sIL-6R is related to weekly training mileage and psychological wellbeing in athletes

Tom Cullen[1,2*], Andrew W. Thomas[3], Richard Webb[3], Thom Phillips[4], and Michael G. Hughes[1].

[1] Cardiff School of Sport, Cardiff Metropolitan University, Cardiff CF23 6XD, UK.

[2] Institute of Sport & Exercise Science, University of Worcester, Henwick Grove, Worcester, WR2 6AJ, UK.

[3] Cardiff School of Health Sciences, Cardiff Metropolitan University, Cardiff CF5 2YB, UK.

[4] University Hospital Wales Cardiff, Cardiff, CF14 4XW, UK.

Corresponding author: Tom Cullen. Institute of Sport & Exercise Science, University of Worcester, Henwick Grove, Worcester, WR2 6AJ, UK. t.cullen@worc.ac.uk.

Abstract

Introduction: IL-6 has been ascribed both positive and negative roles in the context of exercise and training. The dichotomous nature of IL-6 signalling appears to be determined by the respective concentration of its receptors (both membrane-bound (IL-6R) and soluble (sIL-6R) forms). The purpose of the present study was to investigate the response of sIL-6R to long-term training, and to investigate the relationship between sIL-6R, self-reported measures of wellbeing, and upper respiratory illness symptoms (URS) in highly-trained endurance athletes. **Methods:** Twenty-nine athletes provided resting blood samples, and completed wellbeing and illness monitoring questionnaires, on a weekly basis for a period of 18 weeks

during a winter training block. **Results:** URS were not correlated to concentrations of sIL-6R or cortisol, but there was a non-significant trend (P=0.08) for the most illness-prone athletes (as defined by self-reported illness questionnaire data) to exhibit higher average sIL-6R concentrations compared to the least ill (23.7±4.3 Vs 20.1±3.8 ng/ml). Concentrations of sIL-6R were positively correlated to subjective measures of stress (r=0.64, P=0.004) and mood (r=0.49, P=0.02), but were negatively correlated to sleep quality (r=-0.43, P=0.05) and cortisol concentration (r=-0.17, P=0.04). In a sub-group of 10 athletes, weekly training distance was quantified by coaching staff, and this negatively correlated with sIL-6R in the following week (r=-0.74, P<0.005). **Conclusion:** The findings of the current study suggest that sIL-6R is responsive to prolonged periods of exercise training, with sIL-6R levels varying related to the volume of training performed in the preceding week. Importantly, our data indicate that changes in sIL-6R levels could be linked to common symptoms of overreaching such as high levels of stress, and/or depressed mood.

Keywords: sIL-6R; athletes; fatigue; overreaching

Introduction

IL-6 is a pleiotropic cytokine that has multiple functions throughout the body, and which has been ascribed both positive and negative roles in terms of health (29, 40); in the context of exercise IL-6 exerts anti-inflammatory effects primarily by causing the induction of anti-inflammatory mediators such as IL-10, IL-1Ra and cortisol (39). As such, exercise-induced increases in IL-6 are associated with a transient anti-inflammatory state that, if repeated, can lead to health benefits via the reduction of chronic inflammation (11, 29). Conversely, in the context of infection, sepsis or trauma IL-6 can have a pro-inflammatory role with pyrogenic

functions, and indeed IL-6 administration in humans has been shown to induce symptoms of sickness and fever (34). In addition, chronically elevated levels of IL-6 are associated with the development of numerous diseases of an inflammatory aetiology such as type 2 diabetes, rheumatoid arthritis (40) and clinical depression (8).

The dichotomous nature of IL-6 signalling appears to be related to the fact that it has two types of receptor; a membrane bound (IL-6R) and a soluble (sIL-6R) receptor, each of which is associated with distinct signalling pathways termed ‘classical’ and ‘trans-signalling’ respectively (17). Classical signalling is limited to cells and organs that possess IL-6R such as hepatocytes and leukocytes (17), the brain (37) and skeletal muscle (19). In contrast sIL-6R is present in the circulation and allows cells that do not possess IL-6R to respond to IL-6 via the process of trans-signalling (i.e. sIL-6R interacting with the ubiquitously-expressed cell-surface receptor gp130 in order to trigger intracellular responses within target cells (16)). Trans-signalling through sIL-6R is predominantly pro-inflammatory and appears largely responsible for the negative pathological effects associated with IL-6 (18).

