

Using neopterin to monitor stress in hypoxic and normoxic repeated sprint training in rugby players

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ABSTRACT

Objectives: Neopterin has been used as a stress marker in team sport athletes, but its use in monitoring stress in hypoxic training requires further investigation. The objective of this study was to determine whether neopterin measures could detect differences between hypoxic and normoxic training stress and whether such levels could predict subsequent performance. Methods: Nineteen amateur club rugby players completed two repeated sprint (cycling) sessions per week for 3 weeks in either hypoxic (RSH, n = 9, FIO₂ = 0.145) or normoxic (RSN, n = 10, FIO₂ = 0.209) conditions. Repeated sprint ability (RSA, running), and the Yo-Yo Intermittent Recovery Level 1 test (YYIR1) were assessed pre- and post- intervention. Resting neopterin, total neopterin, and the difference between resting and post-exercise neopterin and total neopterin levels (acute change) were monitored during training. Results: Neopterin and total neopterin measurements demonstrated high individual variability in all participants. Neopterin and total neopterin were likely and very likely elevated respectively in RSH vs RSN between weeks 1 and 3 (neopterin, 56.4 %, ± 55.6, p = 0.10; percent change, ± 90% confidence interval, p value; total neopterin, 42.2 %, ± 23.5, p = 0.02). Aside from a moderate correlation between the acute change in total neopterin with YYIR1 (r = -0.38) there were no substantial correlations between neopterin and total neopterin measures and post-intervention performance. Conclusions: Neopterin or total neopterin can distinguish between hypoxic and normoxic training. However, high individual variability and limited predictive ability of subsequent performance may restrict the practical application of this stress marker.

1. Introduction

High intensity interval training has proved useful in the development of both power output and endurance performance (Taylor, Macpherson, Spears, & Weston, 2015) in team sport athletes. To further enhance training outcomes, training in a hypoxic environment is becoming more common place in team sport environments (Hamlin, Lizamore, & Hopkins, 2018). However, the inclusion of this additional environmental stress to

an already-intensive training programme further increases the risk of overreaching or overtraining (Schmitt, Regnard, Coulmy, & Millet, 2018), necessitating effective athlete stress monitoring.

Two potential stress markers are 7,8-dihydroneopterin and neopterin. During immune system activation, monocyte-derived macrophages synthesise the potent cellular antioxidant 7,8-dihydroneopterin (Gieseg et al., 2009). In an oxidative environment, 7,8-dihydroneopterin is oxidized to neopterin (Gieseg et al., 2009). This oxidation can be caused by

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hypochlorite (HOCl) and potential superoxide which are both generated during inflammation (Giesege, Maghzal, & Glubb, 2000; Widner, Mayr, Wirleitner, & Fuchs, 2000). Practically, 7,8-dihydroneopterin is measured as total neopterin (neopterin + 7,8-dihydroneopterin) due to the limitations of detection techniques. The measurement of total neopterin complements neopterin as a biomarker by providing insight into monocyte-derived macrophage activation as well as oxidative stress in an individual (Giesege, Baxter-Parker, & Lindsay, 2018), both of which are relevant to hypoxic exercise training.

While the effect of altitude training on the immune system presents a complex picture (Mishra & Ganju, 2010), there is at least some evidence indicating an immediate and ongoing increase in immunological function following acute hypoxic exposure (Mazzeo, 2005). The effect of hypoxic exposure on the athlete may therefore be reflected in the resting levels of 7,8-dihydroneopterin (measured using total neopterin) and provide insight into the athlete's overall recovery and readiness for performance.

The immediate rise in neopterin and total neopterin levels help detect short-term stress in sports. For example, these markers increase following exhaustive aerobic exercise (Strasser et al., 2016), or muscle damage sustained during strenuous rugby games (Lindsay et al., 2016). Furthermore, the acute difference between a player's resting and their immediate-post game levels of neopterin and total neopterin vary widely depending on the extent of the individual player's recovery (Lindsay, Lewis, Gill, Giesege, & Draper, 2015). Therefore, the acute change in neopterin and total neopterin post-exercise may be a useful biomarker in monitoring training stress associated with hypoxic training.

The aims of this research were first, to examine whether neopterin and total neopterin were effective markers in assessing both cumulative and acute training stress associated with repeated sprint training in normoxic and hypoxic environments. Second, we aimed to determine the ability of these markers to predict subsequent aerobic (YoYo Intermittent Recovery Level 1-YYIR1) and anaerobic (repeated sprint ability-RSA) performance.

