

Research article

The Post-Exercise Inflammatory Response to Repeated-Sprint Running in Hypoxia

Jaime Morrison¹, Brianna Larsen^{1,2,3}, Amanda J. Cox⁴ and Clare Minahan¹✉

¹ Griffith Sports Physiology and Performance, School of Allied Health Sciences, Griffith University, Gold Coast, Queensland, Australia; ² Menzies Health Institute Queensland, Griffith University, Gold Coast, Queensland, Australia;

³ Queensland Academy of Sport, Nathan, Queensland, Australia; ⁴ Griffith University, School of Medical Sciences, Griffith University, Gold Coast, Queensland, Australia

Abstract

This study investigated the acute inflammatory response to a repeat-sprint training session in hypoxia. Eleven amateur team-sport athletes completed a repeat-sprint training in hypoxia (RSH) protocol (4 sets of 4x4-s running sprints) in both normoxia and normobaric hypoxia (F_iO₂ 0.145 to simulate an altitude of 3000 m) on separate days. Participants provided venous blood samples prior to (PRE), immediately after (POST), and 3 h after (3 h) completion of the protocol, and capillary blood lactate samples were taken upon arrival, at PRE, and at POST. Distance was recorded for each sprint. Venous blood samples were analysed to determine plasma concentrations of cytokines IL-1 β , IL-1ra, IL-6, IL-8, IL-10, and TNF α . There was no interaction or main effect of condition for any cytokine ($p > 0.05$). However, time effects indicated that IL-10 was decreased by an average of 19% across the two experimental trials at 3 h compared to POST ($p = 0.04$), IL-6 increased by 55% from PRE to POST ($p = 0.03$) then decreased by 43% from POST to 3 h ($p = 0.02$), and IL-8 decreased by 30% from PRE to POST ($p = 0.04$) and was further reduced at 3 h compared to POST (by an additional 23%; $p = 0.02$). A time \times condition interaction ($p = 0.03$) indicated that lactate was higher in hypoxia. There was no interaction effect or effect of condition for sprint distance ($p > 0.05$). These results suggest that team-sport athletes can perform a RSH session without increasing inflammation when compared to the same training session performed in normoxia.

Key words: Inflammation, cytokines, performance, altitude training.

Introduction

A comprehensive review focusing specifically on altitude training for team-sports suggests that repeat-sprint training performed in hypoxia (RSH) may be a promising avenue for improving match-related performance (Faiss et al., 2013a). Indeed, a number of studies across a range of sports (e.g. football, rugby, lacrosse) have reported performance benefits following a RSH protocol (Brocherie et al., 2015; Faiss et al., 2013b; Galvin et al., 2013; Hamlin et al., 2017; Kasai et al., 2015), and a recent meta-analysis found that RSH induces greater improvements in mean repeated-sprint performance during sea-level repeated sprinting than the same training performed in normoxia (Brocherie et al., 2017). Conversely, select studies have not observed performance benefits following RSH (Goods et al., 2015; Montero and Lundby, 2017). Nevertheless, given the encouraging findings reported in some of the literature and the growing awareness of RSH as a training method

(Girard et al., 2017), it is likely that RSH will be increasingly utilised within the team-sport setting.

To date, performance factors (e.g., power output, repeat-sprint ability) have been the main variable of interest in the RSH literature. However, relatively little is known regarding the effect of RSH on inflammatory markers, despite the fact that studies of the hypoxia signalling pathway consistently indicate that hypoxia induces inflammation (see review by Eltzschig and Carmeliet, 2011). This may be an important consideration for team-sport athletes given that inflammatory status has the potential to influence recovery and subsequent training performance (Davis et al., 2007). Indeed, athletes are already more susceptible to illness and infection during periods of intense training or competition (Gleeson, 2007), and there is recent evidence to suggest that a RSH program may alter immune functioning (Born et al., 2016). While select studies have investigated the acute inflammatory response following exercise in hypoxia (e.g., Badenhorst et al., 2014; Govus et al., 2014; Sumi et al., 2017), to the current authors' knowledge only one previous study has measured an inflammatory biomarker (interleukin-6; IL-6) after the completion of a RSH protocol (Goods et al., 2016). Goods and colleagues (2016) had ten trained male team-sport athletes perform a repeat-sprint session in both simulated altitude (3000 m) and at sea level. This study reported increases in IL-6 concentrations in response to the sprint protocol in both conditions, but a large effect size ($d = 0.80$) indicated a trend for higher IL-6 concentrations one-hour post-exercise in the hypoxic environment. However, the authors noted that the IL-6 response elicited by the protocol was moderate, likely due to the use of cycling as the exercise modality (Nieman et al., 1998). Indeed, a reduced IL-6 response has been observed when comparing cycling exercise to running (Nieman et al., 1998), which is more specific to most team sports. Moreover, this previous study is limited by its sole use of IL-6 as a marker of inflammation; it is well understood that IL-6 has both pro-inflammatory and anti-inflammatory properties, with elevated post-exercise IL-6 concentrations playing a role in inhibiting pro-inflammatory (i.e., tumour necrosis factor- α ; TNF- α) cytokines and facilitating anti-inflammatory cytokine (i.e., IL-1ra, IL-10) production (Petersen and Pedersen, 2005). Thus, the results obtained by Goods et al (2016) could be indicative of either an enhanced inflammatory status following RSH or a positive adaptive response to the training stimulus.

