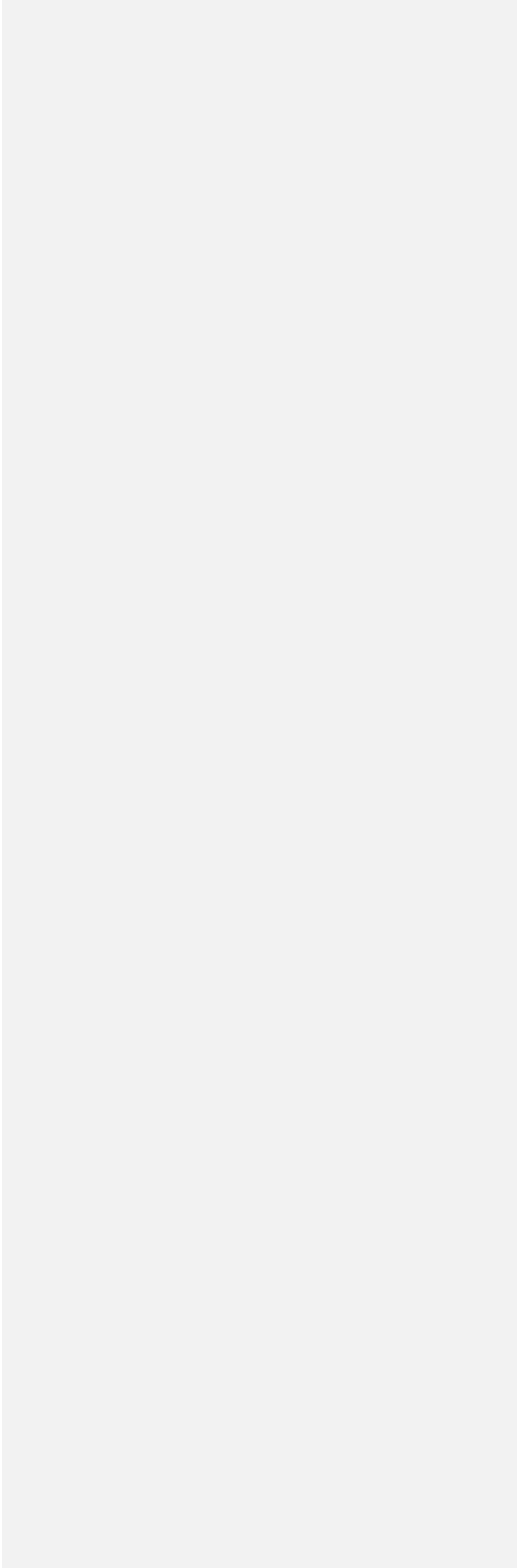**Selected physiological, perceptual and physical performance changes during two bouts of prolonged high intensity intermittent running separated by 72 hours.**

ABSTRACT

This study investigated the effects of performing a second 90-minute intermittent running protocol 72 hours after an initial trial on selected physiological, perceptual and sprint running measures. Eight sub-elite soccer players provided measures of isokinetic muscle function, counter-movement jump (CMJ), 10 m sprinting, and muscle soreness before, and at 0, 24, 48 and 72 h after a 90-minute intermittent high intensity running bout (IHIR 1). A second 90-minute intermittent high intensity running bout (IHIR 2) was performed 72 h after the first. Heart rates, ratings of perceived exertion (RPE), blood lactate concentration ([Bla]) and 10 m sprint times were recorded periodically during both IHIRs. Analysis of effects revealed that in the 72-hour period after IHIR 1 there were *most likely* increases in muscle soreness and *likely to very likely* deteriorations in CMJ, 10 m sprint and isokinetic muscle function. During IHIR 2, heart rates (*possibly* to *likely*) and [Bla] (*possibly* to *very likely*) were lower than IHIR 1, whilst RPE remained unchanged. Sprint times during IHIR 2 were also *likely* to *very likely* higher than in IHIR 1. It was evident that these team sport players exposed to repeat bouts of prolonged high intensity running within 72 hours down-regulated their sprint performances in the second bout despite no change in perceived effort. These findings have implications for managing training and match loads during periods of intense scheduling.

