<u>How does Cryotherapy effect ankle proprioception in healthy individuals?</u>

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**Abstract**

*Objectives:* To investigate how a 15 minute Cryotherapy intervention effects proprioception by measuring Joint positional Sense (JPS) and static single legged balance. *Design:* Repeated measures design. *Setting:* Laboratory. *Participants:* Eighteen healthy university sports team students (11 males, 7 females) aged between 20-21 years. *Main Outcome Measures*: Participants were treated with 15 minutes Aircast Cryo-cuff. The subject's skin temperature was measured before and immediately after 15 minutes Cryotherapy treatment. Ankle active joint positional sense (A-JPS) and passive joint positional sense (P-JPS) was measured at pre-test, immediately post-test and 5 minutes post-test. Static balance was measured by Centre of Pressure (CoP) mean path length, medial-lateral (ML) CoP mean Deviation and anterior-posterior (AP) CoP mean Deviation and mean time-to-boundary (TtB) Minima for AP and ML directions. *Results:* No significant differences found for the variables of JPS and static single balance testing after 15 minutes Cryotherapy treatment. However, mean differences for CoP mean path length and ML mean deviation were shown to improve following Cryotherapy treatment, results not previously found in the literature. *Conclusion:* Results suggest that 15 minute Cryo-cuff treatment doesn't significantly affect proprioception. Although the effect of Cryotherapy on proprioception depends on cooling modality used, time frame applied and joint applied to.

**Keywords**

Cryotherapy; proprioception; JPS; CoP

## Introduction

Cryotherapy is widely used in the sporting world with differing modalities for various reasons and involves the reduction of tissue temperature (Furmanek et al. 2014, Jawantanakul 2004 and Knight, 1995). Cryotherapy results in local analgesia, inhibited oedema formation, decreased blood circulation and reduced nerve conduction velocity amongst others (Knight, 1995).

Proprioception, commonly defined as the 'cumulative neural input from mechanoreceptors into the central nervous system', which is used for aiding joint stability and proper joint function could be effected by cryotherapy, due to the reduction in NCV (Furmanek et al. 2014, Costello and Donnelly 2010 and Wassinger et al. 2007). The effect of cooling has been investigated by various authors and whilst there is a difference between the effect it has on sensory and motor neurones there is an agreed consensus that NCV is affected by cryotherapy which could negatively affect joint control, function and stability (White and Wells 2013, Herrera et al. 2011 and Williams et al. 2001). The predominant factor regarding the effect upon NCV relates to the depth to which the cooling modality can penetrate the tissues which depends on tissue thickness, density and water content (Williams et al. 2013). A Cryocuff which applies cold water and compression has been proven to reduce NCV and effect ankle proprioception when applied for 15 minutes, replicating its potential application in a sporting setting (Fullam et al. 2015 and Herrera et al. 2010). It is seen in some sports such as Rugby and Basketball, where athletes have a cooling method applied to their ankle for a period of time to aid recovery before returning to dynamic play, this can be viewed on the television by the public in broadcasted events such as the RBS Six Nations 2016 or NBA games, where players are substituted off for various reasons and are resting on the bench with a cooling method applied. There is no specific literature investigating these methods and their efficacy due to a wide range of variables that would compromise the evaluation of them.

Proprioception can be measured by measuring JPS as a sub-modality of proprioception, JPS determines the ability of the subject to perceive a presented joint angle and then, after the limb has been moved, to actively or passively reproduce the angle, used by clinicians as an indicator to proprioceptive related injury (Ribeiro et al. 2007 and Bleakley et al. 2004). A negative JPS may be caused by the cryotherapy application reducing the afferent information travelling to the central nervous system (CNS) affecting the efferent signals to the joint to correct JPS (Khanmohammadi et al. 2011). JPS is controlled by mechanoreceptors, located in the joints, referred to as either muscle receptors or joint receptors. These joint receptors are located in joint capsules and structures such as reinforcing ligaments, with muscle spindles being the best known type of such receptors (van der Wal 2009). It is these joint receptors that play the leading role in monitoring joint positional sense, sending afferent information to the CNS to sense the position of the joint within space (Prochazka 2010). Studies have shown that muscle afferents provide the substrate of mid or full-range receptor activity (or both) present in recordings from articular nerves (van der Wal 2009). Mechanosensors and GTO will be referred to as JPS throughout this study. JPS is the most investigated aspect of proprioception, with the effect of cryotherapy on JPS at different joints investigated in nine separate studies, the majority found there to be no effect on JPS and only two studied the effects at the ankle (Lins et al. 2015, Khanmohammadi et al. 2011, Oliviera et al. 2010, Surenkok et al. 2008, Wassinger et al. 2007, Dover et al. 2004, Uchio et al. 2003, Ozmun et al. 1996, Hopper et al. 1997). It is difficult to directly compare the results of these studies as different protocols and modalities were used. The general consensus in the literature is to measure JPS actively, due to being a more functional measurement, only one study measured P-JPS and found it to be negatively affected by cryotherapy, results should be taken with care due to not reporting skin temperature or duration of cooling application (Surenkok et al. 2008, Bennell et al. 2005). When measuring ankle JPS, the studies (Khanmohammadi et al. 2011, Hopper et al. 1997) used a pedal goniometer, an apparatus first designed by Gordan (1988) to measure ankle proprioception, which has been proven to have high reliability, for inter-tester (r=0.96) and intra-tester (r=0.91) reliability. Although this equipment has a proven high reliability it is a piece of equipment not easily accessible. Although the pedal goniometer is highly reliable, choosing to measure the participants JPS using a goniometer is both in keeping with its use in a clinical setting and it is an inexpensive piece of equipment (Konor et al. 2012). Venturni et al. (2006) found the intra-session reliability of a universal goniometer to be high (ICC=0.91-0.97) when measuring active ankle range of motion (ROM). The standard error of measurement for DF ROM is shown to be $2^{o}$ and PF ROM is shown to be $3^{o}$ at the ankle joint, when using a hand-held goniometer (Dickson et al. 2012). The proprioceptive system is an important aspect in the maintenance of static balance, modulated through the integration of visual, vestibular, and somatosensory afferent input (McKeon and Hertel 2007). The somatosensory system relays relevant information about body movements in space in relation to a stable surface to the CNS, in order to remain balanced (Hosseinimehr and Norasteh 2010). The ankle AP and ML movements can be measured during a static single legged balance test to identify what effect Cryotherapy is having on the ankle CoP. CoP has been found to be negatively affected following the effects of Cryotherapy, associating to lateral ankle instability (Kernozek et al. 2008). Various investigations have measured the effects of cryotherapy treatment on static balance and found no differences in AP, ML or overall stability.