2. Methods

In this single-blind, placebo-controlled research project, participants were randomly divided using randomization software (www.randomizer.com) into either a repeat-sprint training in hypoxia (RSH) group (age: 20.3 ± 2.1 years; weight: 77.1 ± 10.2 kg; height: 173.9 ± 4.9 cm) or a repeat-sprint training in normoxia (RSN) group (age: 22.0 ± 4.1 years, weight: 88.3 ± 14.1 kg; height: 177.9 ± 5.4 cm). The repeat sprint protocol was based on the sprinting patterns during typical rugby matches (Austin, Gabbett, & Jenkins, 2011; Jones, West, Crewther, Cook, & Kilduff, 2015). For this study, we investigated the changes that occurred over the first 3 weeks of training (pre versus post for performance and between weeks for stress markers). Pre-season fitness and training routines were similar between groups at baseline and remained so throughout the research project.

2.1. Participants

A total of 19 amateur club rugby players were recruited for participation in this study however, one participant was later

excluded due to outlying performance data indicative of lower level of athletic ability and incompatibility with the rest of the group. Therefore 18 players (age: 21.2 ± 3.5 years; weight: 83.3 ± 14.1 kg; height: 176.1 ± 5.6 cm; >10 years rugby union playing experience) completed this study. All players completed a medical questionnaire and reported no contraindications to maximal exercise.

2.2. Ethical clearance

All players were informed about the possible risks of volunteering for this study and provided written informed consent prior to the study commencing. In accordance with the Declaration of Helsinki, this research was approved by the institutional human ethics committee (reference 2015-46) and was carried out in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

2.3. Baseline and post-intervention performance testing

As described previously (Hamlin, Olsen, Marshall, Lizamore, & Elliot, 2017) players were asked to avoid intense exercise for 24 h and caffeine for 12 h prior to exercise testing. Additionally, athletes were asked to ensure they were hydrated and were asked to record their dietary intake prior to their baseline testing, for repetition in the post-intervention testing. Baseline and post-intervention testing followed identical sequences and were conducted at the same time of day (± 1 h). Following a 15 min warm up (5 min slow jog followed by 5 – 10 mins of dynamic stretching), participants completed a squat jump (data not reported here), repeated sprint, and Yo-Yo Intermittent Recovery Level 1 (YYIR1) test, with approximately 10 - 15 min of recovery between each test. All tests were completed in a covered training facility on a slip-free floor under similar climatic conditions.

2.4. Repeated sprint and YYIR1 performance tests

The running-based repeat-sprint test included 8 maximal effort sprints over 20 m, timed to go every 20 s. Times for these sprints were recorded to the nearest 0.01 s using a set of electronic speed-timing lights (Smartspeed, Fusion Sport, Ltd, Australia). Repeated sprint performance was reported in mean sprint times. The YYIR1 followed standard procedures (Bangsbo, Iaia, & Krustup, 2008) with the total distance covered by the athlete (m) recorded.

2.5. Repeated sprint training

Repeated sprint training was performed on Wattbikes (Wattbike Pro, Nottingham, UK) for two reasons. First, to reduce ground contact work, and so off-feet training was used to avoid overuse injury (Brooks, Fuller, Kemp, & Reddin, 2005). The second reason was due to the sensitive nature of neopterin and total neopterin which increase with impact-related muscle damage (Lindsay et al., 2016). To this end, we attempted to isolate the effect of exhaustive hypoxic exercise rather than impact-related increases in neopterin and total neopterin by using Wattbikes rather than running-based exercise.

All participants completed 6 cycling repeat-sprint training sessions over 3 weeks (2 sessions/week). Training was completed on a calibrated Wattbike set up to participant-specific dimensions (saddle and handle bar height and position). The Wattbike has high reliability with a typical error of measurement of 2.6% (Hopker, Myers, Jobson, Bruce, & Passfield, 2010). Bike resistance was set to air brake resistance level 3, magnetic setting 3 for weeks 1 and 3, but was increased to air brake level 5, magnetic setting 3 to increase overload in week 2. Participants completed their sprints in an upright, but seated position. Prior to each repeat-sprint training, participants warmed up for 5 min at 50 W, with a 5 s sprint at the end of each min. The repeat-sprint training included 4 sets of 5 repetitions of 5 s maximal-intensity sprints. Repetitions and sets were separated by low-intensity (20 – 50 W) active recovery for 25 s and 5 min, respectively. Therefore, each session was comprised of 35 min of exercise, totalling 280 min over the course of the study (i.e. 280 mins of hypoxic exposure for the hypoxic group).