Key words: Team sports**,** repeat efforts, exercise-induced muscle damage, pacing.

INTRODUCTION

The importance of recovery from strenuous exercise has become a topical area of interest in applied sport science research, particularly with respect to team sports and the likelihood of tissue damage that occurs from an increase in intensity or duration of competition or training demands (6,23). In addition, the metabolic stresses associated with the high-intensity intermittent nature of team sports (for example, reduced ATP production (35), release of oxygen free radicals, reduced pH and mitochondrial respiration (9)) are all implicated in the initial aetiology of exercise-induced muscle damage (EIMD). Symptoms of EIMD include an inflammatory response (14, 37) increased stiffness, soreness, muscle swelling (13) and elevations in blood proteins, such as creatine kinase (15). More importantly, detrimental effects of tissue damage on jumping (6, 26, 29, 31, 18), sprinting (29, 18) and agility performances (18) are practically meaningful for team sport athletes who regularly perform high-intensity intermittent exercise as part of training and matches. Markers of EIMD have been documented both after soccer matches and 'simulations' designed to replicate them (2, 23, 29, 24, 36) and reported to peak at around 24 to 48 hours (24, 31) and endure for up to 72 hours post-exercise (23, 24, 36). Ascensão et al. (2) and Fatouros et al. (14) reported that performances of vertical jumping, sprinting, and knee extensor strength were typically 5 - 15% lower in professional soccer players after a soccer match. Indeed, as soccer players of all standards are often required to perform in successive matches or training sessions with only two or three days of recovery (7) means that EIMD might impact negatively on any subsequent running ability.

Interestingly, several studies using time-motion analysis have reported no differences in running characteristics and technical performance during congested fixture periods in soccer

players (1, 10, 11), although more matches were lost and injury rate was higher when these shorter between-match periods occurred (4, 10). However, the sensitivity of motion analysis methods to detect fatigue-related declines in performance and/or the role of pacing during a match has been questioned (7). Moreover, natural variations in events between matches owing to several contextual factors makes it difficult to interpret the causes of differences in running demands between matches (7), particularly with respect to high-intensity running (19.8 – 25.2 $km·h^{-1}$) and sprinting (>25.2 $km·h^{-1}$) which can vary by up to 16% and 30%, respectively (17). Accordingly, the use of intermittent high intensity running simulation protocols using controlled speeds (e.g. 30) provide a suitable model to better understand the effects of prolonged fatigue on an individual's movement characteristics.

Of the limited data that exists, Rollo et al. (34) reported that playing two soccer matches per week over a six-week period resulted in significant reductions in counter-movement jump, 10 and 20 m sprint and Yo-Yo intermittent recovery test performances. While they speculated that playing multiple matches per week had a negative impact on muscle function, possibly due to repeated skeletal muscle fibre damage, the authors were unable to quantify how this impacted on aspects of match performance (from one match to the next). Indeed, no studies have examined how a second match occurring soon after an initial match has been affected in terms of its physiological or perceptual demands (internal load). Understanding how indirect markers of exercise-induced muscle damage (e.g. force loss, muscle soreness) accumulated from prolonged exercise impacts on physical performances during subsequent iterations of the same type would inform athlete management protocols around periods where both training and competition volumes are high. Therefore, the purpose of the present study was to examine the impact of an initial prolonged high intensity running bout (team sport simulation protocol) on measures of

running performance and internal load demonstrated in a subsequent repeat bout presented only 72 hours later.

