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Introduction

The therapeutic technique of cooling commonly used for the treatment of musculoskeletal conditions and recovery in sport is widely debated[1,2,3]. Deliberation around when athletes may return to activity safely, following local cooling applications is recognised[1,2], in consideration of potential neuromuscular deficits[4]. Although methodological differences reduce the strength of consensus across current studies in this area. Within sporting situations, cryotherapeutic application is often associated with pitch-side or half time management of injuries to induce analgesic responses[2]. Other known physiologic effects include; reduced cellular metabolism[5], receptor firing rate[6] nerve conduction velocity[7] and inhibition of muscle spindles[8,9], are well-reported[2,3]. Reduction of tissue temperatures through local cryotherapy applications occur through contact of cryotherapeutic modalities via skin surface initially, to achieve physiological responses[10]. A therapeutic skin surface temperature (T_{sk}) target range of between 10-15°C is essential to initiate those essential responses in order to aid acute injury management[11]. Modalities of cooling differ in thermodynamic properties and therefore cooling efficiency[10]. Efficient in phase change, modalities such as crushed or wetted ice noted numerously throughout cryotherapy literature as the most efficient for inducing physiological changes[12,13,14]. The known effects of cryotherapy on performance and re-injury/further injury risk lack consensus, with methodology difficult to compare outcome measures across studies. Previous studies discuss changes in muscle force depending on cryotherapy location and report increases, decreases or no change[8,15,16,17]. Emerging literature[1] recognised the importance of further study in muscle strength response post local cooling application, applicable to sporting situations.

Although rate of temperature change between modalities presents fluctuations the consensus agrees on a relationship existing[12]; that being, a highly significant quadratic association between T_{sk} and intramuscular temperatures (T_{im}) post local cooling applications[12]. The gold standard protocol to measure T_{sk} is through infrared thermal imaging[18,19]. Due to the multifactorial considerations that can affect deeper soft tissues, such as duration[20], gender[21], adipose tissue levels[22] and location of cryotherapy applications, knowing the optimum protocol for reduction in muscle temperature to induce physiological changes can be challenging. Furthermore, inconsistencies in methods across studies consequently implicate the ability to compare outcomes or effects accurately. This said literature clearly displays physiological changes as a result of various cryotherapy applications[20; 21; 22] and has indicated the importance of exploration of cryotherapy on neuromuscular function[23].

Literature indicates performance deficits as a result of cooling[2] and these have been attributed with decreases in dynamic contractile force[1]. These conclusions were drawn based on measures of ultrasound shear wave elastography and myoelectrical activity, no output measures of strength were ascertained. It is important to note that the changes in dynamic contractile force were strongly related to muscle stiffness, which resulted in acute change in muscle mechanical properties after air-pulsed cryotherapy intervention[1]. The authors propose this may reduce the amount of stretch able to sustain by the muscle without resulting in injury. The evaluation of muscle strength with isokinetic dynamometry (IKD) is commonly utilised in research due to its high test-retest reliability[24]. Measurements of peak torque (PT) and average torque (AvT) to establish muscle function can determine reductions post fatigue protocols[25]. Strength deficits can have implications on knee stability during performance[26].

Significant reductions in PT and AvT quadricep strength compared to baseline measurements following a 20-minute crushed ice application to the knee, which did not fully recover at 20-minutes post cooling intervention in a recent study[27]. The authors suggest reductions in concentric strength can still occur therefore, even when indirectly cooled distal to the muscle belly[27]. Due to no anterior thigh cooling in the previous study, we cannot allude as to whether differences occur regarding cooling location and severity of effects on muscle concentric strength in the lower limb.

In consideration of the available literature, a comparison between joint and direct muscle cooling over the anterior thigh may further develop the evidence base in the understanding of the effects of cooling on muscle strength and subsequent implications on lower limb injury risk in sporting populations. Physiological differences in gender response to cooling due to levels of adipose tissue, with females generally recording higher levels, causes variables in the efficiency of heat withdrawal from deeper tissues[21]. The aim therefore of the current study is to compare superficial anterior thigh vs. knee cooling on concentric quadriceps muscle strength in male and female athletes over a rewarming period of 30 minutes.