2.6. Hypoxic dosage

All participants were fitted with a face mask (Hans-Rudolph 8980, Kansas City, Missouri, USA) attached to one of 2 sets of 2 x 100 L Douglas bags connected in series via a one-way non-rebreathing valve (Hans-Rudolph 2700). All participants were informed that they would be receiving hypoxic air, however, only one set of the 2 x 100 L Douglas bags was fed with a fraction of inspired oxygen (FIO₂) level of 0.145 (~3000 m), while the other was set to 0.209 (sea level) via the GO2Altitude® hypoxicator system (Biomedtech, Victoria, Australia). An FIO₂ of 0.145 was chosen as it has been found to increase physiological stress without impacting the typical attenuation in performance that normally occurs with such exercise (Bowtell, Cooke, Turner, Mileva, & Summers, 2014). Participants were unable to view any data that would indicate the gas compositions, such as blood oxygen saturation level or heart rate. Blinding was effective as when questioned post-intervention only 20% of the participants in the control group suspected they were receiving either a higher oxygen dose or a placebo.

2.7. Neopterin and total neopterin

Urine samples were collected at baseline before any repeated sprints, and then immediately following the repeated sprints training. Using this approach allowed us to detect both the athlete's basal, or resting immunological and oxidative status states as well as the response to the repeated sprints. Urine samples were collected on the second training session of each week. On each collection day participants were asked to provide a mid-stream urine sample pre and immediately post training using a 70 mL collection pottle. The samples were immediately placed on ice and transported to the laboratory. Samples were prepared in darkness where possible to prevent oxidation of 7,8-dihydroneopterin by UV light. Samples were thawed and diluted 1 in 40 with a buffer (20 mM ammonium phosphate pH 2.5). A 100 µL sample was transferred to an autosampler vial for neopterin high-performance liquid chromatography (HPLC) analysis. For total neopterin analysis, an oxidation step was included to convert 7,8-dihydroneopterin to neopterin. Twenty µL

of acidic iodide solution (5.4 % I₂/10.8 % KI in 1 M HCl) was added to 100 µL of the 1 in 40 diluted sample and incubated for 15 min at room temperature in the dark. Then, 10 µL of 0.6 M ascorbate was added to quench the acidic iodide oxidation reaction and 100 µL was transferred to an autosampler vial for HPLC analysis. High-performance liquid chromatography measurement of neopterin was performed using a Shimadzu Sil-20A HPLC with autosampler and RF-20AxIs fluorescence detector. A 10 µL sample was injected onto a Luna 5 µm SCX 100Å, 250 × 4.6 mm column with a mobile phase of 100% 20 mM ammonium phosphate pH 2.5 pumped at 1 mL/min. Neopterin was detected by its native fluorescence at excitation 353 nm and 438 nm emission. The concentration and identity of the eluted neopterin was confirmed by comparison to a standard with an intra-assay CV (coefficient of variation) of 3.04% and inter-assay CV of 5.42% (Lindsay, Janmale, Draper, & Gieseg, 2014). This was made up daily using 1.5 – 2 mg of neopterin dissolved and diluted down to 100 nM in 20 mM ammonium phosphate pH 2.5, before being quantified by peak area using Shimadzu Class VP software.

Specific gravity (SG) was measured using an ATAGO N-20 refractometer. Calculations were derived using the formula described below and based on the normal population (SG1.020) (Goldberger, Loewenthal, Darwin, & Cone, 1995). Before measurement, samples were brought to room temperature to remove temperature-dependent density variation bias (Alessio, Berlin, Dell'Orto, Toffoletto, & Ghezzi, 1985).

$$[\text{neopterin}](\text{nM}/\text{SG}1.020) = (\text{SG}1.020 - 1) / (\text{SG}_{\text{sample}} - 1) \times [\text{neopterin}](\text{nM})$$

Neopterin and total neopterin were taken prior to any exercise to assess the athlete's resting level of immune system activation and oxidative stress. This measure is referred to as "resting neopterin" or "resting total neopterin". Neopterin and total neopterin measured immediately post-training were subtracted from their resting measurement to provide an indication of the acute response of the athletes to the immediate training stress. This measure is referred to as "the acute change in neopterin" or "the acute change in total neopterin".