METHODS

Experimental Approach to the Problem

The study used a repeated measures design, with participants attending the laboratory on five occasions. All participants were required to refrain from strenuous exercise in the two days before the initial visit and during the four subsequent visits, to maintain a normal diet throughout, and abstain from using analgesic agents or recovery strategies during the testing period. Participants completed a multi-stage shuttle run (33) followed by familiarization to the intermittent high intensity running protocol (IHIR) and the measures of muscle function. Participants completed two IHIR bouts (IHIR 1 and IHIR 2) 72 hours apart. During both IHIR trials, sprinting speed was measured for every sprint completed, heart rates were recorded throughout and [Bla] and ratings of perceived exertion recorded immediately after each 15 min block (i.e. during the 3-minute rest periods). Measurements of isokinetic muscle function, muscle soreness, countermovement jump (CMJ) height, and 10 m sprint performance were also recorded before and then immediately, 24, 48 and 72 hours after the IHIR 1. All measurements were performed at the same time of day ($\pm$ 2 h) to minimize the effect of any diurnal variation and recorded in the same order as described for each visit. A period of ~5 minutes was permitted between each measurement to reduce the effect of cumulative fatigue on subsequent performance.

Subjects

After institutional ethics approval, eight male university soccer players (age 23 ± 3 y, stature 176.8 ± 5.7 cm, body mass 77.9 ± 9.4 kg, predicted VO_{2max} 49.1 ± 5.9 ml·kg^{-1}·min^{-1}) volunteered to participate in the study. All participants played in outfield positions, attended training at least twice and played one match per week. Subjects had no history of injury in the previous six months and were free of injury at the time of testing. After receiving verbal and a written explanation of the study, all participants provided written informed consent before taking part.

Procedures

The multi-stage shuttle run test (MSSRT)

After a standardized warm-up, participants completed the MSSRT in an indoor gymnasium (wooden surface). The test consisted of shuttle running between two markers set at 20 m with the speed increasing every minute until exhaustion. The MSSRT score achieved was used to predict maximal oxygen uptake (VO_{2max}) and the speeds equivalent to 55% and 95% VO_{2max} for use during the IHIR (33).

Intermittent high intensity running protocol (IHIR)

To simulate the running demands of soccer, the Loughborough Intermittent Shuttle Test (31) was used for the IHIR. This required participants to alternate between walking, running and sprinting over a distance of 20 m in an indoor gymnasium (wooden surface; $20 \pm 2°C$, $38 \pm 2\%$ relative humidity) for a period of 90 minutes. Briefly, participants completed five blocks that each lasted approximately 15 minutes with a 3-minute rest period between each (where water

Commented [CT1]: Is this right Nick?

was consumed *ad libitum*). Each 15 min block comprised a set pattern of intermittent high-intensity activity as follows (31):

- 3 x 20 m at walking pace

- 1 x 20 m at maximal sprint

- 4 s recovery

- 3 x 20 m at a running speed corresponding to 55% of individual VO_{2max}

- 3 x 20 m at a running speed corresponding to 95% of individual VO_{2max}

In the sixth and final block participants completed shuttle running that alternated every 20 m between speeds corresponding to 55% and 95% VO_{2max} until volitional exhaustion (see Figure 1). Verbal encouragement was given by the same researcher before each sprint and during the time to exhaustion run to encourage a maximal effort. This protocol has been shown closely replicate the movement demands of intermittent team sports with both sprints and running to exhaustion possessing acceptable reliability (31).

***** Insert Figure 1 about here*****

Assessment of isokinetic muscle strength

Peak knee extensor and flexor concentric strength were measured on the dominant limb using a isokinetic dynamometer (Biodex, multi-joint System 3, Biodex Medical, New York, USA) at 60 deg·s^{-1}. Before the test, a standardized 3-minute warm up cycling at 50 W (Lode, Corival, Lode BV, Groningen, Netherlands) was used. The participants were positioned in a

seated position with the lateral femoral epicondyle aligned to the centre of the dynamometer axis of rotation. With the upper body secured, the lever arm was positioned at the distal point of the tibia and above the malleoli. The range of motion (0 to 90 degrees) and limb mass were measured before testing to adjust for gravitational influences (18). Participants were given two warm-up trials before five maximal efforts, with visual and verbal encouragement being given throughout. Peak torque values were calculated by the dynamometer's software as the highest torque generated over the active range of motion for each action. In-house determined test-retest reliability coefficient of variation (CV) for the measurement peak isokinetic extension and flexion torques was 4.2 - 6.8% when measured over a range of 0 (full knee extension) to 90 (knee flexion) degrees.

