability (25), and performance (41) in the cold, very little is known about the changes in skin microcirculation following whole body cold exposure. Several studies have demonstrated that the application of cold in the form of water therapy (12, 40) and ice application (22, 23, 36) reduced blood flow in healthy subjects. Other animal-based studies have shown that cold application decreases microvascular permeability following striated muscle contusion in rats (11). To our knowledge there is no published literature that assesses the changes in skin microcirculation following two different modalities of cryotherapy. Therefore the aim of this investigation was to elucidate the effects of WBC and CWI on thermal comfort, thermal sensation, and on skin microcirculation using the novel and recently developed technology known as Tissue Viability (TiVi) Imaging.

MATERIAL AND METHODS

Five healthy, male participants, with a mean±S.D. age of 21.5±2.3 years, height 178.8±8.3 cm, and mass 79.8±7.5 kg, volunteered for this study. The study was conducted within the standards set by the latest version of the Declaration of Helsinki and approved by the Education and Health Sciences Research Ethics Committee at University of Limerick, Ireland. After being briefed on the requirements of the study, all participants completed a pre-screening questionnaire and provided informed written consent. Demographic variables (age, height, and weight) were ascertained and recorded. The area of interest was a 3cm x 3cm on the mid-thigh, between the most anterior aspects of the inguinal fold and the patella. Participants were instructed to shave this area at least 24 hours before exposure to the cryotherapy protocols. The reason for this was two-fold. First, to allow better contact and signal for the TiVi, and second, hair on this region might otherwise have had physiological consequences with respect to the insulating properties of the skin.

Procedure

In a randomised crossover design participants were exposed to either WBC or cold water immersion. One week later each participant was exposed to the remaining protocol. As previously described by Costello et al. (7, 8), WBC exposures were administered in a specially built, temperature-controlled unit (Zimmer Elektromedizin, Germany), at the Shannon Cryotherapy Clinic in Ennis, County Clare. The chamber consists of two rooms, a pre-cooling room and a cryogenic chamber. The temperature of the therapy room remained at a constant level [$-110 \pm 3^\circ\text{C}$ (mean \pm SD)]. Subjects entered the first room ($-60 \pm 3^\circ\text{C}$) for 20 seconds before entering the second room ($-110 \pm 3^\circ\text{C}$) for 3 min. The duration and temperature of the cold chamber were similar to that utilized elsewhere (7, 8, 16, 19, 20, 33, 42, 44). To negate any potential insulating effects of stationary air, the subjects were instructed to walk slowly in a circular direction and to flex and extend their elbows and fingers. In the chamber, subjects wore two pairs of gloves, had their noses and mouths covered with surgical masks, had their ears covered with a woollen headband, and wore dry shoes and socks. All participants wore shorts and nothing above the waist during the exposure. Glasses, contact lenses, and all jewellery and piercings were removed before entry to the chamber (7, 8).

The cold water immersion protocol took place in a hydrotherapy lab at the University of Limerick and the methodology was similar to that employed by Costello

and Donnelly (9). Participants were seated in a tank and immersed to the level of the sternum for 5 min in water at 8°C. The temperature of the water immersions was measured using a digital aquarium thermometer and the water was stirred at regular intervals by the experimenter. Immediately after the water immersion each participant was asked to towel-dry his body, change into dry shorts and T-shirts, and transfer to a nearby darkened room where the imaging took place. On both occasions, the participants commenced the imaging at 5 min post immersion/exposure.

Post treatment - Tissue Viability (TiVi) Imaging

The TiVi camera used in the current study is based on the technology commercially available through Wheels-Bridge AB, Linköping, Sweden. The camera is a small, portable device that can be used for high-resolution imaging. Each image is subject to a post-processing algorithm, the result of which is a false-coloured image, sensitive to the red blood cell concentration in the superficial skin tissue (30).

The imaging system was set to take an image every 15 s with 3648×2736 pixel resolution at a height of approximately 15 cm from the skin surface. Every effort was made to ensure constant experimental conditions during this study e.g. environment, procedural protocol, and ambient temperature. Imaging commenced almost immediately (5 minutes) following treatment and lasted for 40 minutes resulting in a total of 160 images. During the image collection period subjects lay supine and immobile on a plinth with the head supported. The room temperature was kept controlled at a temperature of 21° C.