When postural deviations are small, the body is often simplified as an inverted pendulum pivoting at the ankles, which describes the "ankle strategy" (Boonstra et al. 2013). It has been stated that ankle joint motion represents CoP sway variability in the sagittal plane, however, it is suggested that quiet standing is a multi-joint motor task, involving contributions of variance from the ankle, knee, hip, and neck joints, with primary control in the AP direction (Gage et al. 2004, Oba et al. 2015 and Kilby et al. 2015). This needs to be considered when analysing postural sway data.

Postural stability is generally controlled at the ankle joint through fine movements. During quiet standing, the muscles crossing the ankle joint provide sensory information required to maintain upright standing, with forward body sway associated with a stretch of the calf muscles maintaining balance (Guilo et al. 2009 and Loram et al. 2005). This is controlled by sensory organs, consisting of Golgi tendon organs (GTO) and muscle spindles. GTO's are responsible for detecting changes in muscle tension, if there is too much muscle tension the GTO will inhibit the muscle from creating any force via autogenic inhibition (Moore 2007). Muscle spindles are sensitive to a change in muscle fibre length and play a central role in the control of movement and posture (Radovanovic et al. 2015). The general consensus is that muscle fibres are the main sensors of joint rotation (Guilo et al. 2009). It is possible that cryotherapy could affect the neural feedback from the muscle sensory organs to help maintain balance at the ankle.

Another more sensitive method for analysing postural control during static balance is through TtB measurements of CoP excursions. TtB measurements estimate the time it would take for the CoP to reach the boundary of the base of support, if CoP were to continue on its trajectory at its instantaneous velocity (Hertel et al. 2006). This is a variable that has yet to be measured following Cryotherapy treatment, to identify any postural sway deficits at the ankle joint. TtB quantifies the theoretical amount of time an individual has to make a postural correction to maintain postural stability. TtB Minima provide more dynamically relevant information about the control of the CoM (Van Wegen et al. 2002). If the CoP reaches the boundary of the foot, then postural control can no longer be maintained, lower TtB measurements indicate a greater postural instability (McKeon & Hertel 2007 and Van Emmerick & Van Wegen 2002). Previously, TtB measures have mostly only been described in postural tasks requiring double leg stance, termed as “virtual time-to-collision” (Slobonuv et al. 1997). TtB has been measured during single leg stance, testing how an administered balance programme effects PC (McKeon et al. 2008). No study has solely looked at the effects of cryotherapy application of the ankle joint and the effects it has on TtB minima. McKeon and Hertel (2007) investigated the effects of ice immersion to the plantar aspects of the feet during single and double leg stance, finding an increase in AP in TtB minima following treatment, but rather investigating how plantar cutaneous sensation is affected. As argued above, a study looking at the effects of cryotherapy on postural sway at the ankle joint, measured at pre, post and 5 minutes post looking at its effect on CoP, ML/AP sway variability and TtB minima may produce findings not previously shown in the literature.

A study is yet to assess proprioception, measuring both JPS and single legged balance after the application of a Cryo-cuff for 15 minutes at the ankle joint. Most studies have also failed to keep a measurement of the skin surface temperature to ensure the cooling affect is occurring correctly. Based on previous research, it was anticipated that proprioception would not be significantly affected by cryotherapy treatment.

## Method

### *Design*

This study is a single group pre and post repeated measures experimental design. Measurements were recorded for JPS and static balance during three separate trials (pre, post and 5 minutes post-test). The order in which the measurements were taken was randomized via a flip of a coin.

### *Participants*

Eighteen healthy university sports team students (mean age, 20 ± 0.59 years) from both gender types (11 males, 8 females) volunteered for this study. All of which are involved in various sporting activities (football, hockey, rugby, cricket, running). The participants were required to take part in at least three hours of exercise a week and be involved with regular team sports, by playing competitive games. This is to ensure that they are used to cooling methods, before returning to dynamic activity. Participants were recruited by approaching university sports teams and asking players if they would take part in the study.