Assessment of sprint performance

After a standardized warm-up, participants completed three 10 m maximal sprints with an initial 5 m acceleration area and a 2-minute active recovery between each. Speed was measured to the nearest 0.01 s using timing gates (Brower, Speedtrap 2, Brower, Utah, USA). In-house determined test-retest reliability CV for the measurement sprint performance was 1.1%.

Assessment of counter-movement jump performance

Two practice jumps followed by three maximal jumps were performed using a jump mat (Just Jump System, Probotics, Inc, Alabama, USA). The participant was instructed to stand upright with hands on the hips, from which they flexed their knees to approximately 90 degrees, before extending into a jump. No arm swing was permitted and participants were required to

ensure that hands remained on hips throughout the jump. Each jump was observed by the researcher to ensure the correct technique was performed and the mean height of the three scores recorded for analysis. In-house determined test-retest reliability CV for the measurement of counter-movement jump height was 2.0%.

Assessment of perceived muscle soreness

Subjective measures of perceived muscle soreness were recorded while the subject performed a squatting action using a '0' – '10' visual analogue scale, where '0' equated to no soreness on movement and '10' meant their muscles were too sore to move. After demonstration of the appropriate technique and familiarization, participants were instructed to perform an unloaded squat to activate the muscles of the lower limbs with their hands on their hips. Subjects flexed to a knee angle of approximately 90 degrees at which point they were asked to indicate the degree of soreness on the visual analogue scale. This has been shown to be a valid and reliable method for assessing the sensation of pain (32).

Assessment of internal and external load during the IHIRs

During each IHIR protocol, heart rate was measured every 15 s using a recordable heart rate monitor (Polar Electro, S810i, Polar Electro, Oy, Finland; CV = 3.8%), the mean for each block being used in the analysis. Rating of perceived exertion (RPE; CV = 2.4%) was recorded using the Borg 6-20 scale (5) after participants were familiarized to the scale and how to use it using a set of standardized instructions (28). Blood lactate concentration ([Bla]; Arkray, Lactate Pro, Arkay, Kyoto, Japan; CV = 8.2%) was also measured from a fingertip capillary sample immediately after each block. Sprint times over 10 m (Brower, Speedtrap 2, Brower, Utah, USA)

were recorded for each block of the IHIR, from which the mean value was later calculated to represent sprint performance.

Statistical Analysis

All dependent variables were log transformed to reduce bias due to non-uniformity of error that enabled our analysis using the effect size (ES) statistic with 90% confidence intervals (CI) and % change to determine the magnitude of effects. Magnitude-based inference statistics were employed to provide information on the size of the differences, allowing a more practical and meaningful explanation of the data (3). The threshold for the magnitude of the observed change for each variable was determined as the within-participant standard deviation (SD) in that variable x 0.2, 0.6 and 1.2 for a small, moderate and large effect, respectively (20). Threshold probabilities for a meaningful effect based on the 90% CI were: <0.5% *most unlikely*, 0.5–5% *very unlikely*, 5–25% *unlikely*, 25–75% *possibly*, 75–95% *likely*, 95–99.5% *very likely*, >99.5% *most likely*. Effects with 90% CI across a likely small positive or negative change were classified as *unclear* (20). All calculations were completed using a predesigned spreadsheet (19) with data presented as mean ± SD.

