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Influence of cold-water immersion on indices of muscle damage following prolonged intermittent shuttle running

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Abstract
The aim of this study was to assess the effects of cold-water immersion (cryotherapy) on indices of muscle damage following a bout of prolonged intermittent exercise. Twenty males (mean age 22.3 years, $s = 3.3$; height 1.80 m, $s = 0.05$; body mass 83.7 kg, $s = 11.9$) completed a 90-min intermittent shuttle run previously shown to result in marked muscle damage and soreness. After exercise, participants were randomly assigned to either 10 min cold-water immersion (mean 10°C, $s = 0.5$) or a non-immersion control group. Ratings of perceived soreness, changes in muscular function and efflux of intracellular proteins were monitored before exercise, during treatment, and at regular intervals up to 7 days post-exercise. Exercise resulted in severe muscle soreness, temporary muscular dysfunction, and elevated serum markers of muscle damage, all peaking within 48 h after exercise. Cryotherapy administered immediately after exercise reduced muscle soreness at 1, 24, and 48 h ($P < 0.05$). Decrements in isometric maximal voluntary contraction of the knee flexors were reduced after cryotherapy treatment at 24 (mean 12%, $s_x = 4$) and 48 h (mean 3%, $s_x = 3$) compared with the control group (mean 21%, $s_x = 5$ and mean 14%, $s_x = 5$ respectively; $P < 0.05$). Exercise-induced increases in serum myoglobin concentration and creatine kinase activity peaked at 1 and 24 h, respectively ($P < 0.05$). Cryotherapy had no effect on the creatine kinase response, but reduced myoglobin 1 h after exercise ($P < 0.05$). The results suggest that cold-water immersion immediately after prolonged intermittent shuttle running reduces some indices of exercise-induced muscle damage.

Keywords: *Cryotherapy, intermittent exercise, muscle soreness, muscular dysfunction*

Introduction

The deleterious effects associated with muscle damage following a bout of unaccustomed or eccentric-based exercise are well documented (Armstrong, 1984; Clarkson & Sayers, 1999; Proske & Allen, 2005). The time course and severity of muscle soreness, muscular dysfunction, and appearance of markers of muscle damage in the systemic circulation can vary considerably depending on the duration, intensity, and type of exercise performed (Clarkson, Byrnes, McCormick, Turcotte, & White, 1986; Eston, Critchley, & Baltzopoulos, 1994; Thompson, Nicholas, & Williams, 1999). These factors may partially explain why the precise aetiology of exercise-induced muscle damage remains elusive. Nevertheless, delayed-onset muscle soreness (DOMS) and associated decrements in muscular function are one of the most commonly reported sport-related injuries (Byrne, Twist, & Eston, 2004).

Many investigations have attempted to alleviate or prevent exercise-induced muscle damage and its associated symptoms. Treatment strategies include stretching, ultrasound, massage, antioxidant supplementation, and administration of non-steroidal anti-inflammatory drugs (for a review, see Cheung, Hume, & Maxwell, 2003). More recently, attention has focused on the effect of cryotherapy in aiding recovery from muscle-damaging exercise (Eston & Peters, 1999; Howatson and van Someren, 2003; Yanagisawa *et al.*, 2003a,b). The role of cryotherapy as a treatment of sport-related injuries is well documented (Bleakley, McDonough, & MacAuley, 2004), although support for its specific application to exercise-induced muscle damage remains predominantly anecdotal.

Cryotherapy is proposed to reduce the inflammatory response to injured tissue as well as decrease

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oedema, haematoma formation, and pain (Swenson, Sward, & Karlsson, 1996). Thus, cryotherapy may be considered a pertinent treatment modality because inflammation is integral in the aetiology of exercise-induced muscle damage (Smith, 1991) and muscle soreness is the most commonly reported symptom of this exercise-related injury (Armstrong, 1984). Additionally, inflammation has been shown to exacerbate existing disruptions to skeletal muscle tissue, as this immune response is coupled with secondary damage via transient hypoxia as well as the non-specific cytotoxic actions of leukocytes (Lapointe, Frenette, & Cote, 2002; MacIntyre, Reid, Lyster, Szasz, & McKenzie, 1996; Merrick, Rankin, Andres, & Hinman, 1999).