### *Ethical Implications*

Participants were students recruited from the University of Worcester. They were informed about the study objectives and protocols. Giving their informed consent in accordance with the University of Worcester Ethical Committee board. The following exclusion criteria was considered; if they have any previous or current fractures to their lower extremities, currently suffer from diabetes, have low blood pressure and decreased blood flow, metal plates in their lower extremities, suffer from chronic ankle instability (CAI), have sensory impairments or suffer from Raynaud's phenomenon, participants who train less than twice a week and play less than one competitive match a week. Participants who reported pain, discomfort or numbness during the procedures for data collection or those that failed to implement the correct assessment procedures were excluded from the study.

***Procedures***

Prior to testing, subjects indicated their dominant leg, only the dominant leg was measured in this study. Leg dominance was classed as the leg they would naturally kick a ball with. Participants removed their shoes and socks to acclimatize to room temperature. An Aircast ankle Cryo/cuff was filled with ice cubes and then filled with tap water to the specified level on the container. The researcher changed the water for every two participant's to ensure that the low temperature of the water was being maintained. Before testing began, the subjects had their sensory sufficiency tested (Tip-Therm thermal sensation pen) to ensure that they were able to comprehend if their ankle became numb from at any point during the testing. The examiner was a 3rd year Sports Therapist who was trained in the use of all the instruments. Each subject had their A-JPS, P-JPS and static balance randomly measured. Before being placed in the Cry-cuff each subject had a skin thermistor placed on the anteromedial aspect of their ankle using 3M tape, which was attached to the Edale thermistor thermometer (accuracy= +/- 0.1 deg C exc probes) to measure their skin surface temperature. Temperature was recorded pre and post-test. Their foot was then placed in the Cryo-cuff. The Cryo-cuff was then filled with the water from the container by lifting the nozzle on the top of the container (Figure 1). Once filled, the Aircast was unattached from the container and the subject had the Aircast applied for 15 minutes. The Aircast fully covered the ankle joint. Following recording the post-test measurements, subjects were asked to walk up and down a ten metre line for five minutes before having their A-JPS, P-JPS and static balance measured at 5 minutes post-test.

Figure 1. Cryo-cuff and skin thermistor

***Pilot trial***

A participant was used in an experimental trial to test the length of time it would take to measure one participant and to ensure that the participant's skin surface temperature could be effectively kept to 15$^{o}$C. The above procedures were followed. The experimental trial found the time it would take to test each participant and the Aircast was effective in cooling the skin to the required temperature found to be effective in past literature (15°C).

***JPS***

JPS was measured with a hand-held goniometer for dorsiflexion and plantarflexion, actively and passively. This was measured pre-test, immediately post-test and 5 minutes post-test. The difference between the perceived angle and the actual angle was recorded as the proprioceptive difference. Subjects lay supine on the electric treatment table with their hips and knees at 0° and heels off the edge of the table. The subjects were also blindfolded to remove any visual cues.

Figure 2. A-JPS DF

The axis of the goniometer was placed 1.5cm inferior to the lateral malleolus, with the stationary arm parallel to the longitudinal axis of the fibula, lining up with the head of the fibula. The moveable arm was parallel to the longitudinal axis of the 5th metatarsal. The subjects were randomly tested for A-JPS or P-JPS first. When testing for A-JPS, the subjects were asked to bring their foot into the target angle in dorsiflexion (20°), the subject was asked to focus on the position of their ankle joint in space for 3 seconds, a time period utilized across the literature (Khanmahammadi et al. 2011). Following this the subjects were asked to bring their foot back into neutral position. Once in neutral, the subjects were asked to actively reproduce the target angle they had just created (Figure 2). This test procedure was repeated for active plantarflexion (60°) (Figure 3). When testing for P-JPS, the subjects were passively moved into 40° of plantarflexion, the subject was again asked to focus on the position of their ankle joint for 3 seconds, before being passively brought back into neutral position. They were then asked to re-create the target angle. The same protocol was repeated for passive dorsiflexion, the target angle produced for this ROM was 10°.

Figure 3. A-JPS PF

***Static single leg Balance Test***

Subjects were familiarized with the protocol and the equipment being used (Force Plate). The Force Plate (AMTI, BP40600, Watertown, MA, USA) consists of a metal square built into the floor in the middle of the lab, with a sensor linked to a computer to measure the force exerted by the subject balancing on one leg for thirty seconds. The Force Plate was calibrated according to manufacturer instructions to ensure accuracy of data. The Force Plate measures the mean centre of pressure (CoP) mean path length, medial-lateral (ML) CoP mean Deviation and anterior-posterior (AP) CoP mean Deviation and TtB for AP and ML. Measuring the deviation in which the CoP is moving in the subject's foot during the thirty second time period, which are reliable measures of static balance (Williams et al. 2013). Static balance was measured three times, pre-test, post-test and five minutes post testing. Before commencing testing, subjects were given the chance to familiarize themselves with balancing on their dominant leg, with their hands on both hips and placing their non-dominant leg in a bent to 45° position. Having their eyes open staring at a black dot on a piece of paper in front of them, placed at eye level. Testing begun once the participant felt balanced. Testing was repeated if the subjects took their hands off their hips or their non-dominant leg touched the ground.

***Statistical analysis***

ANNOVA tests were used to compare JPS and static balance scores at pre, post and five minutes post testing. This was completed for the following variables: CoP path length, ML and AP CoP mean deviation, A-JPS and P-JPS DF and PF, and ML & AP TtB. Tukey's-b test was used to identify the mean values for each homogenous subset. A paired samples T-test was used to find the mean difference between the temperatures recorded pre and immediately post-test. The level of significance was set at 0.05. Descriptive statistics were also produced for each variable. This was completed using IBM SPSS Statistics versions.