RESULTS

Muscle function and soreness changes after IHIR1

There were *most likely* increases in perceived muscle soreness from baseline values after IHIR 1 (see Table 1) at 0, 24, 48 and 72 h (1583%, ES 2.19 ± 0.60; 2152%, ES 2.42 ± 0.61; 1855%, ES 2.31 ± 0.73; 1500%, ES 2.16 ± 1.01, respectively). There were *very likely* reductions in CMJ from baseline at 0 and 24 h (-5.9%, ES -0.81 ± 0.59; and -6.8%, ES -0.94 ± 0.60, respectively) with *likely* reductions at 48 and 72 h (-4.9%, -0.67 ± 0.80; and (-3.2%, 0.91 ± 0.76, respectively). Sprint time over 10 m was *very likely* higher at 0 h (5.7%, 1.61 ± 1.36), *likely* higher at 72 h (3.2%, 0.91 ± 0.76) but *unclear* at 24 and 48 h (2.6%, 0.74 ± 1.08; and 2.7%, 0.76 ± 1.20, respectively). Isokinetic knee extensor peak torque was *very likely* lower than baseline at 0 and 24 h (-9.1%, 0.62 ± 0.27; and -7.8%, 0.53 ± 0.30, respectively), *likely* lower at 48 h (-4.5%, -0.3 ± 0.16) and *possibly* lower at 72 h (-2.5%, 0.16 ± 0.18). Isokinetic knee flexor peak torque was *likely* lower than baseline at 0 h (-7.2%, 0.52 ± 0.48) and *very likely* lower at 24 to 72 h (-10.3%, -0.75 ± 0.50; -11.4%, 0.84 ± 0.49; and -8.9%, 0.65 ± 0.44, respectively).

*****Insert Table 1 about here*****

Differences in internal and external load between IHIR trials

Mean heart rates (Figure 2C) were *likely* lower in IHIR 2 compared to IHIR 1 for Block 1 (-4.8%, ES -0.61 ± 0.45), Block 2 (-4.6%, ES -0.60 ± 0.41), Block 4 (-3.7%, ES -0.52 ± 0.38) and Block 5 (-2.8%, ES -0.44 ± 0.36), *possibly* lower for Block 3 (-2.5%, ES -0.29 ± 0.26) but *unclear* for Block 6 (-0.8%, -0.15 ± 0.60). [Bla] (Figure 2A) was *likely* lower during IHIR 2 at

Blocks 1 (-21.2%, ES -0.44 ± 0.54) and 4 (-38.8%, ES -0.69 ± 0.53), *very likely* lower at Block 2 (-43.9%, ES -0.75 ± 0.51) *possibly* lower at Block 5 (-24.8%, ES -0.39 ± 0.55) and *unclear* at Block 3 (1.2%, 0.02 ± 0.42) and Block 6 (-14.0%, -0.3 ± 0.53). RPE (Figure 2B) was *likely* lower in IHIR 2 at Block 2 (-5.3%, 0.66 ± 0.56) but *unclear* at all other time points. Time to exhaustion was *possibly* shorter (-13.7%, -0.25 ± 0.36) in Block 6 of IHIR 2 (2.89 ± 1.39 min) compared to IHIR 1 (3.38 ± 1.88 min).

*****Insert Figure 2A-C about here*****

Average 10 m sprint time (Figure 3) was *very likely* increased at Block 1 and Block 2 (6.2%, 1.09 ± 0.55; and 4.9%, 0.78 ± 0.53, respectively) and *likely* higher for Block 3-5 (3.9%, 0.6 ± 0.50; 3.3%, 0.47 ± 0.32; and 4.9%, 0.41 ± 0.36, respectively) during IHIR 2 compared to IHIR 1.