Methods

Approved by the Science, Technology, Engineering, Medicine and Health (STEMH) ethical committee, the process of this study commenced according to the Declaration of Helsinki[28]. All participants provided written informed consent to take part in the study. Physiological gender differences reported in literature[21] detail that females have larger adipose tissue however the effects cooling has on biomechanical function between males and females is limited. Twelve participants, 6 males (Height 179.0±4.4cm; Weight 65.4±6.4kg; age 19.2±1.3 years and BMI 20.4±1.3 kg/m^2) and 6 females (Height 163.1±8.7cm; Weight 59.7±6.0kg; age 19.2±0.9 years and BMI 22.4±1.4 kg/m^2) volunteered to take part in the study, based on a priori power calculation to determine optimum sample size (statistical power >0.7; $p<0.05$). Participants adhered to the inclusion criteria; healthy, aged between 18-40 years old, no history of lower limb musculoskeletal injury in the past six months, no neurological disease, and no known contraindications to cryotherapy or cold, such as Raynauds[13]. Advice against the consumption of caffeine/alcohol or partake in physical activity[11], minimised external factors that may affect local cooling intervention and standardised protocol prior to data collection. All data took place in a movement analysis laboratory.

Participants were randomly allocated (randomisation.com) to receiving either anterior thigh or knee cooling, returning one week later for exposure to the opposite intervention location. Both groups followed a clinically relevant cooling dosage of 10-minutes wetted ice, supporting earlier suggestions for investigation into dosages of cooling in line with pitch-side or half time applications of cryotherapy in sport[2]. On arrival, participants underwent a 15-minute acclimatisation period supporting previous study methods[27], to ensure a steady thermal state. Room temperature recorded at hourly intervals throughout testing monitored fluctuations as closely as possible. During the acclimatisation period, anthropometric measurements and dominant leg were established. The dominant limb for testing chosen due to regularly being the limb used to kick in land-based sports and determined by which leg the participant naturally chose to kick a football with[29].

Following gold-standard recommendations in current literature[18,19], T_{sk} data using an infrared thermal imaging camera (ThermoVision A40M, Flir Systems, Danderyd, Sweden) gathered at baseline, immediately post and at 10-minute intervals up to 30 minutes post of either the anterior thigh or knee, dependent on group allocation was facilitated by determining a region of interest (ROI). To create an anatomical region of interest over the anterior thigh, application of thermally inert skin surface markers formed a framework[12]. Location of markers consisted of superior marker placement, 1/3 way between ASIS and Base of Patella; Inferior marker placement 2/3 way between ASIS and Base of Patella. Central thigh was determined by the measure of thigh circumference at centre of thigh (COT), located at 50% between ASIS and Base of Patella. Markers then placed at 10% of this distance in medial and lateral directions from COT completed the ROI for anterior thigh[12]. Markers also placed at the base of patella, medial and lateral border of patella tendon margin at tibiofemoral joint line level and tibial tubercle determined a ROI for local knee cooling[30]. The thermal imaging camera situated at a height of 134cm from the ground, positioned perpendicular to the anterior lower limb, with participants laying supine on a plinth followed standard clinical set up with an emissivity setting of 0.97-0.98.

Following baseline T_{sk} data collection, a measure of concentric quadriceps muscle strength determined baseline strength data using an isokinetic dynamometer (IKD) (Cybex, division of Lumex Inc., Ronkonkoma, NY, USA) chosen due to high reliability (0.9-0.98)[24]. A 10-minute wetted ice application either to the anterior thigh or knee region followed[2], depending on allocation of group. Previous research surrounding cooling techniques recommends use of wetted ice[12,13,14] . Therefore, the protocol for wetted ice intervention consisted of 800ml of cubed ice and 800ml of room-temperature water; then placed into a clear polythene bag size of 22x40cm, with the excess air removed and secured with a knot[13]. The bag of wetted ice held in place securely to the limb with a cling-film wrap, and between skin and the wetted ice bag, a placement of a thin, damp microfiber towel for 10 minutes at either anterior thigh or knee[11,30].