Recent research has focused on the role of cryotherapy on indices of muscle damage following eccentric exercise of isolated muscle groups. Eston and Peters (1999) observed that repeated cold-water immersion (15 min at 15°C every 12 h) was effective in reducing plasma creatine kinase activity and muscle stiffness, indirectly assessed as relaxed arm angle, in the days after repeated eccentric elbow flexion. Using a comparable muscle-damaging exercise protocol, Yanagisawa and co-workers (2003a,b) also reported some beneficial effects of cold-water immersion (15 min at 5°C) on exercise-induced muscle oedema as well as a tendency for reduced muscle soreness and creatine kinase activity. Conversely, Isabell, Durrant, Myrer, and Anderson (1992) observed no effect of cryotherapy (ice-massage) on indices of muscle damage and suggested repeated cryotherapy may be contra-indicatory over a prolonged period.

There is limited evidence to support cryotherapy following more dynamic whole-body exercise, which may be considered more ecologically valid when providing recommendations in a sports performance environment. The aim of this study was to assess the effects of a single administration of cryotherapy on the recovery from a bout of strenuous intermittent shuttle-running exercise.

Methods

Participants

Twenty healthy men (mean age 22.3 years, $s = 3.3$; height 1.80 m, $s = 0.05$; body mass 83.7 kg, $s = 11.9$) volunteered to take part in the study, which had received approval from the university ethics committee. Participants completed a mandatory health questionnaire and provided written informed consent. All participants were habitually active in a variety of sports, but were unfamiliar with the exercise to be performed. Participants were required to abstain from therapeutic treatments including massage and anti-inflammatory drugs for the duration of the investigation.

Experimental design

Having refrained from exercise for at least 2 days, participants arrived at the laboratory in a fasted state ($\sim$10 h). A venous blood sample ($\sim$10 ml) was taken from a vein in the antecubital fossa after participants had been supine for at least 10 min. Next, perceived muscle soreness was recorded and muscular function was assessed using isokinetic dynamometry and a vertical jump test (described in detail below). Subsequently, participants completed the Loughborough Intermittent Shuttle Test (LIST) as described previously (Thompson *et al.*, 1999). Briefly, the LIST is a field test specifically designed to replicate the demands associated with intermittent activity such as soccer (Nicholas, Nuttall, & Williams, 2000). Participants were required to exercise at varying intensities for 90 min, with average exercise intensity equal to 75% maximal oxygen uptake ($\dot{V}O_{2max}$) determined from a progressive shuttle-run test (Ramsbottom, Brewer, & Williams, 1988). Subjective ratings of perceived exertion were recorded every 15 min during the LIST (Borg, 1998), heart rate was monitored every 15 s by short-range telemetry (Polar 8810, Vantaa, Finland), and core body temperature was monitored at regular intervals using an ingestible thermometer pill (CorTemp^{TM}, HQI, Palmetto, USA). Nude body mass was determined immediately before and after exercise. Participants were required to ingest water in a bolus equal to 5 ml $\cdot$ kg^{-1} immediately before exercise and 2 ml $\cdot$ kg^{-1} every 15 min during exercise. A venous blood sample was taken immediately after exercise and additional samples were taken 1, 24, and 48 h after exercise. Participants were instructed not to resume exercising until the conclusion of testing.

Cryotherapy treatment

Before exercise, participants were matched for several anthropometric and physiological characteristics and randomly allocated to either a cryotherapy or control group (Table I). Immediately after exercise, the cryotherapy group immersed their lower limbs (ensuring that the iliac crest was fully submerged) in a cold-water bath for 10 min. The water was maintained at a mean temperature of 10°C ($s = 0.5$) by the addition of crushed ice and was repeatedly agitated to avoid the formation of a warmer boundary layer. This single bout of cryotherapy was similar to that used in previous investigations (Yanagisawa *et al.*, 2003a,b) and has been shown to lower subcutaneous and intramuscular temperature

Table I. Physiological characteristics and physical activity status of groups (mean ± *s*).