*A-JPS*

**Results**

A paired samples t-test was used to analyse skin surface temperature. This test found that the mean skin surface temperature decreased from 21.8°C pre-test, to 15.4°C following the 15 minutes Cryo-cuff treatment (Table 1) (*p*=0.00). The mean difference between the two recorded measurements is shown to be 6.3 °C.

| | *Pre-test* | *Post-test* | *P* |
|---|---|---|---|
| *Skin Temperature (°C)* | 21.8 (0.86) | 15.4 (0.75) | 0.00 |

Table 1. Mean (SD) skin surface temperature before and after 15 minutes of Cryo-cuff treatment

The statistical analysis found no significant difference for JPS using a repeated measures, ANOVA, for active dorsiflexion (*p*=0.080), active plantarflexion (*p*=0.051), passive dorsiflexion (*p*=0.383) and passive plantarflexion (*p*=0.659) following the cryotherapy treatment. A Tukey-b test was carried out to find the mean values for each homogenous subset groups of A-JPS and P-JPS values measured. A change in mean JPS was shown to have occurred for each of the ranges of motion, the changes in positional error are marginal, ranging from 1.22 to 2.11° (Table 2). There is a shown increase from pre to post-test values, that again decreases, 5 minutes post-test, but doesn't return to pre-test values.

| *Range of Motion (°)* | Pre-test | Post-test | 5 Mins Post test | *P* |
|---|---|---|---|---|
| *Dorsiflexion* | 1 ± 0.97 | 2.61 ± 1.33 | 1.38 ± 0.91 | *0.080* |
| *Plantarflexion* | 1.11 ± 1.18 | 2.38 ± 1.42 | 1.61 ± 1.19 | *0.051* |
| | | *P-JPS* | | |
| *Range of Motion* | Pre | Post | 5 Mins Post | *P* |
| *Dorsiflexion* | 1.16 ± 1.46 | 3.61 ± 3.44 | 1.94 ± 1.69 | *0.383* |
| *Plantarflexion* | 2 ± 2.37 | 4.11 ± 2.65 | 2.16 ± 2.66 | *0.659* |

Table 2. Mean (SD) A-JPS and P-JPS scores pre, post, and five minutes post treatment

The results of the static balance test (Table 3) found no significant difference using a repeated measures, ANOVA, for CoP path length ($p$=0.235), ML mean deviation ($p$=0.617), and AP mean deviation ($p$=0.116) following the cryotherapy treatment. A Tukey-b test was carried out to find the mean values for each homogenous subset for CoP path length, ML mean deviation and AP mean deviation.

Table 3. Mean (SD) CoP path length and AP/ ML CoP mean deviation

| | PRE-TEST | POST-TEST | 5 MINS POST TEST | P |
|---|---|---|---|---|
| COP PATH MEAN LENGTH (MM) | 2211.56 ± 838.22 | 2069.58 ± 496.76 | 1857.06 ± 453.40 | 0.235 |
| ML COP MEAN DEVIATION (MM) | 8.24 ± 4.19 | 7.74 ± 1.74 | 7.32 ± 1.77 | 0.617 |
| AP COP MEAN DEVIATION (MM) | 8.91 ± 2.78 | 10.91 ± 3.43 | 9.21 ± 2.89 | 0.116 |

The results show CoP path length (Figure 5) and ML mean deviation (Figure 6), have a gradual decrease in movement following treatment. Both measurements showed a decrease in movement from pre to 5 minutes post. AP mean deviation increases following cryotherapy treatment, which then decreases 5 minutes post-test, but not returning to baseline value following cryotherapy treatment (Figure 6).