2.8. Statistical analysis

Aim 1: Effect of hypoxic vs normoxic environments on neopterin and total neopterin. To negate any differences in the baseline levels of stress responses, change scores in the stress markers from week – to – week were compared between the hypoxic and control groups. In particular, a pre-post parallel-groups spreadsheet (Hopkins, Marshall, Batterham, & Hanin, 2009) was used to assess the between-group differences in change scores from weeks 1 – 3 in resting and in the acute change in neopterin and total neopterin post-training. Cohen's value of 0.2 of the mean difference divided by the between-subject standard deviation was used to assess the smallest worthwhile change. Prior to analysis all data were log-transformed to reduce non-uniformity of error. Results are displayed as a percent change ± 90 % confidence interval. The 90% confidence indicates the range of uncertainty around the true value. All data was assessed using the clinical inference, which is more conservative regarding the risk of harm. In this regard, an odds ratio of benefit: harm was only accepted if

it was above 66%; if not, the effect was considered “unclear”. For clear results, the magnitude of the change was reported using the following scale <0.5% = most unlikely; 0.5–5% = very unlikely; 5–25% = Unlikely; 25–75% = possibly; 75–95% = likely, 95–99.5% = very likely, >99.5% = most likely (Hopkins et al., 2009). The direction of the change (increase, trivial or decreased) was determined, and interpreted according to the variable. In addition, p values are noted for researchers unfamiliar with magnitude-based inferences.

Aim 2: Ability of neopterin and total neopterin to predict post-intervention performance. Resting and the acute change in neopterin and total neopterin following training in Week 3 were correlated with post-training YoYo Intermittent Recovery Test Level 1, and repeated sprint ability performance. Correlations were performed in the Statistical Analysis System using the Proc Corr procedure (Version 9.3; SAS Institute, Cary, NC). Correlations were interpreted using Cohen’s magnitude scale of <0.10, 0.10, 0.30, 0.50, 0.70, and >0.90 for which we used

Hopkins et al. (2009) descriptions (i.e. trivial, small, moderate, large, very large, and extremely large respectively).

3. Results

3.1. Differences in stress markers in hypoxic vs normoxic groups

One participant in the RSH group was excluded due to missing week 1 neopterin data. Both groups demonstrated a high level of individual variation (Figure. 1) in all neopterin measurements. Overall the participants in the hypoxic group had higher resting levels of neopterin and total neopterin than their normoxic counterparts (Table 1). Conversely, the acute difference in resting total neopterin after repeated sprint training was lower in the hypoxic group compared the normoxic group. The acute difference in resting and exercising neopterin was unclear between groups.

Table 1: The between-group differences in the physiological stress markers.

Stress marker	Group	n	Training period		Difference in week 1 and week 3 measures
			Week 1 (Mean ± SD)	Week 3 (Mean ± SD)	
Resting neopterin (nM/SG _{1.020})	RSN	10	3266.0 ± 1102.4	2322.8 ± 811.0	56.4, ± 55.6; p = 0.10 Likely elevated neopterin in RSH
	RSH	7	2516.7 ± 1347.4	2601.3 ± 735.2	
Resting total neopterin (nM/SG _{1.020})	RSN	10	6307.2 ± 1958.9	5592.2 ± 1256.1	42.2, ± 23.5; p = 0.02 Very likely elevated total neopterin in RSH
	RSH	7	4559.6 ± 1368.7	5822.1 ± 1577.7	
Acute change in neopterin (nM/SG _{1.020})	RSN	10	191.5 ± 934.7	340.0 ± 460.2	-66.3 ± 1336.3; p = 0.45 Unclear
	RSH	7	334.2 ± 1208.3	-97.0 ± 478.0	
Acute change in total neopterin (nM/SG _{1.020})	RSN	10	1069.3 ± 1378.8	1241.0 ± 691.0	-78.2 ± 332.6; p = 0.09 Likely reduced total neopterin following in RSH
	RSH	7	1349.4 ± 960.0	-572.1 ± 1004.0	

CL: confidence limits; SD: standard deviation; RSN: Group receiving a normoxic placebo during the repeated sprint intervention; RSH: group receiving hypoxic during the repeated sprint intervention; Resting neopterin and total neopterin: neopterin and total neopterin measurements taken at rest prior to exercise training; Acute change in neopterin and total neopterin: the difference between the post-training and resting levels of neopterin and total neopterin.

