



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Pruscino, CL;Halsen, S;Hargreaves, M

Title:

Effects of compression garments on recovery following intermittent exercise

Date:

2013-06-01

Citation:

Pruscino, C. L., Halsen, S. & Hargreaves, M. (2013). Effects of compression garments on recovery following intermittent exercise. *European Journal of Applied Physiology*, 113 (6), pp.1585-1596. <https://doi.org/10.1007/s00421-012-2576-5>.

Persistent Link:

<https://hdl.handle.net/11343/283110>

Effects of Compression Garments on Recovery following Intermittent Exercise

Cathryn L. Pruscino^{1,2}, Shona Halson³, Mark Hargreaves¹

¹ Department of Physiology, University of Melbourne, Melbourne, Australia;

² Department of Sport Science, Victorian Institute of Sport, Melbourne, Australia;

³ Department of Physiology, Australian Institute of Sport, Canberra, Australia.

Address for correspondence:

Ms. Cathryn Pruscino

Victorian Institute of Sport

PO Box 12608 A'Beckett Street, Melbourne, VIC 8006, Australia

Email: cathryn.pruscino@vis.org.au Phone: +613 9425 0091 Fax: +613 9425 0100

Abstract

Purpose: To examine the effects of wearing compression garments for 24 h post-exercise on the biochemical, physical and perceived recovery of highly-trained athletes. **Method:** Eight field hockey players completed a match-simulation exercise protocol on two occasions separated by 4 wks after which lower-limb compression garments (CG) or loose pants (CON) were worn for 24 h. Blood was collected pre-exercise and 1, 24 and 48 h post-exercise for IL-6, IL-1 β , TNF- α , CRP and CK. Blood lactate was monitored throughout exercise and for 30 min after. A 5 counter-movement jump (5CMJ) and squat jump were performed and perceived soreness rated at pre-exercise and 1, 24 and 48 h post-exercise. Perceived recovery was assessed post-exercise using a questionnaire related to exercise readiness. Repeated measures ANOVA was used to assess changes in blood, perceptual and physical responses to recovery. **Results:** CK and CRP were significantly elevated 24 h post-exercise in both conditions ($p<0.05$). No significant differences were observed for TNF- α , IL-1 β , IL-6 between treatments ($p>0.05$). Power and force production in the 5CMJ was reduced and perceived soreness was highest at 1 h post-exercise ($p<0.05$). Perceived recovery was lowest at 1 h post exercise in both conditions ($p<0.01$), whilst overall, **perceived recovery was greater when CG were worn ($p<0.005$)**. **Conclusion:** None of the blood or physical markers of recovery indicate any benefit of wearing compression garments post-exercise. However, muscle soreness and perceived recovery indicators suggest a psychological benefit may exist.

Key Words: CYTOKINES, INFLAMMATION, CREATINE KINASE, MUSCLE FUNCTION, TEAM SPORTS.

Introduction

The underlying feature of lower body, graduated compression garments is their ability to alter the flow of blood, and in particular, enhance the return of blood from the peripheries to central regions of the body (Sigel et al. 1973; Sigel et al. 1975). The compressive force applied by the garments to the lower limbs reduces the size of the venous bed at the extremities, thereby increasing femoral vein blood flow velocity (Sigel et al. 1973; Sigel et al. 1975). The associated reduction in stasis and increased venous return at rest has proved beneficial in the treatment of patients with chronic venous insufficiency (Horner et al. 1980; Zajkowski et al. 2002) and has reduced the incidence of deep vein thrombosis following major surgery (Holford 1976). Inactive, hospitalised patients and those with venous conditions have traditionally been the beneficiaries of such enhanced haemodynamics, however, the benefit of wearing lower body compression garments on blood flow has also been reported in patients with normal venous systems (Sigel et al. 1973; Sigel et al. 1975).

In recent years, compression garments have been promoted to athletes as a means of optimising circulation during and following exercise. The potential influence of compression therapy on the inflammatory and repair process post-exercise has emerged as a new frontier in the recovery of elite athletes. Physiological recovery and repair of muscle fibres following exercise-induced muscle damage (EIMD) can take up to 4-5 days (Armstrong et al. 1991; Connolly et al. 2003) and can therefore, be a limiting factor for elite team-sport athletes during consecutive days of training or competition (Barnett 2006; Ispirlidis et al. 2008). An acceleration of the muscle repair process, even for minor episodes of muscle damage, is advantageous in their capacity to return to competition or training at the earliest opportunity. Compression garment use is now being incorporated into athlete recovery strategies in an attempt to attenuate the inflammatory response, reduce the effects of EIMD and restore optimal muscle function sooner (Barnett 2006; MacRae et al. 2011). However, the efficacy of such garments to deliver these outcomes in elite athletes is yet to be clearly demonstrated.

Given the effect of compression garments on blood flow, it is anticipated that enhanced circulation may attenuate the increase in the concentration of cytokines in the post-exercise period, and therefore

influence the inflammatory response. However, the effect of compression garments on the post-exercise inflammatory response has been investigated only once before with the impact on the appearance of specific cytokines (IL-6 and IL-10) reported as unclear (Montgomery et al. 2008a). The appearance of a range of cytokines in the blood suggests that an acute-phase inflammatory response has been initiated and is thought to reflect the magnitude of the muscle damage that has occurred (Pedersen et al. 1998). The sequence of cytokine appearance begins with TNF- α and IL-1 β which are pro-inflammatory cytokines and are thought to trigger the release of IL-6, which is restorative rather than pro-inflammatory in nature (McIntyre et al. 1995; Pedersen et al. 1998). IL-6 is also thought to be involved in substrate delivery during exercise through its ability to induce lipolysis and fat oxidation and through its involvement in glucose homeostasis (Petersen and Pedersen 2005). Further along the inflammatory cascade, the presence of IL-6 promotes synthesis of C-reactive protein (Pedersen et al. 1998), a marker of systemic inflammation and tissue damage (Malm et al. 2004) which is involved in the suppression of pro-inflammatory cytokine production (Petersen and Pedersen 2005).

Previous research has provided evidence that different types of exercise can trigger the production of cytokines in the post-exercise period. A 2.3-fold increase in TNF- α , a 2.1-fold increase in IL-1 β and a 128-fold increase in IL-6 was reported within 10 min of completion of a marathon race (Ostrowski et al. 1999). Both soccer (Ispirlidis et al. 2008) and basketball (Montgomery et al. 2008a) match-play resulted in a 3-4 fold elevation of IL-6 concentrations immediately after exercise, and following the soccer match, the peak in IL-6 preceded the peak in CRP which is in line with previous findings (Malm et al. 2000). In contrast, maximal eccentric resistance training produced a peak in IL-6 concentrations 8 h after exercise with an almost 2-fold elevation above resting conditions (Miles et al. 2007a). Maximal isometric contractions produced a 1.5 fold elevation of IL-6 levels at 8 h post-exercise, with no significant change in CRP concentrations (Miles et al. 2007b). Distance-related increases in CRP concentrations after 5 running races of varying durations (15-88km) have been reported, with CRP peaking 24 h after each race regardless of the distance travelled (Strachan et al.