Figure 4. CoP Mean Deviation (mm) measured Pre, Post, and 5 Minutes Post Test

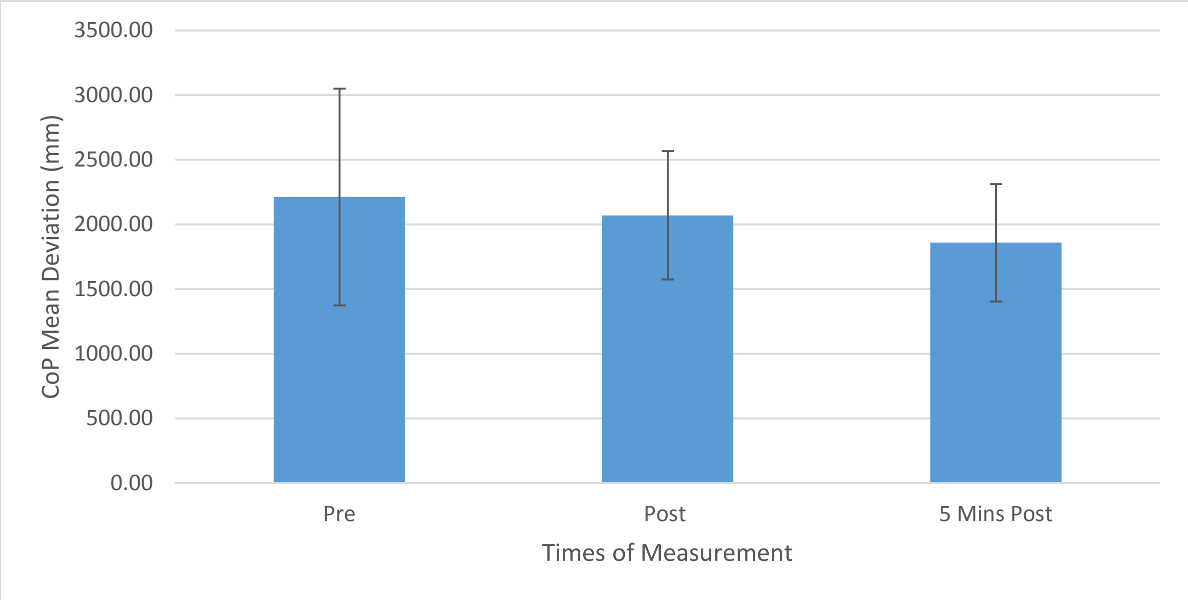



Figure 5. ML CoP Mean Deviation (mm) measured Pre, Post, and 5 Minutes Post Test

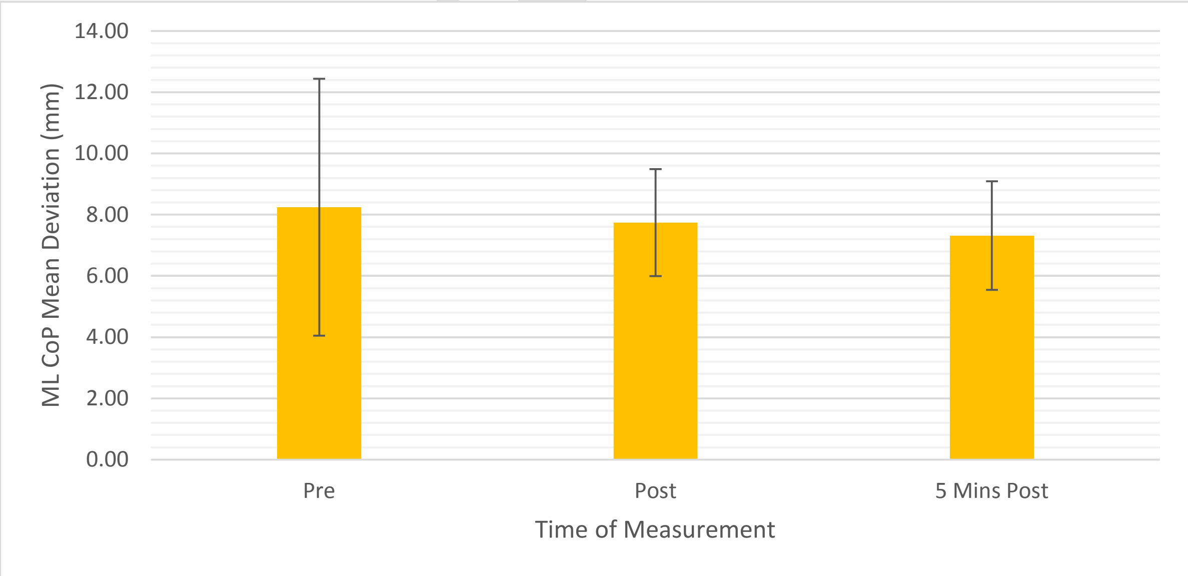
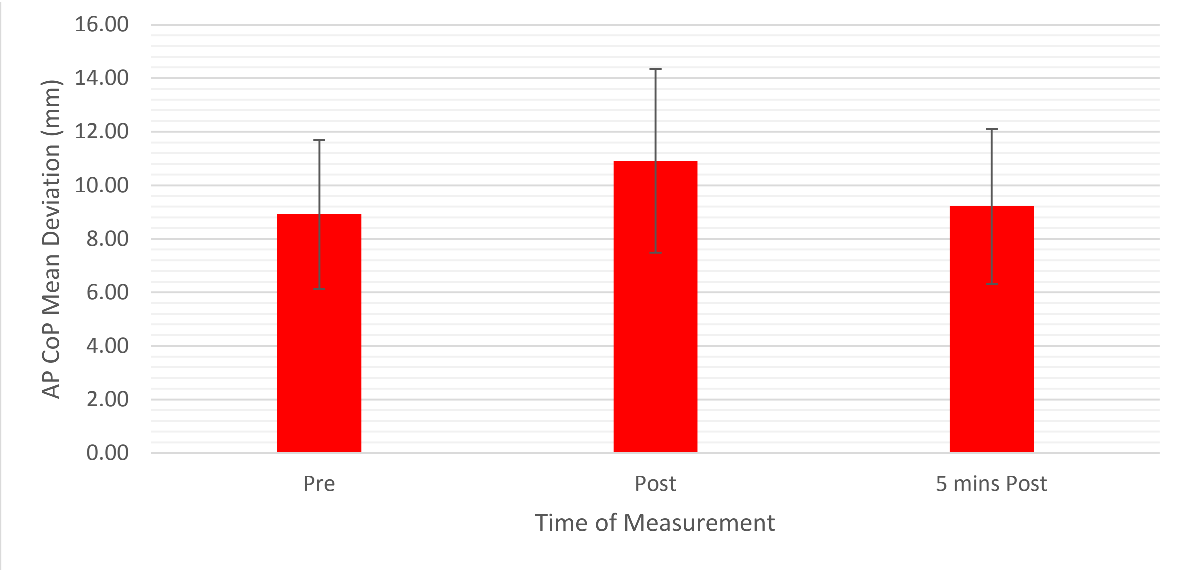


Figure 6. AP CoP Mean Deviation (mm) measured Pre, Post, and 5 Minutes Post Test




The results from the TtB minima calculations show no significant difference using a repeated measures, ANOVA test, for MLTtB Minima (*p*= 0.617) and APTtB Minima (*p*= 0.218). A Tukey-b test was carried out to find the mean values for each homogenous subset for MLTtB Minima and APTtB Minima. Two anomalous results were removed from MLTtB Minima measurements, due to excessive movement on the force platform preventing a valid TtB Minima from being calculated. The minima for both ML and AP are taken from graphs that present the lowest point where there is a shift in the CoP and its direction, effectively when the participant is heading towards the boundary of the foot and corrects themselves.