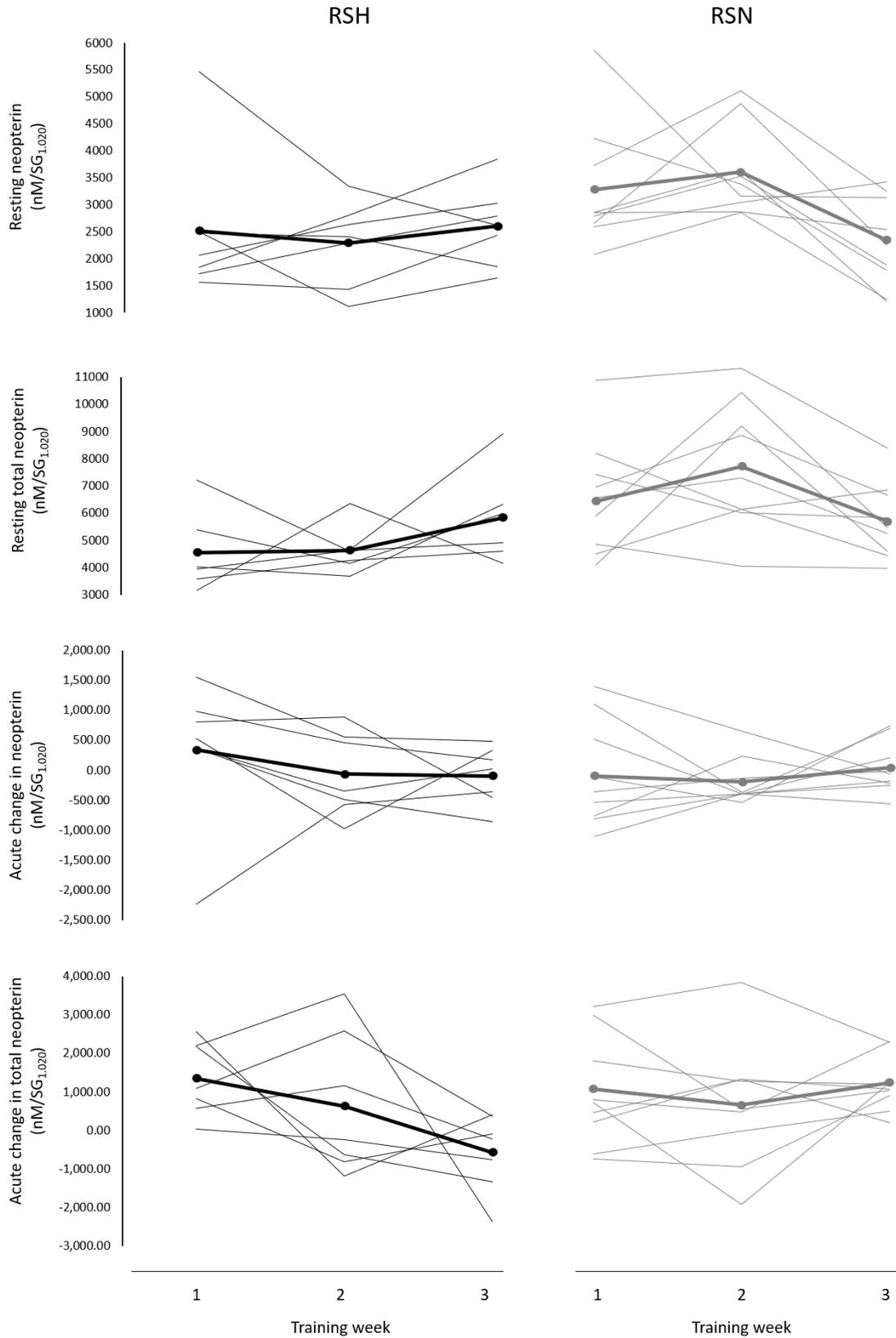


Figure 1: Group mean and individual variation in neopterin, total neopterin and their acute changes from resting immediately post repeated sprint training. Data were collected weekly over 3 weeks of repeated sprint training in hypoxic and normoxic environments. Thin lines represent all individual results while thick lines represent the group mean.