In the same format at T_{sk}, IKD data was collected immediately post intervention and at 10 minute intervals up to 30 minutes post for each group as recommended in a previous study[27]. Between isokinetic measures participants were asked to long sit on a plinth. Previous research within isokinetic testing advocates the use of a range of testing speeds[25]. The gravity corrected torque-angle curve was analysed for each testing speed, with analysis restricted to the isokinetic phase. PT, the corresponding angle (ϴ), and AvT across the isokinetic phase were identified for each player, at each testing speed[25]. Concentric isokinetic torque measurements for the quadriceps performed at three repetitions per time point, into knee extension at $60°.s^{-1}$ and at $150°.s^{-1}$, with passive movement into flexion at $10°.s^{-1}$ between repetitions[27]. The two repetitions eliciting the highest PT value were identified for each time point and utilised for subsequent analysis. Observation of each repetition completed by the same researcher ensured consistent and smooth effort exerted by each participant throughout testing[27]. To minimise participants' extraneous body movement's standard positional setup using chest, pelvis and mid-thigh straps were applied[27] with the tibial strap placed three-quarters distally on the tibia, and rotational axis of the dynamometer aligned to the lateral femoral epicondyle. To isolate torque production at the quadriceps participants crossed their upper limbs across the chest[27].

Statistical Analysis

A univariate repeated measures general linear model quantified main effects for recovery duration post-ice application and isokinetic testing speed. Significant main effects in recovery duration were explored using post hoc pairwise comparisons with a Bonferonni correction factor. The assumptions associated with the statistical model were assessed and met to ensure model adequacy. To assess residual normality for each dependant variable, q-q plots were generated using stacked standardised residuals. Scatterplots of the stacked unstandardized and standardised residuals were also utilised to assess the error of variance associated with the residuals. Mauchly's test of sphericity was also completed for all dependent variables, with a Greenhouse Geisser correction applied if the test was significant. Partial eta squared (η^2) values were calculated to estimate effect sizes for all significant main effects[31]. Partial eta squared was classified as small (0.01–0.059), moderate (0.06-0.137), and large (>0.138). Interactions within the general linear model were also identified within the analysis of data. All statistical analysis was completed using PASW Statistics Editor 24.0 for windows (SPSS Inc, Chicago, USA). Statistical significance was set at $P \leq 0.05$, and all data are presented as mean ± standard deviation.

Results

Skin Surface Temperature (T_{sk}*) (°C)*

Whole group T_{sk} data demonstrated statistical significant decreases at the knee for all timepoints compared to pre application temperatures, IP ($p \leq 0.001$), 10 minutes ($p \leq 0.001$), 20 minutes ($p \leq 0.001$), and 30 minutes post intervention ($p = 0.03$) (Figure 1). Post cryotherapy application to the quadriceps noted statistically significant decreases in T_{sk} at IP ($p \leq 0.001$), 10 ($p \leq 0.001$), and 20 minutes post intervention ($p = 0.04$). No statistically significant changes in T_{sk} were reported at post 30 minutes intervention for the anterior thigh ($p = 0.11$), however T_{sk} did not return to baseline temperatures for whole group (Figure 1).

Statistically significant decreases in T_{sk} were also noted when comparing male and female groups separately across all time points for the knee ($p \leq 0.05$) (Table 1). Comparatively, statistically significant decreases for quadricep T_{sk} were noted in males at each time point up to 20 minutes post ($p \leq 0.05$), however no significant decreases in T_{sk} were noted for males ($p = 0.41$), or females ($p = 0.19$) at the anterior thigh at 30 minutes post (Table 1). Throughout the entire investigation, ambient room temperature was constant (21.1±0.5°C).