	Cryotherapy ($n=10$)	Control ($n=10$)	*P*-value
Age (years)	23.6 ± 4.1	21.7 ± 2.0	0.123
Height (m)	1.80 ± 0.06	1.81 ± 0.05	0.665
Body mass (kg)	85.9 ± 12.8	81.5 ± 11.2	0.517
Body mass index ($kg \cdot m^{-2}$)	26.3 ± 2.8	24.9 ± 2.7	0.487
Sum of 4 skinfolds (mm)[a]	35.3 ± 12.8	31.3 ± 6.3	0.583
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	55.2 ± 4.8	56.2 ± 5.3	0.676
Weekly exercise sessions (*n*)	5 ± 2	4 ± 1	0.265

[a]Sum of four skinfolds (triceps, biceps, suprailiac, subscapular).

by 7–10°C (Meeusen & Lievens, 1986). During this time, control participants remained at rest in the same long seated position as the experimental participants. Heart rate and core body temperature were monitored at regular intervals throughout and for 15 min following the treatment period. Additionally, ratings of perceived coldness were assessed during treatment and recovery using a visual analog scale that ranged from 1 (''not cold'') to 10 (''very, very cold'').

Assessment of muscle damage

Ratings of perceived soreness were assessed using a visual analog scale (Thompson *et al.*, 1999) ranging from 1 (''not sore'') to 10 (''very, very, sore'') before, immediately after (±5 min), and 1, 24, 48, and 168 h after exercise. Participants rated general whole-body soreness while standing in the relaxed state and were encouraged to palpate major muscle groups during assessment.

Maximal voluntary isometric contraction (MVC) of the knee extensors and flexors was assessed while seated using an isokinetic dynamometer (Cybex model 770, LUMEX Inc., Ronkonkoma, USA). Participants were familiarized with the apparatus and protocol on at least two occasions before performing the LIST. Before assessment on the dynamometer, positional adjustments for knee extension and flexion were made to ensure movement was restricted to the saggital plane and that the axis of rotation passed through the femoral condyles. Following a warm-up set of five sub-maximal repetitions of knee extension and flexion ($1.05\ rad \cdot s^{-1}$), participants completed two maximal isometric repetitions of the dominant limb for 5 s for extension at 1.05 rad and flexion at 0.35 rad, where full knee extension was 0 rad. These angles have previously been identified as optimal for peak force generation during isometric knee flexion and extension (Westing & Seger, 1989). Contractions were separated by 60-s rest periods. Participants were verbally encouraged and received visual feedback during each repetition. The greatest peak torque achieved from both repetitions was recorded.

Vertical jump height was recorded as previously described (Byrne & Eston, 2002). Participants performed the squat jump technique with no countermovement to minimize the effects of the stretch–shortening cycle. Participants performed three consecutive jumps on an electronic timing mat (Powertimer 1.0 Testing System, Newtest Oy, Kiviharjuntie, Finland) on each occasion. Jumps were separated by 60 s rest and the highest jump was recorded as the peak height.

Sprint performance was assessed during the LIST and again 48 h after exercise. Sprint times were measured using two infrared photoelectric cells (RS Components Ltd., Zurich, Switzerland) interfaced with a computer. Participants were required to perform 11×15-m maximal sprints during each 15-min exercise block of the LIST. The values recorded during the first 15-min block of the LIST were compared with a subsequent 15-min block performed 48 h after the initial exercise bout.

Blood analysis

Aliquots of blood were used to determine haemoglobin concentration by the cyanomethaemoglobin method (Boehringer Mannheim, GmbH Diagnostica, Mannheim, Germany) and haematocrit by microcentrifugation (Hawksley Ltd., Lancing, UK). Changes in plasma volume were assessed using these haematrocrit and haemoglobin values (Dill & Costill, 1974). The remaining blood was dispensed into a tube, left to clot, and then centrifuged (4°C) at $4000\ rev \cdot min^{-1}$ for 10 min to obtain serum. Serum creatine kinase activity and myoglobin concentration were determined at 37°C using commercially available techniques (Randox, Crumlin, UK) designed specifically for use on an automated system (COBAS Mira Plus, Roche Diagnostics Systems, Rotkreuz, Switzerland).

Statistical analysis

A two-way analysis of variance (ANOVA) with repeated measures on time was used to determine if differences existed between treatment conditions. When significant *F* values were observed, the Holm-Bonferroni step-wise method was used to determine the location of the differences (Atkinson, 2002). Values for creatine kinase activity and myoglobin were not normally distributed and therefore these values were log transformed before ANOVA. Pearson product–moment correlations were used to examine

the relationship between variables. Data analysis was conducted using SPSS version 12.0 and statistical significance was set at $P < 0.05$. Values are expressed as means and standard errors of the mean (s_x) unless otherwise stated.