1984). As such, it is clear that the magnitude and time course of the cytokine response varies with different types of exercise. Whether wearing compression garments in the post-exercise period reduces the time course or magnitude of this biochemical response in the hours and days after exercise will be further investigated in the present study.

The specific effect of wearing compression garments in the post-exercise period on commonly used indicators of EIMD, have previously been investigated with mixed results. Untrained subjects demonstrated an enhanced recovery of force production, reduced creatine kinase (CK) concentrations and decreased perceived soreness in the days following maximal eccentric muscle contractions after wearing a compressive sleeve (Kraemer et al. 2001). Similarly, resistance-trained subjects showed reduced CK response and improved upper body restoration of strength after wearing full body compression garments in the recovery from a whole body heavy resistance exercise protocol (Kraemer et al. 2010). However, subjects ranging from recreational performers to regional level athletes who wore lower-body compression for 12 h following a resistance exercise protocol (French et al. 2008) displayed reduced range of movement through knee and hip joints 48h after exercise, showed no restoration of lower leg power above control conditions and suffered a slower recovery of 30m sprint times compared to control at 48 h after exercise. Furthermore, similar garments worn by trained subjects after a plyometric drop-jump protocol (Davies et al. 2009) and a simulated basketball tournament (Montgomery et al. 2008b) appear to have also impeded recovery of 5-20m sprint performance with sprint times increasing beyond control values in the post-exercise period.

Although a growing body of research has investigated the efficacy of wearing lower body compression garments on post-exercise recovery, the benefit to the highly-trained athlete remains unclear. The main reasons for this include the differences in type and pressure characteristics of the compression garments utilised, as well as the variation in the training status of the subjects used to assess this recovery strategy. Furthermore, much of the research examining the effect of compression garments on exercise recovery employs eccentric resistance exercise regimens traditionally used to elicit muscle damage. Whilst this method is highly effective for measuring and monitoring purposes,

for elite athletes, this may not reflect the extent of typical damage incurred during training or competition. As such, the recovery from this type of exercise may not be specific to the training recovery required for their sport (Bishop et al. 2008).

The present study aimed to investigate the efficacy of wearing commercially available, full-length, lower body compression garments following a hockey-simulation exercise protocol to determine whether this strategy influenced the post-exercise biochemical response and recovery of muscle function in highly-trained athletes. The pressure exerted by the compression garment was measured and a practical post-exercise protocol utilised. The physiological, biochemical and perceptual responses of highly-trained subjects were examined to determine the effect on post-exercise recovery, of wearing versus not wearing lower body compression garments.

Methods

Subjects. Eight highly-trained male hockey players (mean age 21.9 ± 2.3 yrs, height 180.1 ± 8 cm, body mass 77.9 ± 13.9 kg, sum of 7 skinfolds 70.6 ± 21.1 mm) who compete at either National or International level, volunteered to participate in this investigation. Prior to commencing the study, all participants were informed of the requirements, risks and benefits of the investigation and written informed consent was obtained from each. Approval for the procedures of the study was granted by the University of Melbourne Human Research Ethics Committee.

Pre-test protocol. Data were collected during two 3 day trial periods separated by 4 weeks. The 3 day trial procedure is outlined in Figure 1. In the days prior to each trial and throughout the study period, subjects were asked to refrain from taking anti-inflammatory medication (aspirin, ibuprofen). During each trial period subjects were asked to limit the water temperature and duration of showers and refrain from using the following therapeutic modalities: ice, ice baths, contrast water therapy, cold water immersion, heat massage, massage therapy, stretching. A pack containing sports drinks, a urine container, an instruction sheet, a food pack and a food diary was delivered to each subject prior to the commencement of each trial. The food pack contained hydration fluids for the night before each trial

and a normalised breakfast pack for the morning of the trial aimed to deliver 1.2g CHO.kg BM⁻¹. Food diaries were kept by each athlete in the 24 h prior to the start of the first trial and throughout the 3 day trial period and this food intake regimen was then replicated prior to the subsequent trial. Subjects were restricted from performing high intensity exercise in the 24 h prior to each trial.

The Loughborough Intermittent Shuttle Test (LIST) exercise protocol, used in the present study, includes exercising at varying percentages of each individual's maximal oxygen consumption. As such, initial indication of the aerobic capacity of each subject was required prior to testing in order to determine the appropriate protocol to be used. At least 7 days prior to the commencement of the study and in accordance with procedures previously recommended (Nicholas et al. 2000), the predicted VO_{2max} of each subject was ascertained using a progressive multistage shuttle running test (Ramsbottom et al. 1988). Following completion of the multistage shuttle running test, subjects were grouped according to their predicted VO_{2max} scores. Subjects with a predicted VO_{2max} in the range 53-57 ml.kg.min⁻¹ were assigned the 55 ml.kg.min⁻¹ LIST exercise protocol and those in the range 57-62 ml.kg.min⁻¹ were assigned the 60ml.kg.min⁻¹ LIST exercise protocol to perform during the study period. As such, the running speeds required of subjects during testing, were suitable to the aerobic fitness level of each individual.

Each subject also participated in a familiarisation session for the tests of muscle function to be used during the study. Body mass (BM) and waist girth measurements assisted the process of fitting compression garments which was overseen by a representative of the manufacturer to ensure sizing was appropriate. Subjects were fitted into a comfortable size, smaller than that outlined on the packaging, which is in line with current manufacturer's recommendations. Two garments were then labelled and put aside for each subject.

Pressure interface monitoring. Separate to testing, the interface pressure between the compression garment and the skin was monitored for each subject using a portable interface pressure evaluator (Talley Medical, Miami, USA). Measurements were recorded at 3 sites on the lower limb (minimal

ankle, maximal calf and maximal thigh) according to international recommendations (Parsch et al. 2006). Subjects were measured, in the standing position, using a 2.8 cm pressure sensor positioned at each of the 3 designated sites. The pressure sensor was attached to the hand held pressure evaluator via a jack plug and air connector. The sensor was slowly inflated and then deflated whilst the pressure (mm Hg) between two surfaces was displayed on the digital screen. The interface point was indicated by visual and auditory signals.

Experimental protocol. On the first morning of each trial subjects were required to collect a urine sample on waking. Urine specific gravity was measured to assess hydration status of the subject prior to exercise. Subjects arrived at the testing venue at the same time on both trials. Upon arrival, subjects had body mass (BM) measured and were then required to rest seated for 15 min before a baseline venous blood sample was obtained for later analysis of IL1- β , IL-6, TNF- α and CRP, and, a baseline capillary sample was obtained for CK analysis. This blood collection procedure was repeated at 1, 24 and 48 h post-exercise (Fig 1). Following baseline blood measurements, subjects moved to the indoor gymnasium where they completed a standard warm-up consisting of low intensity stationary bike, running drills, 30 m run throughs increasing in intensity and box jumps. A maximum of 5 min rest was allowed before commencing baseline tests of muscle function.

Tests of muscle function. A 5-repetition counter movement jump (5CMJ) and a squat jump (SJ) were performed by each subject at baseline, 1, 24 and 48 h post-exercise to monitor the effect of fatigue and recovery on lower-body force and power production.