*****Insert Figure 3 about here*****

DISCUSSION

This study examined how EIMD caused by an intermittent high intensity running protocol impacted on the physiological, perceptual and physical responses to the same exercise performed 72 hours later. The initial high intensity running bout (IHIR 1) caused moderate impairments of muscle function and large increases in soreness that persisted for up to 72 hours. When repeated 72 hours later, a down-regulation of sprinting performance, lower heart rate and [Bla] during the second high intensity intermittent running protocol (IHIR 2) was accompanied by an unchanged perception of effort.

In the first IHIR, eccentric contractions from the numerous accelerations, decelerations and changes in direction are likely to have provided the main stimulus for the symptoms of EIMD that followed. This is confirmed by the observed reductions in isokinetic muscle function, sprint and jump performances, and elevations in muscle soreness that remained for 72 hours after the initial IHIR bout. These observations are consistent with previous studies reporting the time-course responses of impaired muscle function and muscle soreness after the same prolonged intermittent running protocol (23, 24) and a soccer match (23, 24). With the exception of knee extensor peak torque, small to moderate reductions from baseline values remained for all measures of muscle function alongside very large increases in soreness. These responses reflect EIMD imposed by prolonged intermittent exercise and more importantly, suggest that participants started the second prolonged IHIR running protocol with impaired functional capacity and muscle soreness.

During the second IHIR protocol performed 72 after the first, a key finding was the moderate to large increase in 10 m sprint time during the 90 minutes. This reduction in sprint performance might be attributed to the structural damage to the muscle fibres (28), which is confirmed by the immediate and prolonged losses in muscle function after IHIR1. Preferential damage to type II muscle fibres is known to occur as a consequence of multiple eccentric contractions (6) which can be manifest as a reduction in sprint performance (38). It has also been suggested that a reduced reflex sensitivity could impair the participants' ability to utilize the ground reaction forces during sprinting (18). Moreover, such damage would explain the elevated muscle soreness scores that remained from the first prolonged running bout. Increases in muscle soreness impose an inhibitory response that regulates voluntary activation of the exercising musculature. This mechanism has been reported as a reduced neural drive via stimulation of III

and IV afferents, and subsequent reduction in force generation to prevent further injury to the neuromuscular system (22, 27, 40). Therefore, starting the second prolonged running protocol with sensations of muscle soreness likely played a similar role, such that participants down-regulated the only self-regulated element in an attempt to work within tolerable limits.

A lower blood lactate response observed for Blocks 1-5 during the second IHIR protocol is in contrast to work that has shown an increased [BLa] in the presence of EIMD (16). Our findings however, are in agreement with those of Twist and Eston (38) who reported a lower [BLa] during a cycling time trial performed 48 h after muscle-damaging exercise. Along with a ~3-5% reduction in mean heart rate in Blocks 1-5, a lower [BLa] reaffirms that participants down-regulated their maximal intensity efforts during the second IHIR protocol. That participants were able to increase their exercise intensity from Block 5 to Block 6 supports this notion further, with similar heart rates and [BLa] at Block 6 for both running trials. This 'end spurt' phenomenon has been reported previously for team sport athletes (39) and is indicative of participants conserving energy expenditure before a final increase.

Participants' ratings of perceived exertion did not differ between the two IHIR trials, despite the reductions in the internal and external loads in the second trial. That is to say, participants provided the same perceived ratings of exertion in both IHIR bouts despite a voluntary down-regulation of movement speed (i.e. sprint running) and concomitant reduction in heart rate and blood lactate response during the second IHIR bout. We propose that reductions in muscle force generating capacity and increases in muscle soreness as a consequence of the first IHIR are likely causes of this response. Indeed, these findings are consistent with those studies

that report how symptoms of exercise-induced muscle damage alter the association between effort perception and physiological response (38, 25). Our data also reaffirm the importance of effort perception both in exercise regulation and its role in fatigue (12).