Peak Torque (PT)

Table 1 summarises the effects of wetted ice application and the temporal pattern recovery on PT. There was a significant main effect for time ($p \leq 0.001, \eta^2 = 0.126$), with pre ice application higher than all other time points ($p \leq 0.05$). With the data set collapsed to consider each speed in isolation, PT displayed a significant main effect for time at all speeds (PT_{60}: $p = 0.03, \eta^2 = 0.98$; PT_{150}: $p = 0.001, \eta^2 = 0.177$) (Table 1). There was also significant main effects for isokinetic testing speed ($p \leq 0.001, \eta^2 = 0.264$), sex of the participant ($p \leq 0.001, \eta^2 = 0.269$) and position of the ice application ($p \leq 0.001, \eta^2 = 0.151$) (Table 1). There were no significant interactions found between speed, time, position and gender for PT ($p \geq 0.05$).

Average Peak Torque (AvT)

Table 1 further summarise the effects of wetted ice application and the temporal pattern recovery on AvT. There was a significant main effect for time ($p \leq 0.001, \eta^2 = 0.159$), with pre ice application higher than all other time points ($p \leq 0.02$) except at 30 minutes post ($p = > 0.05$). With the data set collapsed to consider each speed in isolation, AvT displayed a significant main effect for time at all speeds (AvT_{60}: $p = 0.009, \eta^2 = 0.126$; $AvPT_{150}$: $p < 0.001, \eta^2 = 0.234$) (Table 1). There was also significant main effects for isokinetic testing speed ($p \leq 0.001, \eta^2 = 0.301$), sex of the participant ($p \leq 0.001, \eta^2 = 0.246$) and position of the ice application ($p \leq 0.001, \eta^2 = 0.085$) (Table 1). There was a speed *x* position interaction ($p = 0.023, \eta^2 = 0.028$), no other significant interactions were found ($p \geq 0.05$).

Discussion

The current study reports the effects of anterior thigh and knee cooling on PT and AvT isotonic strength of the quadriceps in males and females over a rewarming period. Previous studies have traditionally cooled over the exercising muscle[13] and others only the distal joint[32] or simultaneously[4], to our knowledge no study compares both. It is unclear however as to the extent of positive or deleterious effects of local cooling at different locations over the peripheral lower limb on the mechanical properties of muscle strength, with literature failing to reach a strong consensus. Results demonstrate reductions in PT and AvT concentric quadriceps strength in males and females, of which did not fully recover to baseline at 30 minutes post cryotherapy intervention. Findings from the current study agree with [1,2,27], but also refute[16,17] some evidence. It is problematic however to copiously compare results directly, because of the variability in testing protocols across available literature. Notably, current findings report the need for further enquiry into the immediate and latent effects of common cryotherapeutic applications used pitch side in sport with varied dosage applications on muscle strength.

To mimic closely common applications of cold applied pitch-side or at half time during competitive sport, duration of wetted ice followed a 10-minute dosage in the current study[34]. Although contrasting to longer dosage protocols[3,8,9,] the decision supports the recommendation for investigations in cryotherapy to replicate simulated play and helps understand the extent of effects induced by cryotherapy applications in sporting scenarios[2]. A 10-minute wetted ice exposure initiated whole group average T_{sk} recorded at 9.6±1.6°C (knee) and 12.1±1.4°C (anterior thigh), immediately post intervention in the current study. These results establish a T_{sk} response to within the desired therapeutic range of cooling (10-15°C)[11], expected for physiological response occurred after a 10-minute application (Figure 1). Whole group T_{sk} did not return to baseline levels at 30 minutes over the knee (24.0±1.0°C), or at 20 minutes post over the anterior thigh (29.1±0.6°C) for whole group data (Figure 1), supporting previous literature[27]. In addition, regardless of cooling location, reductions in strength were reported in both male and female groups, for both speeds ($PT_{60/150}$, $AvPT_{60/150}$) (Table 1). The noted reductions in strength coincide with reductions in T_{sk} over the rewarming period and demonstrate a relative incline over 30 minutes post removal (Table 1). Observation of percentage difference in concentric quadriceps strength data between cooling locations noted no definitive pattern when comparing all post data to baseline. Although, a trend is suggestive that concentric strength data immediately post demonstrated greater reductions noted subsequently following knee joint cooling than the anterior thigh in males for both speeds (PT/AvPT: $60°.s^{-1}/150°.s^{-1}$), but not in females. Accordingly at all other timepoints (10, 20 and 30 minutes), data recorded greater reductions for anterior thigh cooling compared to knee for both gender groups, and speeds. Unsurprisingly this supports previously reported quadratic relationship mechanisms between T_{sk} and deeper musculature response to cooling following removal of local cooling[12]; that being that as skin rewarms, muscle continues to cool pertinent to cooling ability of the cryotherapy modality applied. This also supports the findings that strength following cooling at either locations, across both gender groups and speeds does not return to pre-intervention levels at 30 minutes. Although largely data reports different percentages of strength deficit noted following anterior thigh compared to knee cooling; both cooling locations demonstrated statistically significant reductions in concentric strength over the rewarming period (Table 1) regardless of gender or speed compared to baseline measures. Due to the relatively small sample size utilised in the present study, caution when comparing findings between genders may be noted. This may also contribute to the interactions between variables highlighted in the complex study design. Significant interactions were highlighted for speed *x* position in AvT, with a small effect size reported. Consideration must be given to this in future work.