The present study aimed to assess the acute

inflammatory response to one RSH session performed on a non-motorised treadmill when compared to the same exercise performed at sea level. Specifically, TNF α , IL-1 β , IL-1ra, IL-6, IL-8, and IL-10 were measured before and after the performance of a team-sport specific RSH session. It was hypothesised based on the (albeit limited) existing literature that the RSH session would illicit a greater inflammatory response when compared to the same session performed in normoxic conditions.

Methods

Participants

Eleven amateur team-sport athletes (seven Australian rules footballers, three soccer players and a touch football player) volunteered to participate in the present study and provided their written informed consent. The Griffith University Human Research Ethics Committee approved all procedures used in the study (AHS/72/14/HREC). All participants had competed for a minimum of two consecutive years in their respective sport immediately prior to participation in the study, trained for a mean (\pm SD) of 152 ± 35 min \cdot week $^{-1}$, and competed in their sport for a mean (\pm SD) of 108 ± 17 min \cdot week $^{-1}$. Familiarisation commenced 3-4 weeks after each participant's last competitive match for the season (i.e., at the beginning of their off-season). The physical characteristics of the participants are presented in Table 1.

Table 1. Participants' anthropometric characteristics (n = 11). Values are means (\pm SD).

| | |
|---|------------|
| Age (yr) | 24.2 (4.7) |
| Body mass (kg) | 78.7 (6.2) |
| Height (m) | 1.81 (.03) |
| Body mass index (kg \cdot m $^{-2}$) | 24.3 (1.9) |

Procedures

All participants performed a repeat-sprint running test (i.e., the RSR $_{444}$; Morrison et al. (2015)) on two occasions. The RSR $_{444}$ consisted of sixteen (four sets of four) 4-s sprints separated by 26 s (and 2 min 26 s between sets) of passive recovery in a standing position, performed in a commercial normobaric hypoxic chamber (Synergy Physical Conditioning Systems, Yatala, Australia). During one trial participants breathed air with an inspired oxygen fraction (FiO $_2$) of 0.209, and in the other trial a FiO $_2$ of 0.145 was used to simulate an altitude of 3000 m (i.e., hypoxia). This FiO $_2$ at sea level creates a PiO $_2$ of approximately 110 mmHg. The study followed a crossover design with five of the participants performing the RSR $_{444}$ in normoxia and then in hypoxia 7-10 d later at the same time of day, whereas the remaining participants (n = 6) performed the RSR $_{444}$ tests in the reverse order with the same number of days between trials. Participants were required to: 1) refrain from caffeine consumption on testing days; 2) refrain from alcohol consumption 24 h prior to testing; 3) refrain from performing any exercise of moderate intensity or greater for 72 h prior to testing, as well as refrain from performing any exercise between POST and 3h blood samples; 4) consume only food/beverages that they would ordinarily consume during the 24 h prior to testing; 5) keep a detailed food diary so that food/beverage consumed prior

to trial 1 could be replicated for trial 2, and; 6) consume only water in the 4 h prior to testing. Water was consumed ad libitum between POST and 3h, and no food was consumed during this time. The hypoxic environment was created via the extraction of oxygen from air that was subsequently pumped into the chamber. Oxygen concentration was monitored using a gas detector (KB-501, Kingsby Electronics,) which utilises an electrochemical sensor. Relative humidity and temperature were maintained between 45-50% and 19-21°C, respectively. Tests were performed on a non-motorised treadmill (Curve 3.0, Woodway, Waukesha, Wisconsin, USA). While we acknowledge that a passive recovery does not replicate team-sport movement demands, the protocol was designed to allow the maintenance of speed, acceleration, and total work (i.e., distance), as well as the rotation of up to four athletes on one treadmill (thus improving team-sport training efficiency).