The results from the collated mean TtB data (Table 4) shows that MLTTB showed a slight increase from pre (3.01) to post test (3.26) that decreased slightly 5 minutes post-test (3.16). The results from APTTB show a different trend to mean min. MLTTB, with there being a decrease in TTB from pre-test (6.35) to post-test (6.24) and then an increase to the highest value of the three recordings at 5 minutes post-test (6.80).

Table 4. Mean TtB (s) AP/ ML Minima

| | *Pre-test* | *Post-test* | *5 Mins post test* | *P* |
|---|---|---|---|---|
| *Mean MLTTB Min. (s)* | 3.01 ± 0.68 | 3.26 ± 0.77 | 3.16 ± 0.71 | 0.617 |
| *Mean APTTB Min. (s)* | 6.35 ± 1.35 | 6.24 ± 1.51 | 6.80 ± 1.45 | 0.218 |

## Discussion

### *Temperature*

The results show that the Cryo-cuff was able to effectively reduce mean skin surface temperature by 6.4°C to 15°C, Fullam et al. (2015) also found that 15 minutes Aircast Cryo-cuff treatment was sufficient in reducing cutaneous temperature. The skin surface temperature is of paramount importance, if the temperature isn't 15°C or below then NCV won't be affected, according to the findings of Algafly and George (2013). This reduction in NCV should in turn theoretically, effect proprioception by effecting the sensory and motor receptors at the ankle. Algafly and George (2013) found that crushed ice, reducing cutaneous temperature to 15°C on the ankle was effective in reducing NCV by 32.8%. If the skin surface temperature is not below 15°C it suggests that proprioception can't be affected, as the speed at which mechanoreceptors feedback to the CNS will not be effected (Furmanek et al. 2014). Therefore, it is important to get this aspect of the study correct, in order for there to be an effect on proprioception that can be effectively investigated. Unlike in previous investigations (Wassinger et al. 2007, Surenkok et al. 2008,Douglas et al. 2013, Williams et al. 2013, Lins et al. 2015) where skin surface temperature wasn't recorded, you cannot directly say whether the modality was cold enough to affect NCV. This effectively limits the results of the studies from the very beginning, as investigators are merely assuming the cryotherapy application reduced skin surface temperature by enough to effect proprioception. This could be why the results of this study, weren't shown to be significant for either JPS or static balance, due to the mean skin surface temperature being 15.4°C. A slightly lower skin surface temperature, may have decreased NCV further, by decreasing the time it would have taken afferent signals to reach the CNS (Algafly and George 2013).

### *JPS*

This study found that 15 minutes of Cryocuff treatment had no significant effect on A-JPS and P-JPS, this is in agreement with results of previous studies (Khanmohammadi et al. 2011, Wassinger et al. 2007, Ozmun et al. 1996, Dover et al. 2004, Lins et al. 2015). However, the mechanism of proprioception is different at each joint, therefore our study can only be compared with the studies investigating ankle joint proprioception (Khanmohammadi et al. 2011). This is due to the depth of mechanoreceptors at different joints and the depth of the penetration of the different cooling applications investigated (Williams et al. 2013). The ankle joint capsule is more superficial than most joints, meaning the joint receptors should be affected more by a superficial cryotherapy application. JPS is controlled by joint receptors that are located within the ankle joint, it is possible that the Cryo-cuff was ineffective in cooling these receptors, due to the depth of penetration not reaching the joint capsule in the testing time frame (Van der Wal 2009). This may also be true in the context of muscle spindles, as muscle spindles are located mostly in areas of muscular tissue, distal and proximal connective tissue (Van der Wal 2009). It is possible that the Cryo-cuff was ineffective in cooling the muscle spindles to elicit an effect on the afferent signals in regards to sensing the position of the joint within the space. As the participants were able to bring their ankle back to the correct angle meaning the muscle spindles were able to provide receptor activity at mid-range.

Similar to our study, Khanmohammadi et al. (2011) recorded JPS, in three separate trials. Our study had better ecological validity than theirs, by having the athletes walk around following the post-test measurement, emulating the athlete returning to functional activity following cryotherapy treatment. This could have affected the results by there being an increase in blood flow to the feet, increasing skin surface temperature, tissue metabolism, blood flow and heat production (Herrera et al. 2011). Therefore, decreasing NCV in a linear fashion with tissue cooling (Herrera et al. 2011). The results show this happening in reverse, with the mean degree of positional error found within JPS at 5 minutes post-test reducing back towards pre-test values, indicating that the effect of rewarming has taken place. Due to the participant's walking around and the acclimatization to room temperature occurring following treatment (Hopper et al. 1997).