Results

Response to intermittent exercise

Mean heart rate during the LIST was 165 beats · min^{-1} ($s_x = 3$) for both groups. Mean rating of perceived exertion increased from 14 ($s_x = 1$) at 15 min into exercise to 17 ($s_x = 1$) at the end of exercise for both groups ($P < 0.05$). Core body temperature during exercise was available for 15 participants (cryotherapy, $n = 8$; control, $n = 7$). Temperature increased from 37.5°C ($s_x = 0.10$) to 38.1°C ($s_x = 0.13$) after exercise ($P < 0.05$). During exercise, participants drank 1.3 litres ($s_x = 0.1$) of water and lost 1.2 kg ($s_x = 0.3$) of body mass. Mean sprint time during the LIST was 2.70 s ($s_x = 0.03$). Estimated changes in plasma volume did not differ during the testing period for either group.

Response to cryotherapy treatment

Heart rate decreased during the treatment period from 107 beats · min^{-1} ($s_x = 4$) to 94 beats · min^{-1} ($s_x = 3$) ($P < 0.05$) and continued to decline (87 beats · min^{-1}, $s_x = 3$) 15 min after treatment ($P < 0.05$) in both groups. Cryotherapy had no effect on heart rate response when compared with the control group. Core body temperature ($n = 15$) decreased from 37.9°C ($s_x = 0.14$) to 37.7°C ($s_x = 0.13$) during the treatment period and continued to fall 15 min post-treatment (37.4°C, $s_x = 0.11$) ($P < 0.05$) but was not different between groups. Perception of coldness was elevated during cryotherapy (mean 6, $s_x = 1$) compared with the control group (mean 1, $s_x = 1$) and remained elevated during recovery ($P < 0.05$).

Indices of muscle damage

Exercise resulted in severe muscle soreness that peaked immediately after exercise and again 24 h later ($P < 0.05$). Cryotherapy reduced ratings of perceived soreness at 1, 24, and 48 h post-exercise ($P < 0.05$) (Figure 1).

Maximal isometric voluntary contraction for knee extension was unaffected after exercise and treatment. However, MVC for knee flexion was reduced at 24 and 48 h post-exercise ($P < 0.05$) and returned to pre-exercise values at 168 h post-exercise ($P < 0.05$). Cryotherapy reduced decrements in MVC at 24 and 48 h compared with the control group ($P < 0.05$) (Figure 2).

Peak vertical jump height was reduced from pre-exercise values (0.36 m, $s_x = 0.01$) at 24 (0.35 m, $s_x = 0.01$) and 48 h (0.34 m, $s_x = 0.01$) for both groups ($P < 0.05$). Vertical jump height was unaffected by cryotherapy. Mean sprint time during the first 15-min block of the LIST (2.67 s, $s_x = 0.03$) was unaffected 48 h (2.70 s, $s_x = 0.04$) after exercise and treatment.

Creatine kinase activity was elevated immediately after exercise ($P < 0.05$), peaking 24 h later but this response was not influenced by cryotherapy (Figure 3). Myoglobin concentration increased

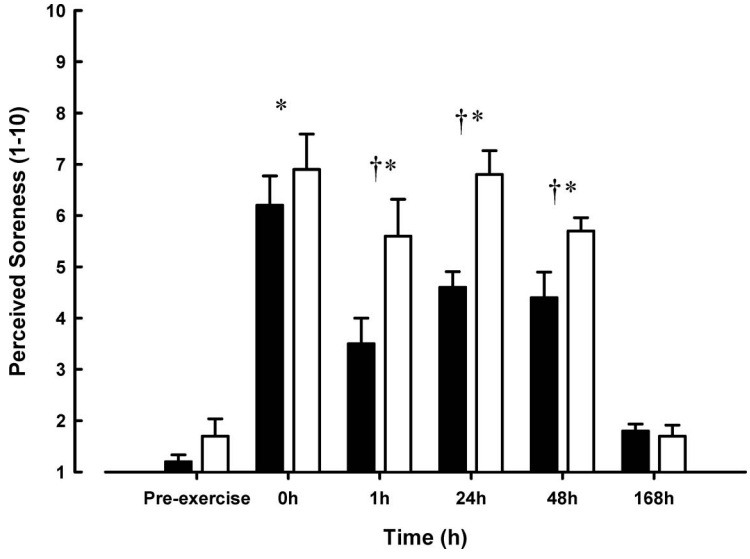

Figure 1. Perceived muscle soreness following exercise for cryotherapy (solid bars) and control (open bars) groups. Values are mean and standard errors. *Different from pre-exercise for both groups ($P < 0.05$); †Different between groups ($P < 0.05$).