Distance (m) data were obtained via a tachometer mounted on the treadmill drum and recorded using Pacer Performance System software (Fitness Technology, Adelaide, Australia). Earlobe capillary blood lactate concentration ([La $^-$]) measurements were obtained using a handheld lactate analyser (Lactate Pro, Arkray Factory Inc., KDK Corporation, Shiga, Japan). Samples were taken upon arrival to the laboratory (baseline; BL) in normoxic conditions, as well as immediately prior to the start of the RSR $_{444}$ (PRE) and immediately after sets 1, 2, 3, and 4 in the environmental condition for that testing day. Measurements of arterial oxygen saturation were made PRE and immediately after sets 1, 2, 3, and 4 using a portable pulse oximeter (Oxive Tech, 300CSE, Beijing Choice Electronic Technology Co., Ltd. Beijing, China) in the environmental condition.

Blood sampling

Prior to the commencement of the RSR $_{444}$, venous blood was sampled from an antecubital vein (PRE) with the participant lying in a supine position with elbow extended, after lying supine for 6 min to account for postural shifts in plasma volume. Subsequent to this, participants were required to complete a standardised warm-up, identical to that described in detail elsewhere (Morrison et al., 2015), with the addition of two 4-s all-out sprints separated by 24 s of recovery, following dynamic stretching. Participants were provided with strong verbal encouragement during all sprints. No feedback was given regarding the speed or distance achieved during each sprint. Subsequent to completion of the final sprint, participants rested in a seated position for 3 min, relocated to the blood sampling room and laid supine for 6 min prior to blood being sampled again (POST). A third blood sample was taken in the same manner 3 h following the completion of the RSR $_{444}$ (3 h).

Blood samples were collected using a 21-gauge needle, deposited into 10 mL EDTA tubes (BD Vacutainer Systems, Plymouth, UK), and stored on ice until being centrifuged at 3000 rev \cdot min $^{-1}$ for 10 min to separate plasma. Plasma was divided into 500 μ L aliquots and stored at -80°C until subsequent analysis. Plasma concentrations of IL-1 β , IL-1ra, IL-6, IL-8, IL-10, and TNF α were determined using a custom 6-plex cytokine suspension array (Bio-Rad Laboratories Pty Ltd; Hercules, CA, USA)

according to the manufacturer's instructions. Assays were completed using a series of six unique bead sets and samples were diluted 1:3 in the sample diluent provided. Samples from individual participants were analysed in a single assay to avoid interassay variation. The Bio-Plex Suspension Array reader (Bio-Rad Laboratories Pty Ltd; Hercules, CA, USA) was calibrated prior to being used to identify bead subsets and measure fluorescence intensity. A standard curve of cytokine concentration as a function of fluorescence units was constructed using Bio-Plex Manager software (Bio-Rad Laboratories Pty Ltd; Hercules, CA, USA). Recorded fluorescence values were used to determine cytokine concentrations by extrapolation from the respective standard curve.

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics 19 software package. As the data were normally distributed, fully-factorial ANOVA with repeated measures were used to determine the interaction between, or main effect of, condition and time for performance and physiological variables. Least squares difference pairwise comparisons were used to detect the specific site of any significant effect identified. The null hypothesis was rejected at $p < 0.05$.

Results

Performance

There was no difference between conditions in sprint distance (interaction: $F = 1.62$, $p = 0.08$; main effect of condition: $F = 2.45$, $p = 0.15$) across the entire RSR₄₄₄; however, sprint distance deteriorated from 24.92 m to 23.48 m across the 16 sprints (decrease of $\sim 6\%$, $F = 7.92$, $p < 0.01$).

Cytokines

Cytokine data are presented in Table 2, with the exception of IL-6 which is presented in Figure 1. There was no interaction effect ($F = 0.35$, $p = 0.71$), or main effect of time ($F = 0.59$, $p = 0.57$) or condition ($F = 0.21$, $p = 0.66$) observed for TNF α , nor IL-1 β ($F = 0.89$, $p = 0.43$ for interaction; F