Although the results from this study weren't deemed to be significant, there was a minimal change in the mean degree of positional error shown for both A-JPS and P-JPS. However, this would have to be larger in order for a proprioceptive injury to occur. Similar to the results from Hopper et al. (1997) who found Cryotherapy to effect proprioception, these were also not deemed clinically significant due to the relatively small degree of positional error ($0.5^{o}$). This suggests that even the relatively small afferent information that travels to the CNS is enough for the efferent signal to correct JPS in this study (Khanmohammdi et al. 2011).

In comparison of the A-JPS/ P-JPS results, there was no pin-point values between the two measurements that point out that one was effected more than the other. However, post-treatment, P-JPS showed a higher mean degree of positional error compared to A-JPS for both dorsiflexion and plantarflexion. This may be due to A-JPS being a more functional movement that is solely controlled by the participant. Whereas, for the passive movement, the participant was passively brought into the target angle, potentially putting a higher stress on the joints mechanoreceptors, disrupting afferent signals from the area, making it harder for the participants to re-create the target angle. This may explain why Surenkok et al. (2008) found P-JPS to be affected, as the stress placed on the knee by the isokinetic dynamometer affected the mechanoreceptors in the joint. Therefore, if the passive angle at which the ankle joint was placed in this study was increased then the mean degree of positional error may also increase.

Similar to this study, no study reviewed measured the effect of cryotherapy on JPS in injured joints by using previously or currently injured participants. This could provide different results to what this study has shown, as injured populations at various stages of healing, will have damaged mechanoreceptors, affecting JPS (Wassinger et al. 2007).

Different cooling techniques will produce different degrees of joint cooling. Hence, why the cooling modality utilised is critical into how JPS will be affected. There is a direct influence and correlation between Cryotherapy and JPS, in terms of soft tissue thickness, contact time, type of modality and skin temperature influencing the results (Khanmohammdi et al. 2012, Surenkok et al. 2008).

***Static Balance***

This study found that 15 minutes of Cryo-cuff treatment had no significant effect on static balance in healthy individuals. Interestingly however this study did find that CoP mean path length showed a gradual decrease in mean path length from pre-treatment to 5 minutes post treatment, showing that balance improved following cryotherapy treatment. This shows that following treatment the participants better controlled their CoG over their base of support, meaning improved PC (Lins et al. 2015). Whereas AP mean deviation shows to have increased following treatment and decreased again

5 minutes post treatment, showing an indication of a return towards baseline value. Indicating that the Cryotherapy treatment affected balance in the AP direction.

The improvement of balance that is shown in these results, doesn't agree with the findings of the literature reviewed. No other study used a Cryo-cuff as the cooling modality. Indicating that this could be the causative factor in the improved balance shown in these results, as the same time frames were used. One study found no difference in CoP mean path length following 15 minutes of crushed ice on the ankle, showing that crushed ice is insufficient in cooling the ankle receptors (Williams et al. 2013). Another assumption that could be made from these results is how quiet standing is shown to be a multi-joint task, incorporating the use of the knees, hips and neck joints to aid better control of the CoM, especially during CKC exercises (Oba et al. 2015 and Kilby et al. 2015). One main assumption that can be attributed to the improvement in static balance could be a learning effect. As most studies (Williams et al. 2013, Douglas et al. 2013, Lins et al. 2015) only recorded measurements pre-treatment and post treatment, meaning the different results found, could be attributed to participants potentially being able to get used to the balancing exercise over the three measurement trials. Further studies could look at applying a Cryocuff to the knee as well as the ankle, to see how PC is then affected. Another factor that could be seen with these results is that static balance was completed with their eyes open. In the study by Williams et al. (2013) they found when measuring CoP mean path length in eyes open and eyes closed conditions, the eyes closed condition was found to have a higher CoP mean path length. Although the eyes open condition is seen as more functional, the eyes closed condition further integrates the vestibular and proprioceptive system for aiding PC (McKeon and Hertel 2008). It was decided to test proprioception in static balance with eyes open to ensure a more functional setting, mirroring that of a sporting environment, enhancing ecological validity and improving inter-session reliability. Previous studies (Douglas et al. 2013, Surenkok et al. 2008) measured the effects of cryotherapy on static balance in an eyes open condition. An external que was also used to ensure validity, having participants with their eyes open staring at a black dot on a piece of paper in front of them, placed at eye level. Also during eyes open the proprioceptive system is still active as the calf muscle is shortening as the body sways forwards and lengthening as the body sways backwards to the vertical during quiet standing (Loram et al. 2005).

Therefore, it would be interesting to see in further research how a Cryocuff effects static balance, with eyes closed compared to eyes open conditions.

The results for ML mean deviation show a gradual decrease in mean deviation from pre-treatment to 5 minutes post treatment, not previously found in the literature. It was stated that poor PC could lead to injury. As studies have shown that proprioception is affected by cryotherapy and Han et al. (2015) states that ankle proprioception is arguably the most vital component to assisting balance in sport, being the only part of the body contacting with the ground during movement. It has been found that impaired ankle proprioception leads to an ankle injury, caused by concurrent contraction of the plantarflexors and dorsiflexors (Han et al. 2015). As found by Kerzonek et al. (2008), following ice immersion of the ankle joint, there was a greater ML sway variability, in participants with Lateral ankle sprain (LAS), showing a higher chance of injury. This cannot be compared directly to the results of this study due to a population with LAS being used. Therefore, it can be assumed from the results of this study that a 15 minute Cryocuff treatment may not lead to a LAS following treatment, indicating a decreased risk of injury.