A repeated counter movement jump was used instead of a single counter movement jump due to the potential for unique high or low scores in a single effort jump test and the likelihood that the measurement of repeated efforts may be more reliable (Hopkins et al. 2001). It has previously been suggested that the 5CMJ is suitable for assessing the training and performance of elite athletes (Cormack et al. 2008) with good overall reliability for peak power and peak force (CV 4.4% and 3.3%, respectively).

For the 5CMJ, subjects were instructed to exert maximal effort for 5 consecutive jumps, without a pause between them. An average of the five peak force and peak power values was calculated for each set of jumps and the highest average value is reported as the 5CMJ mean force and 5CMJ mean power, respectively.

According to previous recommendations for the SJ (Sheppard and Doyle 2008), subjects were required to begin from a 90 degree squat position which was briefly held prior to jumping vertically as high as possible. The brief isometric hold prior to the jump prevents counter movement activity removing the contribution of the stretch-shortening cycle. The peak force for each SJ was recorded and reported as SJ peak force.

Participants performed each jump test at least three times or until maximal force and power values no longer increased. This was to ensure that the maximal values at each time point were recorded. Warm-up procedures and tests of muscle function were repeated at 1, 24 and 48 h post-exercise.

Vertical displacement of each jump was measured with a cable-extension potentiometer (distance transducer) attached to a lightweight pole resting on the subject's shoulders. Peak force data was calculated by Ballistic Measurement System (Fitness Technology, Adelaide, Australia) computer software.

Wearing compression garments during jumping has previously been reported to improve mean force and power production over a 10 counter-movement jump test (Kraemer et al. 1996) and a single maximal vertical jump (Doan et al. 2003) and as such, compression garments were removed prior to jumping in the present study.

Rating of Perceived Soreness. An adapted visual analogue scale (Mattacola et al. 1997) was used to measure the rating of perceived muscle soreness (RPS) that subjects experienced during muscle function testing. Immediately following the 5CMJ and SJ, subjects rated their perceived muscle

soreness (RPS) on the 11 point visual analogue scale (VAS) which had anchors at either end indicating no soreness (0) and extreme soreness (10). Subjects were asked to point to the marker on the line which best indicated their soreness during jumping.

LIST Protocol. The LIST exercise protocol was used to induce fatigue and was performed on day 1 of each trial. The LIST protocol was performed on an indoor running track in moderate conditions (24.8 ± 1.1 deg C; $51.4 \pm 6.1\%$ RH) on both occasions.

The LIST is made up of variable speed running, jogging and walking and was developed to simulate the intermittent nature of sports such as soccer and field hockey (Nicholas et al. 2000). It was selected due to its ability to elicit muscle damage and exercise induced muscle soreness (Thompson et al. 1999).

The exercise bouts are 15 min in duration, interspersed by 3 min rest periods, and include walking, jogging at 55%VO₂max, cruising at 95% VO₂max and sprinting at maximal running speeds up and back on a 20 m course with markers at 0, 10, 15 and 20 m. Five exercise bouts (75 min of exercise in total) were performed in the current study to closely simulate the duration of a field hockey match. Audio cues from a CD indicated when each activity would occur and the activity order was of a repetitive nature. The LIST protocol has been previously outlined in detail (Thompson et al. 1999).

At the conclusion of each 15 min exercise block, average heart rate (HR) and rating of perceived exertion (RPE) was recorded (Borg 1970) and subjects ingested 2 mL.kg⁻¹ BM plain water from clearly labelled, individually prepared drink bottles. At the start of each rest period and after 4, 8, 12, 20 and 30 min of recovery from the LIST, capillary blood samples were obtained from the earlobe of each subject for blood lactate analysis (Lactate Pro, Arkray, Japan).

Post-exercise procedure. Upon completion of the LIST and after obtaining the 4 min post-exercise capillary blood sample, subjects changed into either a full length, lower limb compression garment

(CG; 2XU Compression, Australia) or loose tracksuit pants (CON). The subjects were randomly assigned to the two treatments over two trials in a balanced crossover research design and were blinded to their order of treatment up until the first LIST protocol was completed. During the CG trial, compression tights were worn for the full 24 h post-exercise period and were only allowed to be removed for bathing and during jumping for muscle function testing, ensuring that the total time out of the compression garment did not exceed 30 min.

In the hour following completion of the LIST, subjects rested in a seated position. At the 1 h time point, subjects had blood samples collected and then repeated the standard warm-up, **tests of muscle function** and RPS protocols. Subjects returned to the lab at 24 and 48 h post-exercise to repeat the blood collection, warm-up, **tests of muscle function** and RPS procedures.

Perceived recovery. Whilst resting quietly prior to blood collection at 1, 24 and 48 h post-exercise, participants were asked to respond to the following statement to assess their perceived recovery: “I feel well recovered and physically ready to perform at my best in a match right now”. The participants responded using a scale ranging from strong disagreement (1) to strong agreement (5).

Blood collection and analysis. Venous blood was analysed for inflammatory mediators IL1- β , IL-6, TNF- α and CRP. 5-6 ml of whole blood were collected into a plastic syringe and then separated into blood collection tubes. Following centrifugation for 10 min at 2000rpm, the plasma was collected into four clearly labelled storage tubes and frozen (-80 deg C) until analysis. Plasma concentrations of IL1- β , IL-6 and TNF- α were determined using a standard analysis kit (Human High Sensitivity Multiplex panel, Millipore, USA) whilst CRP was analysed using a separate analysis kit (Human Cardiovascular Disease Panel 2, Millipore, USA). Both assays were carried out according to manufacturer’s instructions using Luminex 100 instrumentation (Bio-Rad Laboratories, Hercules, CA). The reliability (%CV) of measuring each analyte using these kits was 3.11% for IL1- β , 3.51% for IL-6, 3.49% for TNF- α and 8.0% for CRP.

A capillary blood sample was collected from the fingertip of each subject for CK analysis at baseline, 1h, 24h and 48h post-exercise (Fig 1). 32µl of blood was collected into a lithium-heparinised capillary tube then transferred to the Reflotron CK test strip and immediately analysed using a Reflotron Plus diagnostic device (Roche Diagnostics, Basel, Switzerland). The co-efficient of variation for the analysis of CK was 3.5%.

Capillary blood, collected from the earlobe, was used to determine blood lactate concentration immediately following completion of each 15min exercise bout and throughout the 30 min post-exercise period. A portable lactate analyser (Lactate Pro, Arkray, Japan) was used and has previously been shown to be an accurate and reliable portable analyser for blood lactate concentration (Tanner et al. 2010).

Data Analysis. Two-way analysis of variance (ANOVA) with repeated measures (treatment x time) was used to determine differences between the two treatments with regards to blood and muscle function test parameters. Statistical significance is reported when $P < 0.05$. Statistical analysis was carried out using IBM SPSS Statistics (Version 19.0). A Tukey's honestly significant difference post-hoc test was used to establish where any significant differences occurred. Following post-hoc analysis, effect size (ES) data was calculated using Cohen's d to determine the magnitude of the effect. Effect sizes were assessed using the following criteria: <0.2 =trivial; $0.2-0.6$ =small; $0.6-1.2$ =moderate; $1.2-2.0$ = large, >2.0 =very large (Hopkins 2002). All data are expressed as mean $\pm$ standard deviation (SD).