$= 0.61$, $p = 0.56$ for main effect of time; and $F = 2.51$, $p = 0.14$ for main effect of condition) or IL-1ra ($F = 0.73$, $p = 0.49$ for interaction; $F = 2.73$, $p = 0.09$ for main effect of time; and $F = 3.57$, $p = 0.09$ for main effect of condition). There was no interaction effect ($F = 1.22$, $p = 0.32$) or main effect of condition on IL-10 ($F = 3.43$, $p = 0.09$), but a main effect of time indicated a lower IL-10 at 3 h when compared to the concentrations observed POST ($p = 0.037$, CI 0.08 – 2.13). There was no difference in IL-10 concentrations between PRE and POST ($p = 0.10$, CI -1.89 – 0.19). Similarly, there was no interaction effect ($F = 0.56$, $p = 0.58$) or main effect of condition ($F = 0.03$, $p = 0.87$) observed for IL-6; however, a main effect of time showed that IL-6 increased from PRE to POST ($p = 0.03$, CI -1.12 – 0.08), and then decreased from POST to 3 h ($p = 0.02$, CI 0.14 – 1.40) to a value not different from PRE ($p = 0.25$, CI -0.12 – 0.41). There was also no interaction effect ($F = 0.45$, $p = 0.64$) or main effect of condition ($F = 0.07$, $p = 0.79$) for IL-8, but a main effect of time showed that it decreased from PRE to POST ($p = 0.04$, CI 0.13 – 3.26), and was further reduced at 3 h to a value lower than that observed at POST ($p = 0.02$, CI 0.43 – 1.40).

Lactate and SpO₂ data are presented in Table 3.

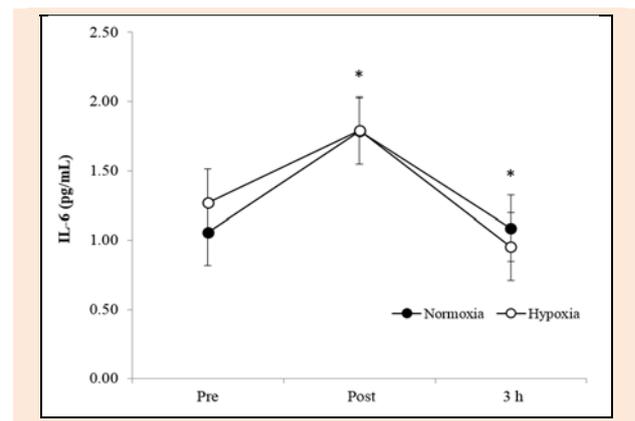


Figure 1. IL-6 concentrations pre, post, and 3 h-post the repeated-sprint running protocol in normoxia and hypoxia. * significantly different ($p < 0.05$) to previous time point.

Table 2. Cytokine data pre, post, and 3 h-post the repeated-sprint running protocol in normoxia and hypoxia. Data are means (\pm SD).

| | Normoxia | | | Hypoxia | | |
|---------------|--------------|---------------|---------------|---------------|---------------|---------------|
| | PRE | POST | 3 h | PRE | POST | 3 h |
| IL-1 β | .44 (.22) | .49 (.28) | .44 (.21) | .63 (.58) | .50 (.21) | .51 (.21) |
| IL-1ra | 24.61 (9.99) | 28.73 (11.67) | 33.69 (20.10) | 31.88 (10.89) | 37.81 (14.79) | 34.73 (20.82) |
| IL-8 | 5.66 (4.38) | 3.67 (1.43) * | 3.22 (1.48) * | 5.38 (1.68) | 4.00 (1.49) * | 2.62 (1.32) * |
| IL-10 | 4.16 (2.09) | 4.68 (2.27) | 3.96 (1.54) * | 5.43 (3.05) | 6.61 (5.32) | 5.12 (3.17) * |
| TNF- α | 4.72 (1.13) | 4.55 (.95) | 4.56 (1.59) | 4.56 (1.59) | 4.72 (1.12) | 4.17 (1.40) |

* Significantly lower than previous time point.

Table 3. Blood lactate concentration [La-] and arterial oxygen saturation (SpO₂) measured in team-sport athletes before, during, and immediately after performing the RSR₄₄₄ (4 sets of 4, 4-s sprints) in normoxic (20.9%) and hypoxic (14.5%) conditions. Data are means (\pm SD).

| Variable | Condition | Baseline | Pre-test | Post-set 1 | Post-set 2 | Post-set 3 | Post-set 4 |
|-------------------------------|-----------|-----------|--------------|--------------|--------------|--------------|--------------|
| [La-] mmol·L ⁻¹ | Normoxia | 1.1(.3) | 2.9 (1.1) | 6.7 (1.5) | 9.1(2.3) | 10.1(2.9) | 10.5 (2.9) |
| | Hypoxia | 1.0 (.3) | 3.2 (1.3) | 8.0 (2.1) * | 10.5 (3.0) | 11.2 (2.7) | 11.5 (2.6) |
| SpO ₂ (%) | Normoxia | 98.0 (.2) | 97.7 (1.0) | 95.7 (2.9) | 95.7 (2.0) | 94.8 (2.0) | 94.9 (1.2) |
| | Hypoxia | 97.9 (.2) | 90.9 (1.4) * | 83.6 (3.0) * | 81.1 (3.4) * | 79.7 (3.7) * | 79.1 (4.0) * |

* Different from normoxia ($p < 0.05$). Baseline measurements were obtained in normoxic conditions before the warm-up. Pre-test measurements were performed in the environmental condition, after the warm-up and immediately prior to the commencement of the RSR₄₄₄.