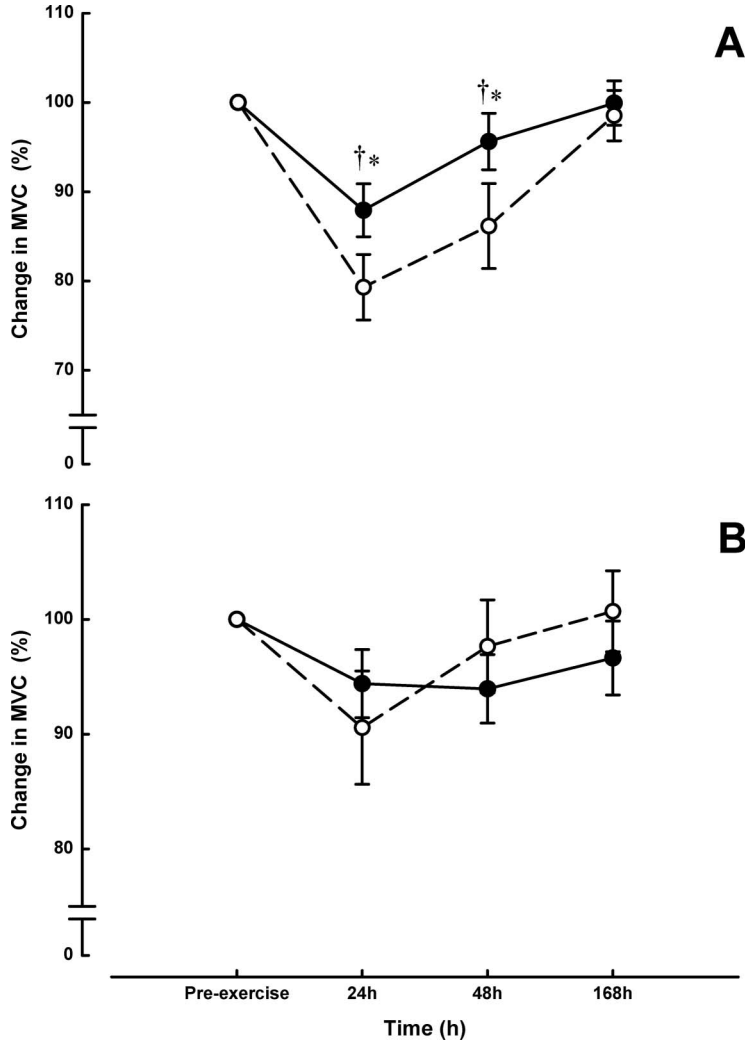

Figure 2. Isometric maximal voluntary contraction of the knee flexors (A) and extensors (B) following exercise for cryotherapy (solid line) and control (broken line) groups. Values are mean and standard errors. *Different from pre-exercise for both groups ($P < 0.05$); †Different between groups ($P < 0.05$).

immediately after exercise in both groups ($P < 0.05$). Concentrations peaked 1 h after exercise in the control group but were reduced at this time in the cryotherapy group ($P < 0.05$) (Figure 3).

Discussion

The main findings of this study were that individuals who received cryotherapy treatment after exercise reported a diminished perception of muscle soreness up to 48 h later, a lower decrement in MVC at both 24 h and 48 h post-exercise, and a reduced serum myoglobin response 1 h after exercise. These findings are consistent with those of similar investigations using cryotherapy as a modality to treat exercise-induced muscle damage (Eston & Peters, 1999; Howatson & van Someren, 2003; Yanagisawa *et al.*, 2003b).