3.2. Relationship between resting neopterin and total neopterin with post-intervention exercise performance

One participant in each of the groups (RSH and RSN) was excluded due to missing post-intervention performance data. The

week 3 neopterin and total neopterin markers and post-intervention Yo-Yo intermittent recovery and repeated sprint ability performances are presented in Table 2 and indicate at best a moderate relationship between the acute change in total neopterin following exercise and YoYo intermittent recovery test performance.

Table 2. Correlation between Week 3 stress markers with post-intervention Yo-Yo intermittent running performance and repeated sprint ability performances.

Stress marker	Yo-Yo intermittent recovery test			Repeated sprint ability		
	Correlation (r-value)	Level of significance (p-value)	Correlation magnitude	Correlation (r-value)	Level of significance (p-value)	Correlation magnitude
Neopterin						
Resting	0.16	0.56	Small	-0.23	0.41	Small
Acute change	-0.04	0.90	Trivial	-0.02	0.93	Trivial
Total Neopterin						
Resting	-0.18	0.53	Small	0.16	0.56	Small
Acute change	-0.38	0.17	Moderate	0.12	0.67	Small

Resting: neopterin and total neopterin measurements taken at rest prior to exercise training; Acute change: the difference between the post-training and resting levels of neopterin and total neopterin respectively.

4. Discussion

While neopterin and total neopterin have been used to assess stresses associated with rugby (Lindsay et al., 2015; Lindsay et al., 2016), exhaustive cycling (Strasser et al., 2016), and body building (Lindsay et al., 2014), this is the first study we are aware of that has investigated its use in repeated sprint training with and without hypoxia. The results of the present study demonstrate large variability in individual neopterin and total neopterin responses to repeated sprint training in both normoxic and hypoxic groups (Figure. 1). Even so, it is likely that repeated sprint training in hypoxia results in elevated neopterin and total neopterin compared to repeated sprint training in normoxia. More research is needed to determine whether neopterin and total neopterin can be used to predict subsequent performance.

4.1. Between group differences in resting neopterin and total neopterin levels

Despite large individual variation, there was a clear decrease in resting neopterin and total neopterin in the normoxic group, while the group training in hypoxia showed a slight increase in these measures. This discrepancy likely indicates that training was well tolerated by the athletes in the normoxic group. However, during repeated sprint training in hypoxia, it is possible that additional

reactive oxygen and nitrogen species were produced, which may have temporarily overwhelmed the body’s antioxidant capacity, resulting in greater cellular damage than repeated sprint training in normoxia (Morales-Alamo & Calbet, 2014). The potential increase in reactive oxygen and nitrogen species may be responsible for the sustained resting neopterin (from the higher oxidative stress) and total neopterin levels (in response to the suspected muscle damage) seen in week 3 vs week 1 in the hypoxic repeated sprint group compared to the normoxic group (see Table 1).

To the best of our knowledge, there is no research on the responses of neopterin or 7,8-dihydroneopterin to hypoxia; however, there has been some research regarding the effect of altitude on general cytokine behaviour, including interferon-γ (Pyne et al., 2000). Interferon-γ is a cytokine that triggers monocyte-derived macrophage activity which results in the increased production of 7,8-dihydroneopterin, or total neopterin (Hamerlinck, 1999). During altitude training, Ermolao et al. (2009) reported a decrease in interferon-γ after 21 days at high altitude (5,050m), whereas Pyne et al. (2000) reported increased interferon-γ after 21 days at 2,100 m. Therefore, interferon-γ appears to respond differently depending on the hypoxic stress. In the present study, the hypoxic intensity was between the intensities used by Pyne et al. (2000) and Ermolao et al. (2009), but the exposure time was vastly shorter than either. Despite the

considerably shorter exposure interval in this study the received hypoxic dosage was still able to elicit a comparative increase in total neopterin, possibly caused by an increase in interferon- γ . Further research is required to support this theory. As hypoxic exposure is associated with both increased generation of reactive oxygen species (Pialoux, Hanly, et al., 2009) as well as systemic inflammation (Hartmann et al., 2000), it is likely that the environmental conditions in the hypoxic group were responsible for the increase in both neopterin and total neopterin. The results of our analysis therefore support the findings of Pyne and colleagues (2000) who found that increases in cytokines in general (including interferon- γ) were primarily due to the environment rather than the training condition.