Discussion

This study primarily aimed to investigate the inflammatory response after performing a repeat-sprint running protocol under both normoxic and hypoxic conditions. There was a time effect observed for certain cytokines (i.e., IL-6, IL-8, IL-10) which indicates a response to the exercise stimulus; however, hypoxia did not alter the pro- or anti-inflammatory cytokine response, which is in opposition to the proposed hypothesis. This finding suggests that performing RSH elicits a comparable inflammatory response to RSN. However, blood lactate was higher, and blood oxygen saturation was lower, in RSH when compared to RSN.

Although a handful of studies have investigated the inflammatory response to performing exercise under hypoxic conditions (e.g., Badenhorst et al., 2014; Govus et al., 2014; Sumi et al., 2017), only one previous study (Goods et al., 2016) has measured an inflammatory biomarker (i.e., IL-6) after completion of a RSH protocol. Goods and colleagues (2016) reported an increase in IL-6 concentrations both immediately and 60-min post exercise, with a trend for higher IL-6 in the hypoxic condition 60-min after completion of the exercise protocol. In line with these findings (Goods et al., 2016) and those reported by the previous interval training studies conducted in hypoxia (Badenhorst et al., 2014; Govus et al., 2014; Sumi et al., 2017), the current study observed an increase in IL-6 concentrations at the cessation of exercise when compared to baseline levels. However, unlike the prior RSH study (Goods et al., 2016), no difference was observed in IL-6 concentrations between the hypoxic and normoxic conditions at any time point. Interestingly, only the study by Goods et al (2016) reported a difference in IL-6 concentrations between the hypoxic and normoxic trials; the studies utilising longer intervals (3–4 min) all reported a comparable IL-6 response irrespective of condition (Badenhorst et al., 2014; Govus et al., 2014; Sumi et al., 2017). When measured 3-h post-exercise, IL-6 had returned to pre-exercise levels in the present study, which supports the findings of Govus et al (2014) who observed IL-6 concentrations comparable to baseline levels 3 h after completion of an interval training protocol in hypoxia. Conversely, Goods et al (2016) and Sumi et al (2017) reported elevated IL-6 concentrations (when compared to pre-exercise values) 60 min and 120 min post-exercise, respectively. This disparity is likely due to the different sampling times utilised; differences may have been observed in the present study had the blood been sampled at 60-min or 120-min post exercise as opposed to 3-h post-exercise. However, the fact that IL-6 concentrations returned to baseline levels within 3 h, and that comparable concentrations were observed for both conditions, suggests that performing RSH does not elicit an exacerbated IL-6 response. Interestingly, the magnitude of the increase in IL-6 in the present study was similar to that observed by Goods et al (2016), despite the present study utilising a running protocol which typically induces a greater inflammatory response when compared with cycling exercise (Nieman et al., 1998). Indeed, greater increases in IL-6 concentrations were reported after each of the longer interval-training studies (Badenhorst et al., 2014; Govus et al., 2014; Sumi et al., 2017), all of which

were performed on a treadmill. Thus, the relatively modest increase in IL-6 concentrations (~75%) observed after the current protocol can most likely be attributed to the relatively short duration of the exercise bouts rather than the mode of exercise utilised.