The intermittent shuttle-running protocol used to elicit muscle damage resulted in severe muscle soreness and an associated period of muscular dysfunction comparable to that previously documented (Bailey *et al.*, 2002; Bailey, Williams, Hurst, & Powell, 2003; Thompson *et al.*, 1999). Additionally, the increase in intracellular proteins was similar and over the same time course as observed in previous investigations using both this exercise protocol (Bailey *et al.*, 2002; Thompson *et al.*, 1999) and other analogous eccentric-based exercise models (Byrnes *et al.*, 1985; Thompson *et al.*, 2004). The greatest soreness was generally reported in the weight-bearing musculature of the lower limbs, specifically the hamstrings (Bailey *et al.*, 2003; Thompson *et al.*, 1999), conceivably related to the eccentric actions of this muscle group during intermittent running. The moderate relationship ($r = -0.58$; $P < 0.05$) between decrements in MVC of the knee flexors and muscle soreness at 48 h post-exercise provides some support for the proposed association between muscle injury, dysfunction, and soreness that is not well

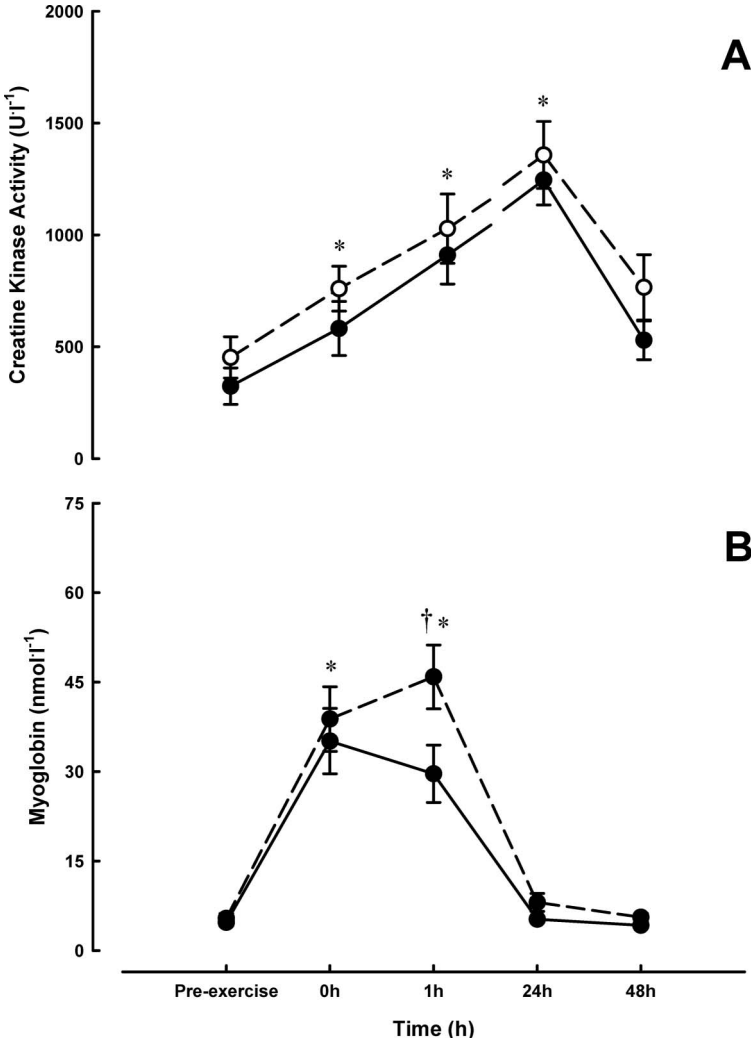

Figure 3. Serum creatine kinase activity (A) and myoglobin concentration (B) following exercise for cryotherapy (solid line) and control (broken line) groups. Values are mean and standard errors. *Different from pre-exercise for both groups ($P < 0.05$); †Different between groups ($P < 0.05$).

documented (Nosaka, Newton, & Sacco, 2002; Warren, Lowe, & Armstrong, 1999).

The acute onset of muscle soreness observed immediately after exercise is related to the accumulation of by-products that are either metabolic or contraction induced (Miles & Clarkson, 1994) rather than DOMS, which is more commonly associated with muscle damage (Cheung *et al.*, 2003). This could account for the biphasic increase in muscle soreness observed following exercise and support the proposal that cryotherapy was effective in reducing muscle injury rather than facilitating removal of exercise-induced accumulation of by-products. The observed reductions in DOMS at 24 and 48 h post-exercise with cryotherapy is consistent with similar previous investigations (Denegar & Perrin, 1992; Prentice, 1982; Yanagisawa *et al.*, 2003b). Some authors attribute this reduced pain perception to the analgesic effects of cooling rather than inhibition of muscle damage (Denegar & Perrin, 1992; Gulick, Kimura, Sitler, Paolone, & Kelly, 1996; Meeusen & Lievens, 1986). The application of cold, sufficient to lower muscle tissue to temperatures around 10–15°C, reduces nerve conduction velocity, muscle spindle activity, the stretch-reflex response, and spasticity, thus inhibiting the pain–spasm cycle (Meeusen & Lievens, 1986). However, the duration of this analgesia is limited to 1–3 h (Meeusen & Lievens, 1986), so this mechanism might only account for the initial reductions in muscle soreness observed 1 h after exercise. Denegar and Perrin (1992) observed similar beneficial effects of cryotherapy (ice packs) on DOMS. These authors documented a further reduction in perceived soreness when the treatment was supplemented with a period of stretching. They proposed that stretching results in stimulation of the Golgi tendon organ, motor inhibition, and reduced muscular tension resulting in