Results

Pre-exercise hydration variables. There were no significant differences in baseline body mass (78.0 ± 13.6 kg v 77.6 ± 12.7 kg for CG v CON, respectively) or urine specific gravity (1.019 ± 0.008 v 1.019 ± 0.005 for CG v CON, respectively) between trials.

Pressure Interface Data. The interface pressure between the lower body compression garment and the skin was measured at three sites of the lower limb. There was a significant difference between the pressure exerted by the compression garment at the ankle and the calf (19.1 ± 3.6 v 7.2 ± 2.8 mm Hg, $p < 0.01$), ankle and thigh (19.1 ± 3.6 v 4.8 ± 1.6 mm Hg, $p < 0.01$), but not between the calf and thigh (7.2 ± 2.8 v 4.8 ± 1.6 mm Hg, $p > 0.05$). Rather than providing uniform pressure along the length of the limb, the garments provide greater pressure at the ankle than at the thigh and therefore can be categorized as a graduated compression garment.

HR, Blood Lactate and RPE during the LIST. The average HR, blood lactate concentration and RPE were recorded following every 15 min bout of the LIST exercise protocol. A main effect for time was found for RPE across the five bouts ($p < 0.01$) with RPE increasing as exercise duration increased. At the completion of the final stage of exercise, regardless of the treatment that was to proceed exercise, there was no difference in average HR (CG: 167 ± 10 bpm v CON: 167 ± 10 bpm, $p > 0.05$), blood lactate concentration (CG: 2.7 ± 1.2 mmol.L⁻¹ v CON: 2.6 ± 0.8 mmol.L⁻¹, $p > 0.05$) or RPE (CG: 15 ± 1 v CON: 14 ± 1 , $p > 0.05$). As such, prior to commencing either CG or CON, the amount of work performed by subjects in the LIST, their physiological response to this work, and their physiological state at the conclusion of the LIST exercise protocol was the same.

Post-exercise Blood Lactate. Table 1 shows the blood lactate concentration of subjects from 0-30 min post-exercise under both conditions. No differences were observed between trials for post-exercise blood lactate concentration ($p > 0.05$).

Cytokines and Proteins. No significant differences were observed for TNF- α or IL-1 β or IL-6 between the two treatments ($p > 0.05$, Figure 2). The C-reactive protein (CRP) response revealed a significant interaction ($p = 0.030$, Figure 2) and post-hoc analysis identified a difference between the two treatments only at baseline. A main effect for time ($p = 0.001$) also confirmed that CRP was significantly higher at 24 h post-exercise than all other time points. For CG, CRP at 24h was higher than baseline, 1h and 48h ($p < 0.01$), whilst for CON, CRP at 24h was higher than at 48h ($p < 0.05$).

CK. A main effect for time identified that CK concentration was significantly elevated in the post-exercise period in both CG and CON ($p=0.001$, Figure 3), however, there were no differences in the concentration of CK when comparing CG with CON ($p>0.05$). CK values peaked 24 h after the completion of exercise at which time they were higher than all other time points ($p<0.05$) indicating that the LIST protocol successfully induced some muscle damage, however, the leakage of CK into the blood, appears to have been similar between trials regardless of the recovery technique used.

Tests of Muscle Function. A significant interaction ($p=0.039$) and a main effect for time ($p=0.002$) were observed for mean power production during the 5CMJ (Table 2). Power production had decreased significantly ($p<0.05$) by 1h after exercise in both trials and was significantly higher at 48h compared with 1h and 24h post-exercise ($p<0.01$). The post-hoc analysis following the significant interaction, revealed a difference between CG and CON only at 48h post-exercise ($p<0.05$), however, the magnitude of the difference between treatments at this time point (158w) and that which was observed across time within each treatment, were generally smaller than the typical error associated with this test (210w intraday, 278w interday) (Cormack et al. 2008). For mean force in the 5CMJ, a significant time effect ($p<0.05$) confirmed that mean force production was significantly lower at 1h post-exercise than at baseline, 24h and 48h under both conditions, and the difference was generally greater than the typical error (69N) associated with this parameter (Cormack et al. 2008). There were no significant differences observed between trials for mean force or mean power output in the SJ (Table 2).

Perceived recovery. Although no interaction effect was observed ($p>0.05$), significant main effects for both treatment ($p=0.005$) and time ($p=0.001$) were found. At 1 h after the end of exercise in both CG and CON, subjects disagreed with the notion that they were “well recovered” and “ready to perform at their best”. They felt significantly less recovered at 1h ($p<0.05$) compared with 24h and 48h post-exercise. **Given the treatment main effect, averaged across all time points it is evident that perceived recovery was reported as superior when CG were worn.** Individual data show that after

wearing CG for 48 h, six out of eight subjects strongly agreed they were “ready to perform at their best”, compared to one out of eight for CON.

Rating of Perceived Soreness. Rating of perceived muscle soreness in the post-exercise period is displayed in Figure 4 (0=no soreness; 10=extreme soreness). There was no significant interaction effect, however, the main effect for time was $p=0.003$ as perceived soreness was higher at 1 h post-exercise in both conditions compared to all other time points ($p<0.05$). **There was a trend for a treatment main effect ($p=0.053$) with soreness reported to be less in the CG versus control condition averaged over all recovery time points.**

Discussion

The focus of the present study was on the efficacy of wearing full length lower body compression garments for 24 h post-exercise as a recovery aid for highly-trained athletes. Additionally, it provided a unique comparison of blood variables, perceptual responses and muscle function in the 48 h following intermittent activity. The majority of subjects in the present study felt better recovered and reported earlier exercise readiness after 24 h of wearing compression garments following a hockey match simulation exercise. Despite this, blood markers and tests of muscle function monitored in this study revealed that compression garments provided minimal assistance to the recovery process.

Compression garments have been shown to increase femoral vein flow velocity by reducing the pooling of blood in the lower extremities and encouraging venous return of blood to the heart. Sigel et al. (1975) found that garments which apply a compressive force of 18 mm Hg around the ankle, reducing to 8 mm Hg around the thigh, generate the fastest average flow velocity of 38.4% above baseline, in inactive recumbent subjects. A garment which applies graduated compression in this way has been shown to increase blood flow to a greater degree than compression distributed evenly over the lower extremity in patients with normal venous systems (Sigel et al. 1975). The commercial garments used in the present study provided interface pressure of 19.1 mm Hg at the ankle, 7.2 mm

Hg at the calf and 4.9 mm Hg at the thigh. Given the pressure applied at the extremities and the graduated nature of the garments used in this study, the expected influence on blood flow would be similar to what has previously been described (Sigel et al. 1975).

In the present study, a range of blood variables were monitored in the post-exercise period to determine whether any influence of the garments on the recovery process could be observed. The effect of circulation on the removal of blood lactate may have provided some insight into the effect of compression garments worn in the immediate post-exercise period. However, the blood lactate response of our subjects in the present study (average 2.7 mmol.L^{-1} across the five bouts) was much lower than previously reported for this protocol (Nicholas et al. 2000) and as such, it is difficult to elucidate the specific impact on blood lactate removal post-exercise. Although the LIST was chosen and has previously been defined as a “high-intensity” exercise protocol (Nicholas et al. 2000), our highly-trained subjects were well adapted to this type of exercise and tolerated the exercise well as a result. We may have seen more changes in the HR, RPE and blood lactate response during exercise in less well-trained subjects.