The previous studies investigating the inflammatory response to training under hypoxic conditions have used IL-6 as the sole inflammatory biomarker, which may be problematic when trying to apply the findings given IL-6 exhibits both pro-inflammatory and anti-inflammatory properties (Petersen and Pedersen, 2005). Thus, the current research sought to provide a more nuanced understanding of the inflammatory response to RSH through the inclusion of other key pro-inflammatory (IL-1 β , IL-8, TNF- α) and anti-inflammatory (IL-1ra, and IL-10) cytokines. There was no apparent effect of the exercise protocol (during either condition) on the production of TNF α or IL-1 β . This finding is perhaps unsurprising, as although TNF α and IL-1 β are the first cytokines to be released during sepsis/infection, generally speaking they are not induced in response to an exercise stimulus alone (Pedersen et al., 1998; Petersen and Pedersen, 2005). Interestingly, no change was detected in IL-1ra concentrations across the course of the protocol, which could be considered unusual given that IL-6 was elevated and IL-1ra typically acts as a cytokine inhibitor (Petersen and Pedersen, 2005). Furthermore, there was no difference in the anti-inflammatory cytokine IL-10 between pre- and immediately post exercise; in studies investigating longer exercise durations (e.g., distance running), increased concentrations of IL-10 have typically been reported immediately post-exercise (Nieman et al., 2001; Ostrowski et al., 1999; Peake et al., 2005) in response to dramatic increases in IL-6 (Pedersen, 2000). These results may be explained, at least in part, by the relatively modest increase observed for IL-6 (IL-6 concentrations can be increased up to 100-fold after a marathon, for example; Pedersen, 2000) and the short duration of the exercise protocol, both of which play an important role in determining the extent of the post-exercise cytokine response (Flynn et al., 2007; Petersen and Pedersen, 2005). Finally, the chemokine IL-8 decreased at each time point throughout the protocol. Exercise does not typically increase the concentrations of circulatory IL-8 (Moldoveanu et al., 2001), although there is relatively strong evidence to suggest that IL-8 is released from working skeletal muscle during exercise and acts locally (Frydelund-Larsen et al., 2007). The reason behind the observed decrease in plasma IL-8 from pre- to immediately post-exercise, and again from immediately post-exercise to 3 h post-exercise, is not immediately clear, and thus requires further investigation. However, the addition of hypoxia did not differentially influence the IL-8 response, which was the primary focus of the present research. Indeed, the current findings suggest that the relative risk of a repeat-sprint training session inducing significant inflammation is low regardless of environment, given the protocol did not elicit significant inflammatory responses in either hypoxic or normoxic conditions.

As expected, blood oxygen saturation was lower in the hypoxic trial when compared with the normoxic trial (Goods et al., 2016; West et al., 1962), whereas blood

lactate concentrations were higher. The findings for blood lactate corroborate the findings reported by previous studies performed in hypoxia utilising longer exercise intervals (Sumi et al., 2017) and continuous incremental exercise (Hughson et al., 1995), as well as another RSH study performed on a non-motorised treadmill (Goods et al., 2014). However, they differ from the findings reported by Goods and colleagues (2016) who observed no difference in blood lactate in hypoxia when compared to sea-level during a repeated-sprint cycling protocol. This is likely due to the fact that subjects in the Goods et al (2016) study performed less work in the hypoxic trial, whereas there was no effect of condition on sprint distance observed in the current study. It should also be noted that there is a ~10% (non-significant) difference in pre-test [La] that may reflect slight differences in warm-up intensity or pacing, which may have influenced the overall lactate kinetics throughout the protocol. Nevertheless, the magnitude of the difference in peak blood lactate in the present study was small (10.5 compared to 11.5 mmol/L) and well below the limits typically achieved during repeated-sprint training (Spencer et al., 2005). Thus, the observed differences between the hypoxic and normoxic condition are unlikely to be practically meaningful in terms of the athletes' recovery.

The ability of athletes to maintain sprint distance despite the lower F_iO_2 during RSH in the present study may be explained, at least in part, by the increase in anaerobic energy release that has been suggested to compensate for a reduction in aerobic ATP production during sprinting in hypoxia (Calbet et al., 2003; Girard et al., 2017). Furthermore, in comparison to the Goods et al (2016) study, the present study utilised shorter (4 v 5 s) and fewer (16 v 27) sprints, a lower within-set work-to-rest ratio (1:6 v 1:4), and a passive recovery, all of which are likely to have contributed to the maintenance of sprint distance during RSH when compared with RSN.

Conclusions

There was no difference between environmental conditions for any of the pro- or anti-inflammatory cytokines measured in the present study, which indicates a comparable inflammatory response between RSN and RSH. Furthermore, while IL-6 increased in both trials immediately after the performance of the RSR₄₄₄ test, the increase was very small relative to what has been previously observed after intense endurance exercise, and blood samples collected 3 h post-exercise indicated comparable plasma IL-6 concentrations to those reported at baseline. This suggests that RSH elicits only a modest inflammatory response, and thus, inflammation should not have any negative impact on subsequent training and performance in team-sport athletes performing RSH.

Acknowledgements

This work was supported by Griffith Sports Physiology and Performance. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The study complied with the laws of the country of the authors' affiliation.