4.2. Between group differences in the acute change in neopterin and total neopterin immediately post-training

When comparing the acute change in neopterin and total neopterin following repeated sprint training, the RSH group had smaller changes compared to the normoxic group. Speculatively, the lower post-exercise rise in total neopterin (and possibly neopterin) in the hypoxic group may be a result of training-induced improvements in oxidative stress-dependant mechanisms (Lindsay et al., 2017) in response to heightened oxidative stress associated with training in hypoxia (Pialoux, Brugniaux, et al., 2009). Additionally, hypoxic-induced adaptations such as enhanced muscle perfusion that acts to delay fatigue via reduction in anaerobic energy dependence and improved waste removal (Faiss et al., 2013), has also been associated with repeated sprint training in hypoxia (Faiss et al., 2013).

4.3. Neopterin and total neopterin as predictors for performance

Aside from a moderate, negative correlation between the week 3 total neopterin and YYIR1 performance, none of the other neopterin or total neopterin measurements were able to accurately predict subsequent performance (Table 2). This is likely due to the well-tolerated interventions and absence of any overtraining for either the RSH or RSN groups as there was no meaningful difference in relative power output between groups over the course of the study (Hamlin et al., 2017). As such, the increased resting neopterin and total neopterin in the hypoxic group is likely still within a well-tolerated level of immune activation and oxidative stress.

The moderate, negative correlation between the acute change in total neopterin immediately post-exercise with the YYIR1 performance may provide support for the speculated improvement in the RSH athlete's oxidative stress tolerance. In this correlation, relatively lower acute post-exercise changes in total neopterin were moderately associated with better YYIR1 performance. Interestingly, the RSH group did not perform better in their immediate post-intervention YYIR1 compared to the RSN group (Hamlin et al., 2017). This suggests that while on average there was a greater reduction in post-training vs resting total neopterin in the RSH group, this was not necessarily true for all participants in the hypoxic group. As this correlation was only in the total neopterin measure (monocyte-derived macrophage activation), and not neopterin (oxidised compound), this correlation may reflect a more tolerant immune response to oxidative stress and

may be able to detect "responders" to altitude training. However, given the considerable individual variation in the present study, more research in larger training groups is needed to verify these findings.

Being able to accurately monitor stress is an important part of assisting athletes in positively adapting to training and recovery loads. We found neopterin and total neopterin can distinguish between hypoxic and normoxic training stress, with higher levels of neopterin and total neopterin in the RSH group compared to RSN. The acute change in total neopterin immediately following the training session in week 1 was attenuated in week 3 in the hypoxic group, but not in the normoxic group. This dampened rise in total neopterin post-exercise is possibly due to improved oxidation stress management induced by the more intensive repeated sprint training in the hypoxic environment. The acute change and resting measurements of total neopterin and neopterin had limited ability to predict subsequent performance.

5. Conclusion

In conclusion, neopterin and total neopterin can distinguish between hypoxic and normoxic training stress, with higher levels of neopterin and total neopterin in the RSH group compared to RSN. The acute change in total neopterin immediately following the training session in week 1 was attenuated in week 3 in the hypoxic group, but not in the normoxic group. This dampened rise in total neopterin post-exercise is possibly due to improved oxidation stress management induced by the more intensive repeated sprint training in the hypoxic environment. The acute change and resting measurements of total neopterin and neopterin had limited ability to predict subsequent performance.

Accurately monitoring stress is an important part of assisting athletes and this study found levels of neopterin and total neopterin in the urine were able to distinguish between athletes completing hypoxic compared to normoxic training and therefore are useful in detecting heightened levels of stress. However, the limited ability for neopterin and total neopterin to predict performance in rugby players, the wide individual variability in the results, and the somewhat more complex nature of the measurement (requiring laboratory analysis), are all barriers to ongoing use of these markers in club-level athletes. Practically, monitoring neopterin and total neopterin may be better suited to elite athletes undergoing intensive training periods where a battery of stress monitoring assessments are used to prevent overtraining. However, as the data in this study may not be generalizable to elite-level rugby players, future research should assess the usefulness of measuring neopterin and total neopterin in elite athletes. Furthermore, the athletes in this study were all participating in preseason training which focusses primarily on increasing fitness, rather than contact-based drills. Future research should explore the use of neopterin and total neopterin during the competitive season when increased tackling and physical contact may heighten the immune response to training.

Conflict of Interest

The authors declare no conflict of interests.

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