Following EIMD, the inflammatory response is generally accepted as part of the post-exercise recovery process and it has been previously documented that eccentric exercise with an endurance component produces a robust cytokine response (Sayers and Hubal 2008, p43). However, whether the post-exercise cytokine response is actually associated with a fully-developed inflammatory response has been questioned. Malm et al. (2004) found that, although there is an increase in cytokines following eccentric exercise, this does not indicate skeletal muscle inflammation. Instead the relationship between cytokines, growth factors and hormones post-exercise may be more closely related to the regeneration and adaptation of human skeletal muscle rather than the inflammatory process (Malm et al. 2004). Ostrowski et al. (1999) suggested that the magnitude and duration of the inflammatory response following endurance exercise is restricted; cytokines are produced locally and are rapidly cleared from circulation following exercise. Previous studies demonstrate that IL-6, IL-1 β and TNF- α reach their peak and are then rapidly removed from the circulation possibly within the first

hour following exercise, much quicker than would be expected of a traditional inflammatory response (Ispirlidis et al. 2008; Montgomery et al. 2008a; Ostrowski et al. 1999; Ostrowski et al. 1998; Peake et al. 2008). IL-1 β and TNF- α have been shown to increase only modestly post-marathon but decrease by 2 h (Ostrowski et al. 1999; Ostrowski et al. 1998). This was not the case in the present study, as there was no main effect for time observed for either IL-1 β or TNF- α following intermittent exercise.

Endurance exercise has been shown to bring about an increase in IL-6 levels immediately after a marathon (128-fold increase), after 60 min of treadmill running (10-fold increase) and following 120 min of cycling (12-fold increase), with values declining dramatically by 1-2 h post-exercise (Cox et al. 2007; Ostrowski et al. 1999; Ostrowski et al. 1998; Peake et al. 2008). Intermittent exercise has also been shown to increase IL-6 with 3-4-fold elevations reported immediately after basketball (Montgomery et al. 2008a) and 4-fold elevations immediately following soccer (Ispirlidis et al. 2008). This is greater than in the present study where IL-6 levels were only 1.5-fold above baseline values at 1 h post-LIST. The baseline IL-6 levels in the present study, however, were higher than reported elsewhere (Cox et al. 2007; Ispirlidis et al. 2008; Montgomery et al. 2008a; Ostrowski et al. 1998) and may have influenced the magnitude of the post-exercise increase observed. This is likely due to the pre-exercise snack that was ingested prior to the LIST protocol which delivered 1.2g.kg BM⁻¹ carbohydrate 2.5 h prior to exercise. Whilst this was intended to ensure that pre-exercise glycogen stores were similar between the two trials, it may actually explain the elevated IL-6 levels at baseline as glycogen ingestion has previously been shown to cause an increased in baseline pro-inflammatory factors (Aljada et al. 2006).

Depending on the trigger for production, it is possible that the post-exercise peak of IL-6 in the present study occurred either earlier than 1 h post-exercise, or closer to 8 h post-exercise which has been reported previously (Miles et al. 2007a; Miles et al. 2007b). The two independent pathways of IL-6 production in response to exercise, as specified by Miles et al. (2007b), suggest that IL-6 which is produced as a normal response to exercise peaks during or immediately after exercise and declines very quickly following exercise. In contrast, the IL-6 peak in response to muscle damage may be

smaller, occurring hours after the completion of exercise (Miles et al. 2007b). Although the IL-6 peak observed in the present study was small and not significant, a significant elevation of CRP at 24 h post-exercise in both trials was observed. Given that IL-6 is a trigger for CRP production (Pedersen et al. 1998) it is possible that, although not detected in the present study, a significant increase in IL-6 may have occurred at some stage throughout the recovery period. However, given the timing of sampling in the present study, the magnitude of the IL-6 response, and any impact that the potential circulatory benefits of CG had on this, remains unclear.

The LIST exercise protocol brought about some changes at the cellular and muscular levels in the post-exercise period. CK increased significantly and peaked 24 h after the conclusion of exercise under both conditions suggesting cell membrane permeability had been disturbed, but there was no impact of the garment worn. In addition there is evidence that muscle function was reduced at 1 h after exercise when the 5CMJ was performed in the present study. However, similar to the impact on CK, the lower body CG worn for 24 h post-exercise in the present study did not enhance the recovery of muscle function in the lower limbs. Several previous studies investigating the use of lower body garments on recovery, have found similar results with regards to CK activity (Davies et al. 2009; Duffield et al. 2010) and recovery of muscle function (Davies et al. 2009; Duffield et al. 2010; French et al. 2008; Montgomery et al. 2008b). Kraemer et al. (2010), however, found that a whole body compression garment reduced CK values by half at 24 h post-exercise in men and women after an upper and lower body resistance workout, and Kraemer et al. (2001) reported reduced CK values after subjects wore a compression sleeve over the arm for 5 days following eccentric exercise. Both studies reported that recovery of upper body muscle function was significantly improved after wearing compression compared with control conditions, however there was no impact on the lower body measures of muscle function when whole body compression was worn (Kraemer et al. 2010). This may be because eccentric exercise of the arm has been shown to induce much larger increases in CK activity and have a greater impact on muscle function compared to lower body exercise of the same relative intensity (Jamurtus et al. 2005). As such, the effect on CK activity and muscle function reported by Kraemer et al. (2001) when an arm sleeve was worn, and Kraemer et al. (2010) when a

whole body garment was worn, may be primarily due to the specific characteristics of the upper body response and may be why the same outcome was not evident in the present or previous studies investigating lower body garments.

In contrast to blood and muscle function results, the perceived response of subjects in the present study suggests that there may be a psychological benefit of wearing the garment in the post exercise period. Muscle soreness was rated higher in CON throughout the entire trial period and peaked at 1 h post-exercise in both conditions. This is much earlier than the observed peak in CK activity at 24 h post exercise in the present study. The poor relationship between plasma CK activity and muscle soreness has previously been reported with suggestion that the muscle soreness commonly associated with EIMD may not directly reflect the magnitude of damage to the muscle itself (Malm et al. 2004; Nosaka 2008, p66). Malm et al. (2004) provided evidence that damage to the connective tissue rather than the muscle itself may be more likely associated with delayed onset muscle soreness following EIMD, and furthermore, the increase in CK activity is not related to muscle inflammation post-exercise. This may explain the evident dissociation between muscle soreness, plasma CK activity and changes in muscle function observed in the current and previous research (Nosaka et al. 2002). Although muscle soreness may not specifically reflect the magnitude of exercise-induced muscle damage, it does provide further information regarding the state of the muscle environment (Nosaka et al. 2002) and likely contributes to the athlete's perceived readiness for exercise.