References

Badenhorst, C.E., Dawson, B., Goodman, C., Sim, M., Cox, G.R., Gore,

- C.J., Tjalsma, H., Swinkels, D.W. and Peeling, P. (2014) Influence of post-exercise hypoxic exposure on hepcidin response in athletes. *European Journal of Applied Physiology* **114**, 951-959.
- Born, D.-P., Faiss, R., Willis, S.J., Strahler, J., Millet, G.P., Holmberg, H.-C. and Sperlich, B. (2016) Circadian variation of salivary immunoglobulin A, alpha-amylase activity and mood in response to repeated double-poling sprints in hypoxia. *European Journal of Applied Physiology* **116**, 1-10.
- Brocherie, F., Girard, O., Faiss, R. and Millet, G.P. (2015) High-intensity intermittent training in hypoxia: a double-blinded, placebo-controlled field study in youth football players. *The Journal of Strength & Conditioning Research* **29**, 226-237.
- Brocherie, F., Girard, O., Faiss, R. and Millet, G.P. (2017) Effects of repeated-sprint training in hypoxia on sea-level performance: a meta-analysis. *Sports Medicine* **47**, 1651-1660.
- Calbet, J.A., De Paz, J.A., Garatachea, N., Cabeza de Vaca, S. and Chavarren, J. (2003) Anaerobic energy provision does not limit Wingate exercise performance in endurance-trained cyclists. *Journal of Applied Physiology* **94**, 668-676.
- Davis, J.M., Murphy, E.A., Carmichael, M.D., Zielinski, M.R., Groschwitz, C.M., Brown, A.S., Gangemi, J.D., Ghaffar, A. and Mayer, E.P. (2007) Curcumin effects on inflammation and performance recovery following eccentric exercise-induced muscle damage. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* **292**, R2168-R2173.
- Eltzschig, H.K. and Carmeliet, P. (2011) Hypoxia and inflammation. *New England Journal of Medicine* **364**, 656-665.
- Faiss, R., Girard, O. and Millet, G.P. (2013a) Advancing hypoxic training in team sports: from intermittent hypoxic training to repeated sprint training in hypoxia. *British Journal of Sports Medicine* **47**, i45-i50.
- Faiss, R., Léger, B., Vesin, J.-M., Fournier, P.-E., Eggel, Y., Dériaz, O. and Millet, G.P. (2013b) Significant molecular and systemic adaptations after repeated sprint training in hypoxia. *PLoS One* **8**, e56522.
- Flynn, M.G., McFarlin, B.K. and Markofski, M.M. (2007) State of the art reviews: The anti-inflammatory actions of exercise training. *American Journal of Lifestyle Medicine* **1**, 220-235.
- Frydelund-Larsen, L., Penkowa, M., Akerstrom, T., Zankari, A., Nielsen, S. and Pedersen, B.K. (2007) Exercise induces interleukin-8 receptor (CXCR2) expression in human skeletal muscle. *Experimental Physiology* **92**, 233-240.
- Galvin, H.M., Cooke, K., Summers, D.P., Mileva, K.N. and Bowtell, J.L. (2013) Repeated sprint training in normobaric hypoxia. *British Journal of Sports Medicine* **47**, i74-i79.
- Girard, O., Brocherie, F. and Millet, G.P. (2017) Effects of altitude/hypoxia on single-and multiple-sprint performance: a comprehensive review. *Sports Medicine* **47**, 1931-1949.
- Gleeson, M. (2007) Immune function in sport and exercise. *Journal of Applied Physiology* **103**, 693-699.
- Goods, P.S., Dawson, B., Landers, G.J., Gore, C.J., Croft, K. and Peeling, P. (2016) Effect of repeat-sprint training in hypoxia on post-exercise interleukin-6 and f2-isoprostanes. *European Journal of Sport Science* **16**, 1047-1054.
- Goods, P.S., Dawson, B., Landers, G.J., Gore, C.J. and Peeling, P. (2015) No additional benefit of repeat-sprint training in hypoxia than in normoxia on sea-level repeat-sprint ability. *Journal of Sports Science & Medicine* **14**, 681-688.
- Goods, P.S., Dawson, B.T., Landers, G.J., Gore, C.J. and Peeling, P. (2014) Effect of different simulated altitudes on repeat-sprint performance in team-sport athletes. *International Journal of Sports Physiology and Performance* **9**, 857-862.
- Govus, A.D., Abbiss, C.R., Garvican-Lewis, L.A., Swinkels, D.W., Laarakkers, C.M., Gore, C.J. and Peeling, P. (2014) Acute hypoxic exercise does not alter post-exercise iron metabolism in moderately trained endurance athletes. *European Journal of Applied Physiology* **114**, 2183-2191.
- Hamlin, M.J., Olsen, P.D., Marshall, H.C., Lizamore, C.A. and Elliot, C.A. (2017) Hypoxic Repeat Sprint Training Improves Rugby Player's Repeated Sprint but Not Endurance Performance. *Frontiers in Physiology* **8**, 24.
- Hughson, R., Green, H. and Sharratt, M. (1995) Gas exchange, blood lactate, and plasma catecholamines during incremental exercise in hypoxia and normoxia. *Journal of Applied Physiology* **79**, 1134-1141.
- Kasai, N., Mizuno, S., Ishimoto, S., Sakamoto, E., Maruta, M. and Goto,