After the tests were completed each of the 160 images for both modalities was subjected to a spectroscopic TiVi algorithm, which allows the contrast information to be plotted as a function of time (27). For each image, the equation applied was: $M_{out} = ((M_{red} - M_{green}) \cdot e^{-p((M_{red} - M_{green}) / M_{red})}) / M_{red}$, (26) where M_{red} and M_{green} represent the red and green colour planes of the image, and p represents an empirical factor to produce the best linear fit between output variable (called tissue viability index—TiVi_{index}) and RBC concentration. M_{out} represents a matrix of maximum 3648×2736 TiVi_{index} values for single-image acquisition (24). The region of interest, corresponding to the selected 3 cm \times 3 cm area was chosen from every processed image, and the average TiVi_{index} for the area was found. To monitor the response of the microcirculation, the average TiVi_{index} value was plotted as a function of time. The individual data for each of the five subjects is being presented for analysis.

Thermal Comfort Scales

The subjects rated their thermal sensation with a nine-point standard scale before and immediately after WBC and CWI. The question the subjects were asked was 'How are you feeling now?' The subjects then answered by pointing to a scale from 4 to -4 (4 = very hot, 3 = hot, 2 = warm, 1 = slightly warm, 0 = neutral, -1 = slightly cool, -2 = cool, -3 = cold, -4 = very cold). Thermal comfort (18) was also assessed immediately after exposures with a five-point scale ('Do you find this,' 0 = comfortable, 1 = slightly uncomfortable, 2 = uncomfortable, 3 = very uncomfortable, 4 = extremely uncomfortable). In the dark room, each subject had a diary in which they self-recorded the ratings. The subjects were instructed to relate their sensations to the time of reporting.

Statistical Analysis

The subjective assessment of thermal comfort and thermal sensation were analysed using the Statistical Package for the Social Sciences software (SPSS version 16, SPSS Inc., Chicago, IL, USA). Differences between treatments (thermal comfort) were analysed using the Wilcoxon signed-rank test. The Friedman analysis of variance (ANOVA) was used to detect differences across time for the nonparametric data obtained from the Likert-type measurement scale for thermal sensation. A follow-up analysis using the Wilcoxon signed-rank procedure to examine differences between baseline data and data obtained during each follow-up was done for all items. Statistical significance was accepted at $p < 0.05$, and all data are presented as means \pm S.D unless otherwise stated.

RESULTS

Tissue Viability (TiVi) Imaging

All subjects completed the interventions successfully. Figures 1-5 display the time series for each of the five individuals. All 5 graphs display similar trends. Both WBC and CWI appear to have induced vasoconstriction and these post-treatment data sets display an increase in the concentration on RBC until approximately 40 minutes after both treatments. For four of the five graphs (Figure 1, 2, 4, 5) the greatest reduction in the TiVi Index, for both treatment modalities, occurs in the first five minutes, with all four subjects showing at least a 50% reduction. Approximately 15 minutes after the first image was recorded the normalised TiVi Index declined to below

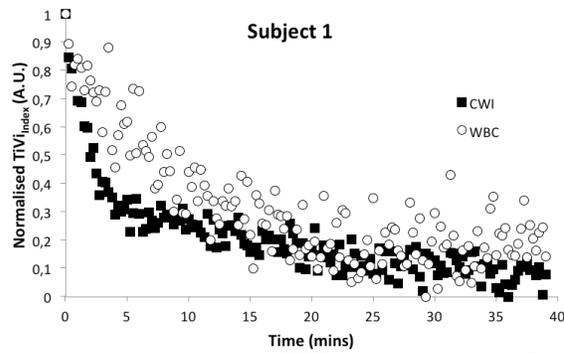


Figure 1. Comparisons of the skin microcirculation using the TiVi index, following the two cooling modalities, for subject 1.

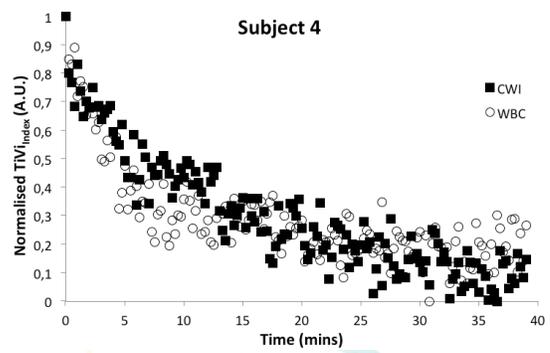


Figure 4. Comparisons of the skin microcirculation using the TiVi index, following the two cooling modalities, for subject 4.