While we used an intermittent test that has been widely adopted to simulate the activity patterns of various team sports (31), we are aware that the protocol did not involve any sideways and backwards movements. Moreover, we did not include any assessment of player skill during the intermittent test. This means we are unable to understand the interactions between physiological, movement and technical capabilities during prolonged exercise bouts separated by short recovery periods. Having each participant wear a global positioning system (GPS) device would also have enabled a more intricate understanding of the movement characteristics during the intermittent running protocols. However, performing the trials indoors to standardize the running surface and avoid any influence of outdoor environmental conditions prevented the use of GPS.

Practical Applications

This study has implications for practitioners concerned with athlete management during periods where two or more matches and/or high intensity training sessions are performed within close proximity. EIMD caused by an initial bout of prolonged intermittent exercise (a simulated soccer running protocol) appeared to encourage sub-elite team sport players to down-regulate their efforts during a subsequent bout of the same duration and intensity. Coaches should therefore consider appropriate nutrition, recovery and tactical strategies when the second bout of activity requires optimal performance. Where the focus is on training, coaches should be mindful

of under-recovery from the initial bout of prolonged intermittent exercise and anticipate impaired sprinting performance in any subsequent bout as a consequence of an athlete's altered sense of effort influenced by increased muscle soreness.

Conclusion

Team sport athletes are frequently required to perform bouts of prolonged intermittent exercise separated by short recovery periods. Simulating the running intensities of match play we present novel data on how the physiological and perceptual responses to a second bout of prolonged intermittent exercise are altered after an initial bout of the same exercise performed 72 hour before. We report that sub-elite team sport players do not recover enough after an initial high intensity intermittent running bout to enable replication of the same exercise intensity 72 h later. Reductions in muscle force generating capacity and increases in muscle soreness appear immediately after the initial bout of intermittent high intensity exercise and remain such that the players start the second bout still experiencing these symptoms. Consequently, players down-regulate their all-out exercise efforts in an attempt to work within tolerable limits, the outcome of which is a lower physiological internal response with the same perception of effort. These data can be used to inform coaching practice during intense periods of competition or training.

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Figure legends

Figure 1. Schematic representation of the IHIR protocol (adapted from Nicholas et al. (30)).

Figure 2. Blood lactate concentration ([Bla]; panel A), rating of perceived exertion (RPE; panel B) and average heart rate (panel C) during Blocks 1 to 6 of IHIR1 (black triangles) and IHIR 2 (white squares). ≈ Denotes trivial difference; ♯ denotes a small difference and * denotes moderate difference. Values expressed as mean ± SD.

Figure 3. Mean sprint times (s) during Blocks 1, 2, 3, 4 and 5 of IHIR 1 and IHIR2. Trial 1 is denoted by solid line and trial 2 by dashed line. ♯ Denotes a small difference; * denotes moderate difference. Values expressed as mean ± SD.

Table 1. Changes in muscle soreness and function after IHIR 1.

	Baseline	0 h	24 h	48 h	72 h
Perceived muscle soreness	0.3 ± 0.6	3.6 ± 2.0*	4.4 ± 1.3*	4.4 ± 1.8*	3.4 ± 2.1#
CMJ height (cm)	42.9 ± 2.8	40.5 ± 3.7*	40.2 ± 5.0*	41.2 ± 5.9*	41.2 ± 5.2#
10 m sprint (s)	1.55 ± 0.05	1.64 ± 0.1‡	1.59 ± 0.09*	1.59 ± 0.08*	1.60 ± 0.07*
Knee extensors peak torque (N·m)	238.7 ± 32.4	217.1 ± 30.7*	218.9 ± 27.6#	227.7 ± 29.0#	232.9 ± 33.1
Knee flexors peak torque (N·m)	135.2 ± 17.1	119.9 ± 15.6#	120.9 ± 9.9*	119.6 ± 12.3*	122.7 ± 10.1*

Values are presented as mean ± standard deviation. # denotes a small difference to baseline value; * denotes moderate difference to baseline value; ‡ denotes large difference to baseline value. CMJ = countermovement jump.