This was certainly the case in this study whereby perceived recovery was significantly greater at 24 and 48 h after exercise in both conditions. Perceived recovery responses were only collected in the post-exercise period and although it is unclear as to which treatment was superior, subjects always felt better recovered when CG were worn. One possible explanation for this may be that the garments felt comfortable to wear and the subjective response of the subjects reflected this notion. In addition, compression garments have previously been found to reduce muscle oscillation during jumping (Doan et al. 2003) and as such, it is possible that a reduction in the displacement of the functioning muscles may attenuate the sensation of pain and stiffness following EIMD. This may suggest that by providing

mechanical stability to exhausted limbs, compression garments enhance the functionality of the connective tissue following EIMD, however we have no specific data to support this notion.

Conclusions

Typical indicators of EIMD including muscle soreness, loss of muscle function, elevated CK and cytokine activity were evident in the present study, even though the level of muscle damage appears to have been low and the inflammatory response was subtle. This may in part be due to the training status of our subjects and their high level of adaptation to the exercise stimulus performed, and the timing of the blood sampling which may have precluded complete capture of the cytokine changes and any potential impact of altered circulation. Even so, muscle soreness was reported, mild loss of muscle function was evident and there was some indication of an acute-phase inflammatory response. Based on the perceptual data, subjects always felt better recovered when CG were worn, however, the restoration of muscle function post-exercise and the biomarkers investigated showed no evidence of enhanced physiological recovery. Additional information in the initial hour and between 1 and 24 h post-exercise, may provide further insight into the true cytokine response to the intermittent exercise and the impact, if any, of the garments worn. Given the heavy promotion of compression garments to athletes and the general belief that they are of benefit, any positive psychological aspect of wearing the garments should be viewed with caution. As such, in the absence of a completely blinded control trial, the potential for an overestimation of the treatment effectiveness should also be considered. Apart from the perceived response of subjects, the biochemical and physical responses observed in this study provide no evidence of a benefit on the recovery process of highly-trained team-sport athletes wearing lower body CG for 24 h following exercise.

Acknowledgements

The authors would like to acknowledge the cooperation of the dedicated subjects involved in the study. We also thank 2XU Compression Australia for providing the compression garments for the research, Nicholas Gant for providing the LIST protocol and Dr. John Wentworth from the Walter and

Eliza Hall Institute for assistance with blood analyses. This project was funded by the National Elite Sports Council Discretionary Research Fund, Australian Sports Commission.

Conflict of Interest

The authors acknowledge that sponsorship agreements exist with the Victorian and Australian Institutes of Sport and 2XU Compression, however, in no way did this influence the results of this study.

Ethical Standards

The experiments carried out in this study comply with the current laws of Australia.

References

- Aljada A, Friedman J, Ghanim H (2006) Glucose ingestion induces an increase in intranuclear nuclear factor kappaB, a fall in cellular inhibitor kappaB, and an increase in tumour necrosis factor alpha messenger RNA by mononuclear cells in healthy human subjects. *Metabolism* 55: 1177-1185
- Armstrong RB, Warren GL, Warren JA (1991) Mechanisms of exercise-induced muscle fibre injury. *Sports Med* 12: 184-207
- Barnett A (2006) Using recovery modalities between training sessions in elite athletes. Does it Help? *Sports Med* 36: 781-796
- Bishop P, Jones E, Woods AK (2008) Recovery from training: a brief review. *J of Strength & Cond Res* 22: 1015-1024
- Borg G (1970) Perceived exertion as an indicator of somatic stress. 2–3:92–98. *Scandinavian Jorنال of Rehabilitation Medicine* 2: 92-98
- Connolly DAJ, Sayers SP, McHugh MP (2003) Treatment and prevention of delayed onset muscle soreness. *J Strength Cond Res* 17: 197-208
- Cormack SJ, Newton RU, McGuigan MR, Doyle TLA (2008) Reliability of measures obtained during single and repeated countermovement jumps. *Int J of Sports Physiol and Perf* 3: 131-144
- Cox AJ, Pyne DB, Saunders PU, Callister R, Gleeson M (2007) Cytokine response to treadmill running in healthy and illness-prone athletes. *Med Sci Sports Exerc* 39: 1918-1926
- Davies V, Thompson KG, Cooper SM (2009) The effects of compression garments on recovery. *J Strength Cond Res* 23: 1786-1794
- Doan BK, Kwon Y, Newton R, Shim J, Popper EM, Rogers RA, Bolt LR, Robertson M, Kraemer WJ (2003) Evaluation of a lower-body compression garment. *J Sports Sci* 21: 601-610
- Duffield R, Cannon J, King M (2010) The effects of compression garments on recovery of muscle performance following high-intensity sprint and plyometric exercise. *J Sci Med Sport* 12: 136-140
- French DN, Thomson KG, Garland SW, Barnes CA, Portas MD, Hood PE, Wilkes G (2008) The effects of contrast bathing and compression therapy on muscular performance *Med Sci Sports Exerc* 40: 1297-1306
- Holford CP (1976) Graded compression for preventing deep venous thrombosis. *BMJ* 2: 969-970
- Hopkins W, Schabert E, Hawley J (2001) Reliability of power in physical performance tests. *Sports Med* 31: 211-234
- Hopkins WG (2002) A scale of magnitudes for effect statistics. A new view of statistics, <http://www.sportsci.org/resource/stats/index.html>

Horner J, Fernandes e Fernandes J, Nicolaides AN (1980) Value of graduated compression stockings in deep venous insufficiency. *BMJ* Mar 22: 820-821

Ispirlidis I, Fatouros I, Jamurtas A, Nikolaidis MG, I M, Douroudos I, Margonis K, Chatzinikolaou A, Kalistratos E, Katrabasas I, Alexiou V, Taxildaris K (2008) Time-course of changes in inflammatory and performance responses following a soccer game. *Clin J Sport Med* 18: 423-431

Jamurtas AZ, Theocaris V, Tofas T, Tsiokanos A, Yfanti C, Paschalis V, Koutedakis Y, Nosaka K (2005) Comparison between leg and arm eccentric exercises of the same relative intensity on indices of muscle damage. *Eur J Appl Physiol* 95: 179-185

Kraemer WJ, Bush JA, Bauer JA, Triplett-McBride NT, Paxton NJ, Clemson A, Koziris LP, Mangino LC, Fry AC, Newton RU (1996) Influence of compression garments on vertical jump performance in NCAA Division 1 Volleyball players. *J Strength Cond Res* 10: 180-183

Kraemer WJ, Bush JA, Wickham RB, Denegar CR, Gomez AL, Gotshalt LA, Duncan ND, Volek JS, Putukian M, Sebastianelli WJ (2001) Influence of compression therapy on symptoms following soft tissue injury from maximal eccentric exercise. *J Orthop Sports Phys Ther* 31: 282-290

Kraemer WJ, Flanagan SD, Comstock BA, Fragala MS, Earp JE, Dunn-Lewis C, Ho JY, Thomas GA, Solomon-Hill G, Penwell ZR, Powell MD, Wolf MR, Volek JS, Denegar CR, Maresh CM (2010) Effects of a whole body compression garment on markers of recovery after a heavy resistance workout in men and women. *J Strength Cond Res* 24: 804-814

MacRae BA, Cotter JD, Laing RM (2011) Compression Garments and Exercise. *Garment Considerations, Physiology and Performance. Sports Med* 41: 815-843