a concurrent reduction in the pain–spasm cycle (Denegar & Perrin, 1992). Although cooling, either alone or accompanied by passive stretching, has inhibitory influences on pain perception, some researchers reporting beneficial effects of cryotherapy on exercise-induced muscle damage have not observed a concomitant effect on muscle soreness (Eston & Peters, 1999; Howatson & van Someren, 2003).

Cryotherapy improved recovery of MVC of the knee flexors 24–48 h after exercise. Exercise resulted in a reduction of knee flexion peak torque at 24 (12%, $s_x = 4$) and 48 h (3%, $s_x = 3$) in the cryotherapy group, which was markedly less than that experienced by the control group at 24 (21%, $s_x = 5$) and 48 h (14%, $s_x = 5$). Values had returned to pre-exercise values 7 days after exercise in both groups. This pattern of strength loss and recovery is similar to that previously reported following this exercise protocol (Bailey *et al.*, 2002; Thompson *et al.*, 1999), although decrements were lower compared with previous studies (Bailey *et al.*, 2002; Thompson *et al.*, 2003). Additionally, these findings provide further support for the use of muscle function as an applicable and reliable measurement tool for quantifying exercise-induced muscle damage (Warren *et al.*, 1999). However, Warren and coworkers' (1999) endorsement of specificity when measuring muscle function was not supported, as assessment of isometric maximal voluntary contraction was more sensitive to decrements in muscular function than sprint and vertical jump assessments.

The effects of cryotherapy on the appearance of intracellular proteins are similar to those reported previously (Eston & Peters, 1999; Howatson & van Someren, 2003). It is still unclear what mechanism is responsible for the difference in myoglobin concentration following cryotherapy treatment. Others have postulated that cryotherapy might reduce post-exercise muscle damage via a decreased permeability of blood and lymph vessels due to an attenuated inflammatory response. These investigations employed creatine kinase activity as the sole marker for intracellular protein release (Eston & Peters, 1999; Howatson & van Someren, 2003). This particular marker is subject to large variability between individuals and caution is advised when interpreting the response of this intracellular protein (Clarkson & Ebbeling, 1988; Warren *et al.*, 1999). This explanation could, in part, account for the lack of a treatment effect observed with creatine kinase activity. Also, as secondary damage to skeletal muscle resulting from inflammation may be more pronounced in the hours rather than days after exercise (Lapointe *et al.*, 2002; Merrick *et al.*, 1999), it is possible that myoglobin is a more accurate indicator of subsequent injury. Although cryotherapy treatment had no effect on core body temperature compared with the control group, as cooling rates were $0.03^{\circ}\mathrm{C} \cdot \mathrm{min}^{-1}$ ($s_x = 0.01$) for both groups, previous investigations have reported reductions in subcutaneous and intramuscular temperatures during similar cryotherapy treatments (for a review, see Meeusen & Lievens, 1986). Therefore, it is reasonable to assume that cold-water immersion was effective in lowering intramuscular temperature. With this in mind, it is possible that cryotherapy mediated a reduced inflammatory response and subsequent secondary muscle damage attenuating the efflux of myoglobin. However, it is also conceivable that cold-water immersion elicited profound haemodynamic changes (Stocks, Taylor, Tipton, & Greenleaf, 2004) that could provide an alternative explanation for the differing appearance in this systemic marker of muscle damage.

The results of this study suggest that cryotherapy applied as a single bout of cold-water immersion immediately after exercise is effective in reducing some of the deleterious symptoms associated with exercise-induced muscle damage. The precise mechanisms responsible for this benefit requires further clarification but findings highlight the multitude of factors involved in the aetiology of exercise-induced muscle damage.

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