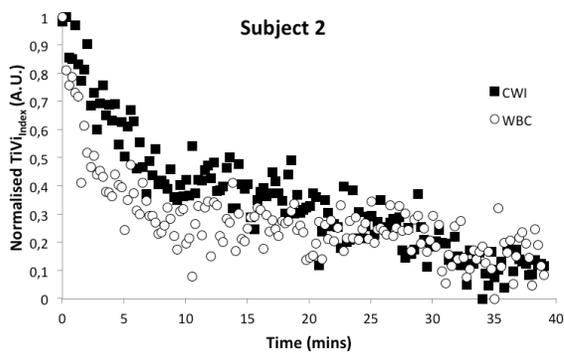


Figure 2. Comparisons of the skin microcirculation using the TiVi index, following the two cooling modalities, for subject 2.

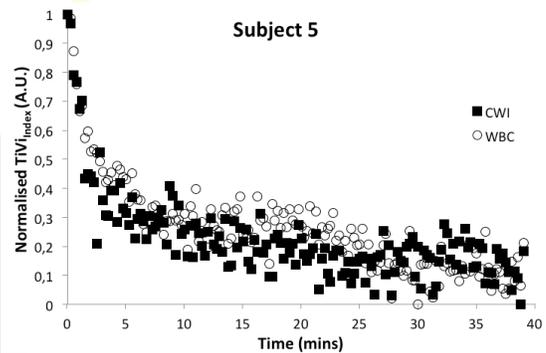


Figure 5. Comparisons of the skin microcirculation using the TiVi index, following the two cooling modalities, for subject 5.

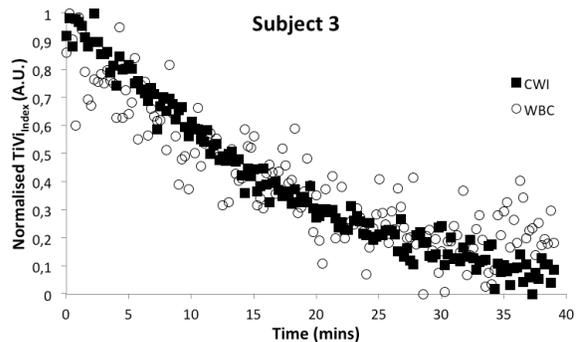


Figure 3. Comparisons of the skin microcirculation using the TiVi index, following the two cooling modalities, for subject 3.

30% of the value in the first image, for both the WBC and the CWI, in all five subjects. From the 30th to the 40th minutes all five graphs depict a similar trend of a horizontal linear tendency.

Thermal Comfort and Sensation

The distribution of thermal sensation and comfort votes before and after WBC and CWI is displayed in Figures 6 and 7. Thermal sensation before both exposures ranged from slightly hot to slightly cool,

with 60% reporting a sensation of slightly warm or neutral. Following both the WBC and the CWI the subjects' thermal sensation ranged from cool to very cold. There was a significant time effect for both the WBC and CWI ($p = 0.003$), with the follow-up analysis showing significant differences ($p < 0.05$) immediately after and 5 minutes post both treatments when compared to baseline. There was no significant treatment effect ($p > 0.05$) between the two groups. Following both the CWI and the WBC, subjects' assessment of thermal comfort ranged from slightly uncomfortable to very uncomfortable, with no individual reporting the exposure as either comfortable or extremely uncomfortable. There were no significant differences in thermal comfort between the two treatment modalities ($p = 0.56$).

DISCUSSION

The present study demonstrates that both cooling treatments (CWI and WBC) induced a reaction in the skin microcirculation. Despite the widespread use of cryotherapy in both a sporting and a clinical setting, little is known regarding the effects of cold on skin microcirculation (34). The human skin circulation is best known for its role in thermoregulation (4) and

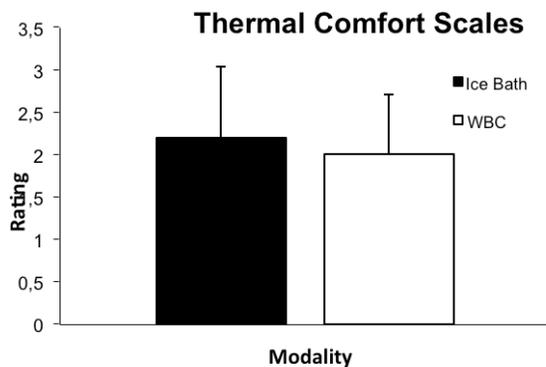


Figure 6. Subjects' ($n = 5$) rating of thermal comfort immediately after exposure to both the Whole Body Cryotherapy (WBC) and the Cold Water Immersion (CWI) ($P > 0.05$). Data is mean \pm SD.