- K. (2015) Effect of training in hypoxia on repeated sprint performance in female athletes. *Springerplus* 4, Jul 2.
- Moldoveanu, A.I., Shephard, R.J. and Shek, P.N. (2001) The cytokine response to physical activity and training. *Sports Medicine* 31, 115-144.
- Montero, D. and Lundby, C. (2017) No improved performance with repeated-sprint training in hypoxia versus normoxia: a double-blind and crossover study. *International Journal of Sports Physiology and Performance* 12, 161-167.
- Morrison, J., McLellan, C. and Minahan, C. (2015) A clustered repeated-sprint running protocol for team-sport athletes performed in normobaric hypoxia. *Journal of Sports Science & Medicine* 14, 857-863.
- Nieman, D.C., Henson, D.A., Smith, L.L., Utter, A.C., Vinci, D.M., Davis, J.M., Kaminsky, D.E. and Shute, M. (2001) Cytokine changes after a marathon race. *Journal of Applied Physiology* 91, 109-114.
- Nieman, D.C., Nehlsen-Cannarella, S.L., Fagoaga, O.R., Henson, D.A., Utter, A., Davis, J.M., Williams, F. and Butterworth, D.E. (1998) Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Medicine and Science in Sports and Exercise* 30, 671-678.
- Ostrowski, K., Rohde, T., Asp, S., Schjerling, P. and Pedersen, B.K. (1999) Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *The Journal of Physiology* 515, 287-291.
- Peake, J.M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K. and Coombes, J.S. (2005) Plasma cytokine changes in relation to exercise intensity and muscle damage. *European Journal of Applied Physiology* 95, 514-521.
- Pedersen, B.K. (2000) Exercise and cytokines. *Immunology and Cell Biology* 78, 532-535.
- Pedersen, B.K., Ostrowski, K., Rohde, T. and Bruunsgaard, H. (1998) The cytokine response to strenuous exercise. *Canadian Journal of Physiology and Pharmacology* 76, 505-511.
- Petersen, A.M.W. and Pedersen, B.K. (2005) The anti-inflammatory effect of exercise. *Journal of Applied Physiology* 98, 1154-1162.
- Spencer, M., Bishop, D., Dawson, B. and Goodman, C. (2005) Physiological and metabolic responses of repeated-sprint activities. *Sports Medicine* 35, 1025-1044.
- Sumi, D., Kojima, C. and Goto, K. (2017) Impact of Endurance Exercise in Hypoxia on Muscle Damage, Inflammatory and Performance Responses. *The Journal of Strength & Conditioning Research*.
- West, J., Lahiri, S., Gill, M., Milledge, J., Pugh, L. and Ward, M. (1962) Arterial oxygen saturation during exercise at high altitude. *Journal of Applied Physiology* 17, 617-621.

Key points

- Repeat-sprint training in hypoxia is being increasingly utilised by team sport athletes, however little is known regarding the effect of RSH on the acute inflammatory response.
- The results obtained in the present study suggest that team-sport athletes can perform RSH without increasing inflammation when compared to the same training performed in normoxia.
- RSH elicited only a modest inflammatory response that was comparable to the same session performed in normoxia, and thus, inflammation should not have any negative impact on subsequent training and performance in team-sport athletes performing RSH.

AUTHOR BIOGRAPHY

Jaime MORRISON

Employment

PhD candidate at Griffith University, in the Griffith Sports Physiology and Performance research group

Degree

MSc

Research interest

Repeated-sprinting in simulated altitude in team sport athletes

E-mail: jaime.morrison@griffith.edu.au

Brianna LARSEN

Employment

Postdoctoral research fellow at Griffith University, a member of Menzies Health Institute Queensland, and a Queensland Academy of Sport Scholar.

Degree

PhD

Research interest

Thermoregulation and performance in elite female athletes.

E-mail: b.larsen@griffith.edu.au

Amanda COX

Employment

Research fellow at Griffith University and a member of Menzies Health Institute Queensland

Degree

PhD

Research interest

The effects of exercise on immune and inflammatory control

E-mail: a.cox@griffith.edu.au

Clare MINAHAN

Employment

Assoc. Prof., Griffith Sports Physiology and Performance, School of Allied Health Sciences, Griffith University, Gold Coast, Queensland, Australia

Degree

PhD

Research interest

The unique and distinct response of the female athlete to exercise and training.

E-mail: c.minahan@griffith.edu.au

✉ Clare Minahan

Griffith Sports Physiology and Performance, School of Allied Health Sciences, Griffith University, Gold Coast, Queensland, Australia