Local cryotherapy in athletic practice, particularly prior to returning to activities that expose muscle tissue to exercise induced damage should consider the findings from the current investigation. Results agree with those conclusions of previous authors, that ≥10-minutes of cooling reduces muscle contractility and subsequently, performance[8]. Furthermore the authors agree that desensitization of deep joint mechanoreceptors following knee joint cooling may affect neuromuscular response, proposing a change in proprioceptive feedback[33], but importantly reaffirm the detrimental effects distal cooling has on the strength of musculature as much as that of direct cooling over the anterior thigh. Reductions in torque production ability of the quadriceps, are formerly reported immediately following a 20-minute cooling application over the anterior knee joint, and highlighted the importance of investigating rewarming periods prior to returning to sport[27]. The implications of reduced muscle strength of the quadriceps or surrounding musculature may predispose an increased risk of non-contact injury at the knee complex[34]. Investigations report acute changes in the mechanical properties of muscle following cryotherapy consequently lowers the amount of stretch that muscle tissue is able to sustain without subsequent injury[1]. Cooling over regions susceptible to strain injury, such as myotendinous junction, may present an increased risk of injury by returning to activity soon after cryotherapy applications. Point et al (2018) considers this heightened risk is due to the reduced capacity of the muscle tendon unit to sustain external strain following cooling caused by increased stiffness in the cooled tissues[35]. Muscle fibres therefore more prone to damage[1] due to known mechanisms predisposing to soft-tissue injury, such as reductions in available range of motion[37] and increases in contractile tissue stiffness[37]. Assumed putative changes in global viscoelastic and myoelectrical activity initiated by lower temperatures may be factors that contribute to the reduction noted in isotonic PK and AvPK in the current study, supporting previous suggestions[1,7]. Reduced muscle deformation, passively, have been reported in cold muscles, prior to rupture, following exposure to cold-water immersion[38]. Although consideration that cold-water immersion is more likely to alter properties of multiple structures that cross over the joint including agonists and antagonist muscles, tendons and articular structures[1]. It is difficult therefore to compare directly current results to CWI or air-pulsed cryotherapy modalities directly, on that basis.

Conclusion

Local cooling over superficial joint or muscles in males and females may result in performance deficits due to reductions in concentric muscle strength. Future studies are essential, in order to establish margins whereby safe return to sport following cooling exposures to the lower limb. Sports medicine practitioners should consider reductions in strength ability of the quadriceps even after shorter application durations (<20') of wetted ice, and regardless cooling location (joint/muscle) or gender. To advance safe rationale for pitch-side cryotherapy applications, comparison of other commonly applied cryotherapy modalities are necessary and observation of multiple variables that may affect the development of optimum dose duration and return to activity panaceas.

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