these results demonstrate that red blood cell concentration in the mid-thigh was altered following exposure to both cryotherapy modalities. Although it is well known that a decrease in skin and/or core temperature causes a reflex activation of sympathetic vasoconstrictor nerves, with a resultant decrease in cutaneous vaso-constriction and skin blood flow, the detailed microcirculatory pathways underlying the effects of cooling in the skin or skeletal muscle remain to be established (34). The thermoregulation system is also assisted by thermal receptors of peripheral (skin) and internal organs. When the body temperature drops rapidly (during cryotherapy), adaptation mechanisms are required to avoid such stress (6). Physiological mechanisms such as surface vessels' contraction, muscle trembling, and shivers protect organisms against excessive cooling. This is the first study that has aimed to assess skin microcirculation following two methods of whole body cooling.

Tissue Viability (TiVi) Imaging

Previous work outlined the operating principle and validation of the Tissue Viability (TiVi) imager as a means of assessing RBC concentration in the skin microcirculation in healthy subjects (27, 29, 30). The current study outlines the similar effect that two different modalities of cryotherapy have on the skin concentration of red blood cells. Despite the large discrepancies in terms of temperature, application, and duration, all five subjects display very similar trends following vasoconstriction, as a result of the cold application. Of the 5 subjects, 4 display a similar pattern of changes to skin blood concentration. A steep decline in the normalised TiVi index for the first 5 minutes to below 50%, followed by a more gentle reduction from 15 – 30 minutes was observed. For all five subjects, a baseline appears to be reached between 30 and 40 minutes following the start of data

collection (5 minutes after exiting either the chamber or the water bath).

The reported TiVi skin measurement in all 5 volunteers immediately after cold corresponds to an observable reddening of the skin visible after both forms of cold exposure. Essentially, skin reddening was highest immediately on return to warm air from the cold treatment, and declined thereafter. The TiVi technique allows detailed measurements of the time course of skin blood flow changes associated with recovery from cold exposure. Cold exposure is likely to induce cutaneous vaso-constriction, with a reactive hyperaemia occurring on return to warm air. This vaso-dilation is likely to be endothelium-derived vasodilation in response to skin temperature changes (4). This preliminary data appears to indicate an initial high skin content of red blood cells when measurement commenced (5 minutes after the end of cold exposure). In four out of 5 volunteers, skin blood cell content declined rapidly initially, with a slower rate of decline after approximately 5 minutes. Since the methodology did not allow measurement during and immediately after the cooling protocols, the timing of the increase in skin blood flow during or immediately after cold exposure was not recorded.

Although the present study only included 5 subjects it is important to note the large variation in the individual skin microcirculatory responses to cold. However, similar variations are not uncommon and previous research has reported large variation in similar physiological assessment following cold exposure (5, 10, 43). This variation may be attributed to factors as body size, fitness level, amount of subcutaneous fat, and gender, although the current study only included healthy males. It has been previously reported that in an individual with a larger body size and good aerobic capacity the rate of body cooling slows down and that leaner subjects have higher skin temperatures and lower muscle temperatures after cooling than subjects with more subcutaneous fat (31, 43).

Thermal Comfort and Thermal Sensation

Previously, similar techniques have been used to assess participants' perceptions of both thermal comfort and sensation following exposure to cold (37, 43). The results of the current study demonstrate subjects' subjective assessment of cold to be significantly lower following both WBC and CWI. However, CWI was only significantly different from pre-treatment five minutes after exposure while WBC was only different immediately after exposure. In relation to thermal comfort, there were no significant differences observed between treatments. Our results are similar to that of Somolander and colleagues (37) and Westerlund and colleagues (43) who assessed