Although CoP mean path length and ML sway variability was shown to improve, AP sway variability was shown to be negatively affected following treatment. Showing, that following treatment there was an increase in AP sway variability and then a slight decrease 5 minutes post treatment, returning towards baseline values. Indicating that the Cryo-cuff was effective in cooling the muscle spindles of the gastrocnemius and calf muscles, the main muscles that provide sensory info to maintain upright standing (Loram et al. 2005 and Guilo et al. 2009). This explains why there was a negative effect on AP sway variability rather than ML sway variability, due to the placement of the Cryo-cuff on the ankle, effectively cooling the calf musculature. This also explains why previous studies didn't find an effect on AP sway variability due to the placements of the cooling modalities not efficiently cooling the calf muscle spindles, which play a central role in the control of movement and posture (Radovanic et al. 2015). It can be assumed in this study that the GTO are responsible for detecting changes in muscle tension (Moore 2007), therefore during the static balance test, if the participant's CoP was moving forward the GTO in the calf will sense this change in muscle tension in the calf and send afferent signals to the CNS to correct posture.

The results from further investigation of postural stability on TtB Minima, show that after treatment, there was no significant effect on ML or AP Mean TtB Minima. However, both ML and AP TtB Minima show similar trends to the mean path length data, further backing the results.

This is the first known investigation investigating TtB Minima following a cryotherapy treatment intervention. It is proposed that TtB Minima is a sensitive and more reliable way of measuring PC (McKeon and Hertel 2007). Only one study can be compared to this one, however, they assessed planter cutaneous sensation rather than proprioception with ice immersion of the plantar aspects of the feet (McKeon and Hertel 2007). Their results indicated that cooling the plantar aspects of the foot increased APTTB Minima, most likely due to the muscle spindles of the calf not being cooled like in this study. The results from the TtB Minima can help to further back the results from the force moments data. Again showing how APTTB Minima was negatively affected by treatment. Showing an increase in the instantaneous velocity of the CoP to the foot boundary, meaning more chance of toppling over, as the closer the value is to zero the higher the loss of PC (McKeon and Hertel 2007). When comparing the results from this study to McKeon et al. (2008) who used healthy participants with a self-reported history of CAI. The control group used by McKeon et al. (2008) have lower TtB Minima than the results found in this study which could indicate that the participants in this study had better PC and can assume the participants are less likely to sustain an ankle injury. Showing a link to Linens et al. (2014) stating that lower TtB Minima could be linked with identifying CAI, indicating episodes of instability. Comparing the overall results of the static single legged balance test it is unlikely following 15 minutes Cryo-cuff treatment that participants will sustain an ankle injury. Linking MLTTB with ML sway variability, it can be assumed that participants are unlikely to develop a LAS, due to PS in this direction.

Both TtB Minima and mean CoP mean path length data showed similar trends showing an increase at measurements 5 minutes post, being higher than baseline recordings. This further backs the assumption of the learning effect taking place over the separate trials or possibly the walking around has acted similar to a warm up and increased blood flow to the ankle area, increasing the rewarming effect (Herrera et al. 2011).

**Limitations**

One notable limitation of this study is that the results can only be compared and applied to a healthy and active population. It cannot be applied to an injured population, as an injured population may be in the inflammation process of healing, which would notably have an effect on the outcome on the proprioceptive system. Also, only eighteen participants took part in this study, a relatively small sample that with an increase in participants may find a significance within the data set. This small sample size was due to the very specific exclusion criteria set, as finding people within University teams who train more than twice a week and play a match more than once week and no previous lower extremity fractures were difficult to find. This led to this study having a relatively smaller sample size compared to most other studies. Another possible limitation with the sample is the use of both males and females in the study, due to the potential for physiological differences. However, in respect to the sporting world both men and women are involved in playing sports and past studies have used both men and women in their investigations. Another notable limitation of this study is not being able to control the room temperature, as it may have been at slightly different temperatures for each participant that may have affected the results due to acclimatization that would have occurred following treatment. Also if proprioception is to be affected then skin surface temperature needs to be lowered to 15°C or below to have any significant effect on proprioception by reducing NCV.

**Conclusion**

This was the first study to look at both the effects of Cryotherapy at the ankle joint on both JPS and static single leg balance. This study found that 15 minutes of Cryo-cuff treatment had no significant effect on proprioception. JPS was shown to be affected differently to static single legged balance. However, when the results are compared with the reviewed literature it can be seen that many variables are involved in having an effect on proprioception with cryotherapy. For proprioception to be effected by cryotherapy it relies on the type of Cryotherapy modality used, timing of application, and depth of penetration of the Cryotherapy application. A minor effect on the proprioceptive system was shown, however, it cannot be deemed significant enough to cause an injury. Further investigations are needed to see how a Cryo-cuff will affect proprioception at other joints and the effect on injured participants, with CAI and previous LAS. More research needs to be undertaken investigating the effects of cryotherapy on functional performance at the ankle, as this will relate more to the functional movement of most sports and show how PS is affected during functional movement. It cannot yet be pin-pointed to what is the most effective component for effecting proprioception, either temperature, time-frame, or modality. Further research is needed into directly comparing these components against each other.

**Conflict of Interests Statement**

I certify that all authors have seen and approved the final version of the manuscript being submitted. I warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

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