Malm C, Nyberg P, Engstrom M, Sjodin B, Lenkei R, Ekblom B, Lundberg I (2000) Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *J Physiol* 529: 243-262

Malm C, Sjodin B, Sjoberg B, Lenkei R, Renstrom P, Lundberg I, Ekblom B (2004) Leukocytes, cytokines, growth factors and hormones in human skeletal muscle and blood after uphill or downhill running. *J Physiol* 556: 983-1000

Mattacola C, Perrin D, Gansneder B, Allen J, Mickey C (1997) A comparison of visual analogue and graphic rating scales for assessing pain following delayed onset muscle soreness. *J Sport Rehab* 6: 38-46

McIntyre DL, Reid WD, McKenzie DC (1995) Delayed muscle soreness: The inflammatory response to muscle injury and its clinical implications. *Sports Med* 20: 25-40

Miles MP, Andring JM, Pearson SD, Gordon LK, Kasper C, Depner CM, Kidd JR (2007a) Diurnal variation, response to eccentric exercise, and association of inflammatory mediators with muscle damage variables. *J Appl Physiol* 104: 451-458

Miles MP, Pearson SD, Andring JM, Kidd JR, Volpe SL (2007b) Effect of carbohydrate intake during recovery from eccentric exercise on interleukin-6 and muscle-damage markers. *Int J Sports Nut Exer Metab* 17: 507-520

Montgomery PG, Pyne DB, Cox AJ, Hopkins WG, Minahan CL, Hunt PH (2008a) Muscle damage, inflammation, and recovery interventions during a 3-day basketball tournament. *Eur J Sport Sci* 8: 241-250

Montgomery PG, Pyne DB, Hopkins WG, Dorman JC, Cook K, Minahan CL (2008b) The effect of recovery strategies on physical performance and cumulative fatigue in competitive basketball. *J Sports Sci* 26: 1-11

Nicholas CW, Nuttall FE, Williams C (2000) The Loughborough Intermittent Shuttle Test: A field test that stimulates the activity pattern of soccer. *J Sports Sci* 18: 97-104

Nosaka K (2008) Muscle soreness and damage and the repeated-bout effect. In: Tiidus PM (ed) *Skeletal muscle damage and repair*. Human Kinetics, Champaign, pp. 59-76

Nosaka K, Newton M, Sacco P (2002) Delayed-onset muscle soreness does not reflect the magnitude of eccentric exercise-induced muscle damage. *Scand J Med Sci Sports*: 337-346

Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK (1999) Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol* 515: 287-291

Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen BK (1998) Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J Physiol* 508: 949-953

Partsch H, Clark M, Bassez S, Benigni JP, Becker F, Blazek V, Caprini J, Cornu-Thenard A, Hafner J, Flour M, Junger M, Moffatt C, Neumann M (2006) Measurement of lower leg compression in vivo: Recommendations for the performance of measurements of interface pressure stiffness. *Dermatol Surg* 32: 224-233

Peake J, Peiffer JJ, Abbiss CR, Nosaka K, Okutsu M, Laursen PB, Suzuki K (2008) Body temperature and its effect on leukocyte mobilization, cytokines and markers of neutrophil activation during and after exercise. *Eur J Appl Physiol* 102: 391-401

Pedersen B, Ostrowski K, Rohde T, Bruunsgaard H (1998) The cytokine response to strenuous exercise. *Can J Physiol Pharmacol* 76: 505-511

Petersen A, Pedersen BK (2005) The anti-inflammatory effects of exercise. *J Appl Physiol* 98: 1154-1162

Ramsbottom R, Brewer J, Williams C (1988) A progressive shuttle run test to estimate maximal oxygen uptake. *British J Sports Med* 22: 141-144

Sayers SP, Hubal MJ (2008) Histological, chemical, and functional manifestations of muscle damage. In: Tiidus PM (ed) *Skeletal Muscle Damage and Repair*. Human Kinetics, Champaign, pp. 37-48

Sheppard J, Doyle T (2008) Increasing compliance to instructions in the squat jump. *J Strength Cond Res* 22: 648-651

Sigel B, Edelstein AL, Felix WR, Memhardt CR (1973) Compression of the deep venous system of the lower leg during inactive recumbency. *Arch Surg* 106: 38-43

Sigel B, Edelstein AL, Savitch L (1975) Type of Compression for Reducing Venous Stasis. Arch Surg 110: 171-175

Strachan AF, Noakes TD, Kotzenberg G, Nel AE, De Beer FC (1984) C Reactive protein concentrations during long distance running. Br Med J 289: 1249-1251

Tanner R, Fuller K, Ross M (2010) Evaluation of three portable lactate analysers: lactate pro, lactate scout and lactate plus. Eur J Appl Physiol 109: 551-559

Thompson D, Nicholas CW, Williams C (1999) Muscular soreness following prolonged intermittent shuttle running. J Sports Sci 17: 387-395

Zajkowski PJ, Proctor MC, Wakefield MD, Bloom J, Blessing B, Greenfield LJ (2002) Compression stockings and venous function. Arch Surg 137: 1064-1068