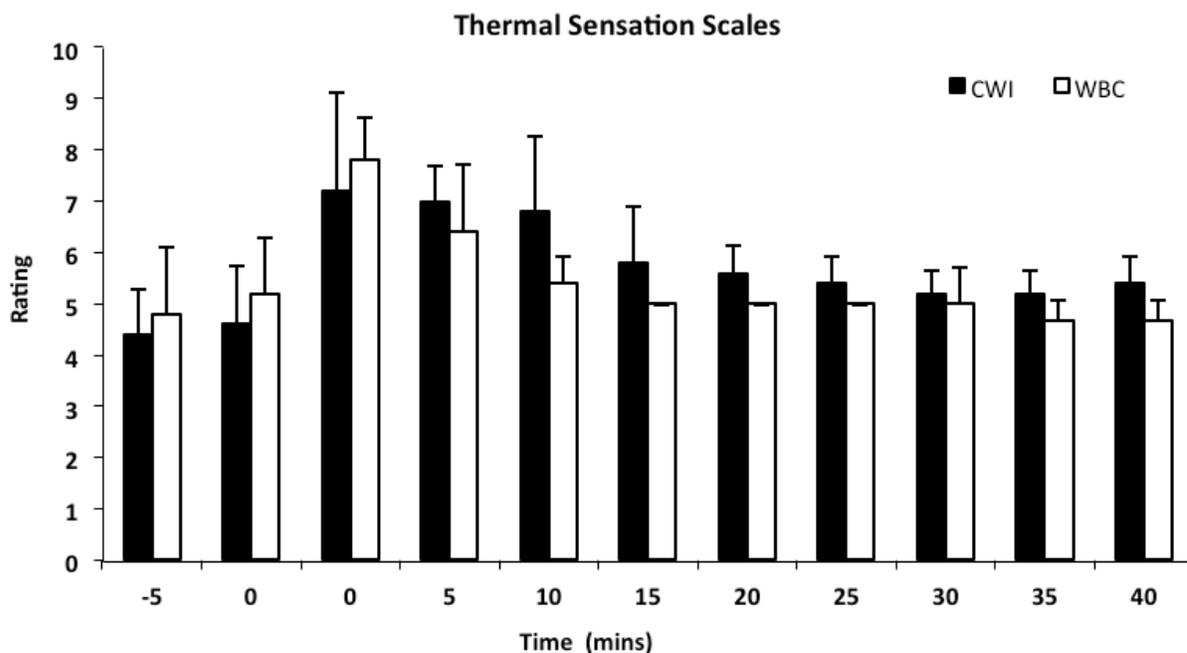


Figure 7. Subjects' (n = 5) rating of thermal sensation before and after exposure to both the Whole Body Cryotherapy (WBC) and the Cold Water Immersion (CWI). Data is mean ± SD. * signifies a significant increase versus pre-treatment for CWI ($P < 0.05$). # signifies a significant increase versus pre-treatment for WBC ($P < 0.05$).

thermal sensation and comfort after exposure to WBC. Somolander et al. (37) also reported that thermal sensation and comfort become habituated following repeated exposure to both WBC and winter swimming. It is of interest to note that the participants in the current study did not report any pain or ill effects after exposure to either treatment.

Study limitations

First, the present results were limited to a small sample size of young healthy males. Second, the cooling treatments used in the present study were limited to either a commonly used sports-based recovery treatment (CWI), or an exposure time and temperature recommended by the manufacturers of the WBC chamber (7, 8). It is possible that increased cooling durations or temperatures may alter the skin microcirculation to a larger extent. Therefore, post-tests in further studies should be repeated for longer than the current study's 40 minutes to ascertain a baseline. Third, baseline data was not recorded in the current study as any discrepancy in the placement of the camera would affect the resolution of the TiVi imaging and give erroneous data. Consequently, pre-data could not be compared to post-treatment data. Moreover, the thickness of the subcutaneous fat in the thigh region was not assessed in the current study. Finally, although assessed elsewhere (6, 7, 8, 43), the

current study did not assess shivering or record the temperature of skin, muscle, core or joint and further research is required to determine whether either of these particular protocols alter these temperatures.

CONCLUSION

In conclusion the present study demonstrated that RBC concentration in the skin microcirculation was altered, in a similar fashion, following two different modalities of whole body cryotherapy, CWI and WBC, which are frequently used by patients and athletes alike in rehabilitative and sporting settings. This was established using a novel, portable, and low-cost piece of technology called TiVi imaging. The result of this current study also demonstrated that both treatments significantly reduced participants' subjective assessment of thermal sensation, assessed using a questionnaire. Further study is necessary to examine the mechanism for this alteration in skin microcirculation during whole body cooling, as the physiologic basis for this effect is still not completely understood.

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