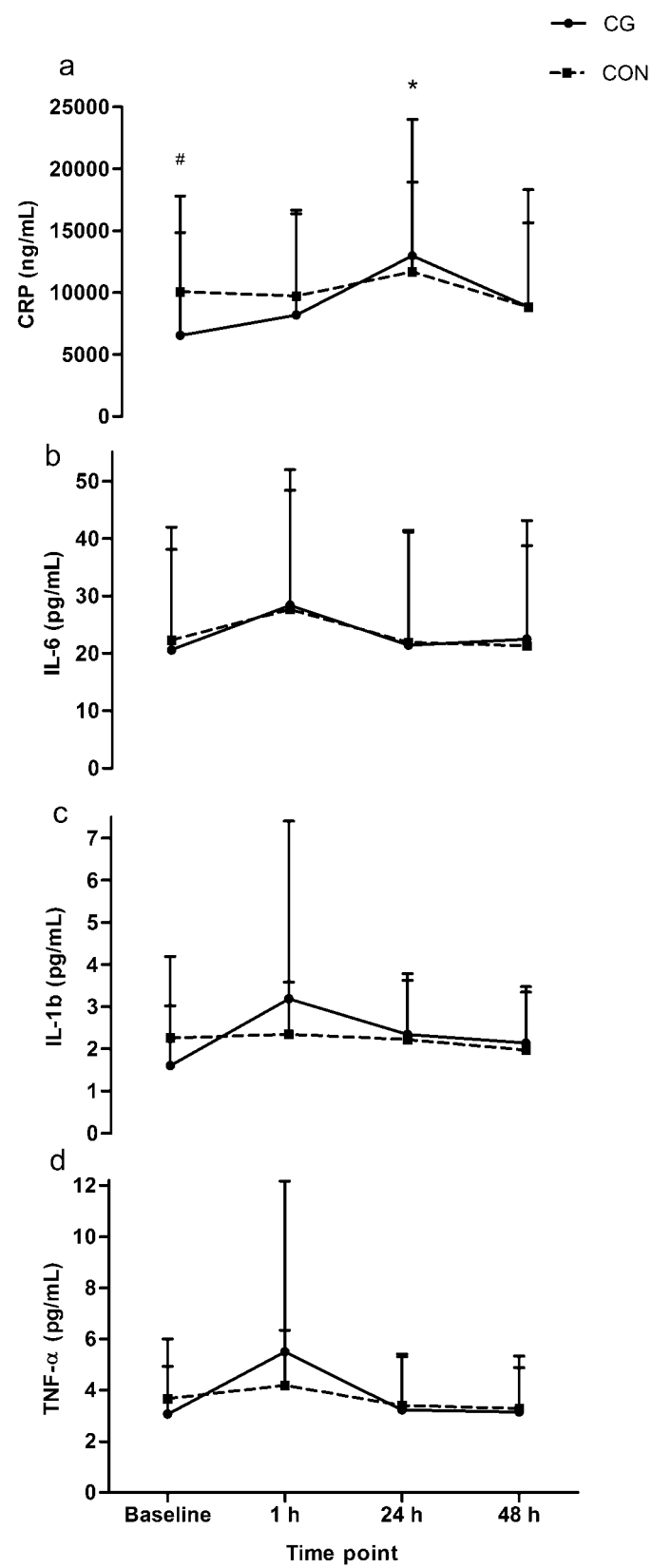
Figure Captions

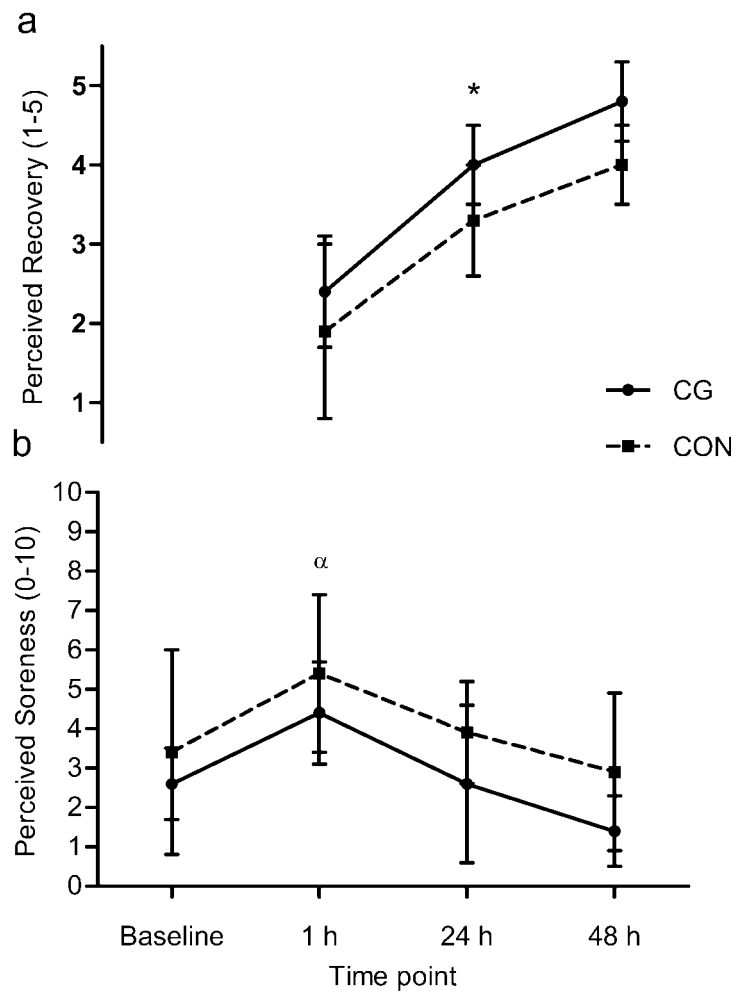
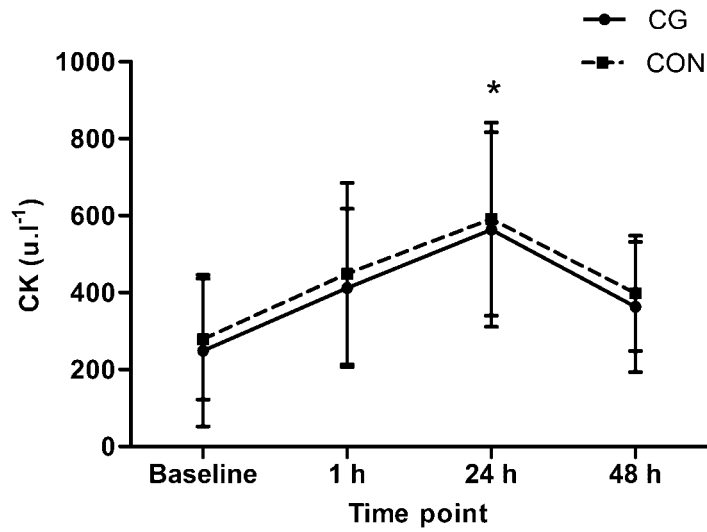
Fig1 Experimental design and study timeline

Fig2 Cytokine and Protein response following intermittent exercise: CRP (A), IL-6 (B), IL-1 β (C), TNF- α (D). *P < 0.05 from baseline, 1h and 24h for CG, and, different from 48h for CON. #P < 0.05 CG different from CON. Values are presented as mean $\pm$ SD.

Fig3 CK concentration. *P<0.01 from all other time points for CG and CON. Values are presented as mean $\pm$ SD.

Fig4 Perceived recovery (A) from strongly disagree (1) to strongly agree (5) at 1 h, 24 h and 48 h post-exercise, and Rating of Perceived Soreness (B) from no soreness (0) to extreme soreness (10) at baseline, 1 h , 24 h and 48 h post-exercise. *P<0.01 from 1h and 48h for CG and CON. α P<0.05 from all other time points for CG and CON. Main effect for treatment observed for both Perceived recovery (P<0.01) and RPS (P=0.053). Values are presented as mean $\pm$ SD.





Time						
Post-LIST	0 min	4 min	8 min	12 min	20 min	30min
CG	2.9 (1.4)	2.4 (0.8)	2.0 (0.7)	1.6 (0.6)	1.3 (0.5)	1.0 (0.2)
CON	2.7 (0.7)	1.8 (0.6)	1.5 (0.5)	1.3 (0.4)	1.0 (0.2)	0.9 (0.1)

Table 1 Blood lactate concentration in the 30 min following exercise (n=8). CG worn from 4 min post-exercise until 24 h post-exercise.
Values are presented as mean (SD).

	CG					
	Baseline	1 h	24 h	48 h	Baseline	1 h
5CMJ Mean Power (w)	3711 (790)	3576 (680)#	3610 (706)	3666 (668) ^a	3648 (776)	3541 (76)
5CMJ Mean Force (N)	2000 (421)	1923 (361)^	1941 (356)	2016 (431)	2032 (420)	1950 (40)
SJ Peak Force (N)	1876 (341)	1838 (340)	1834 (325)	1834 (331)	1848 (320)	1829 (36)

Table 2 Tests of muscle function (5CMJ and SJ) at baseline, 1 h, 24 h and 48 h following exercise.

Values are presented as mean (SD).

* P<0.05 from CG

P<0.05 from baseline

^a P<0.01 from 1h and 24h

^ P<0.05 from baseline, 24h and 48h