It has previously been postulated that IL-6 might also play a role in the development of overtraining (35) and immunosuppression (10), both of which are associated with the increased rate of upper respiratory illnesses (URI), and/or reporting of upper respiratory symptoms (URS) by athletes undertaking high volumes of training. While there is some convincing evidence that supports the role of IL-6 with common aspects of overreaching such as increased perception of training overload, a depressed mood (23), fatigue (31, 33) and decreased performance (34), there is less empirical evidence to support the role of IL-6 in the increased rate of URI in athletes. There is also debate over whether the symptoms associated with URIs are purely due to infections or are in fact a reflection of inflammatory factors that

could be induced by exercise (41). In the case of IL-6, it may be difficult to ascertain whether an increase in IL-6 may increase susceptibility to a possible future infection, or whether such an increase may actually be a response to an infection to which the body is now responding via an inflammatory response. While some studies have reported no elevation in resting IL-6 levels in response to intensified training in athletes (16), others have reported greater exercise-induced increases in IL-6 in URI-prone athletes and have suggested that greater increases in IL-6 in illness-prone athletes could be due to excessive inflammatory responses (3).

Importantly, several studies have suggested that some of the negative, pro-inflammatory effects of IL-6 signalling could be explained by differences in sensitivity to IL-6 that are mediated by changes in the relative concentration of sIL-6R (32). In contrast to IL-6, only a limited number of studies have reported the effect of exercise on the circulating concentration of sIL-6R. Small increases in sIL-6R (approximately 10%) have been reported immediately following aerobic exercise (13, 14, 21, 42); however, other studies have reported no change (14, 28). This contradictory evidence and small number of studies make it difficult to reach a clear conclusion as to how sIL-6R responds to acute endurance exercise. However, it does appear that the circulating concentration of sIL-6R is significantly reduced following a period of prolonged exercise training (1, 44), and a recent study reported a significant reduction in sIL-6R following only 2 weeks of high intensity training (20). To our knowledge no study has yet investigated changes in sIL-6R throughout prolonged training programmes, but given the apparent importance of sIL-6R signalling to the negative effects of IL-6 it is important to gain an improved understanding of how exercise can modulate sIL-6R during prolonged periods of exercise training.

Therefore, the aims of the current study were: a) to investigate circulating sIL-6R responses to long-term training in endurance trained athletes; and b) to investigate the relationship between sIL-6R, subjective measures of wellbeing and the reported rate of URS over the course of an 18-week winter training period.

Methods

Participants

Twenty-nine (16 male; 13 female) endurance-trained athletes volunteered to participate in the study and provided informed consent prior to taking part. The participants consisted of four separate squads including triathletes (n=6), swimmers (two squads, n=10 and n=5), and rowers (n=8), all of which were receiving physiological support from Sport Wales (the organisation responsible for sports science support services to elite athletes in Wales). All athletes within the study were typically training for approximately 20 hours per week with the aim of competing to the highest level, including competing at a national and/or international standard. Ethical approval was obtained from the Cardiff Metropolitan University School of Sport Ethics committee and all procedures conformed to the declaration of Helsinki.

Study Design

All athletes were studied for an 18-week winter training period (October 2013-February 2014), which took place after a period of relative rest following the end of the previous competitive season. Throughout the study, athletes carried out their normal training regimens as directed by their individual coaching staff. All data were collected at the training location

of each squad; sample donation was considered part of their normal routine and no aspect of training was altered as a result of their taking part in the study. Athletes provided blood samples in a non-fasted state, and completed an illness and wellbeing questionnaire on a weekly basis. Data was not collected during weeks 15 and 16 of the study as these dates coincided with a period of reduced training volume and also fell on Christmas Day and New Year's Day (please see Figure 1 for a schematic illustration of the study design).

In order to avoid the acute effects of individual exercise bouts, these measurements were obtained a minimum of 24hrs after the most recent training session. For each squad, data collection took place at the same time of day prior to training on the same day of the week (individual squads were tested on different days of the week in order to comply with their normal training routine). The Triathletes and Swimmers B provided blood samples prior to their morning training sessions at 6:00 whereas the Rowers and Swimmers A provided samples in the afternoon between 14:00-16:00. While athletes from 4 separate squads were used, the entire study was carried out over the same time scale to avoid seasonal differences in respiratory illness.

Capillary blood was used to determine the plasma concentrations of sIL-6R and cortisol, which were compared with self-reported measures of illness and wellbeing. The concentration of sIL-6R was measured every week, while cortisol was measured every month. In a sub group of 10 athletes, all of whom were from the same squad of swimmers (swimmers A), weekly prescribed training distance was correlated with sIL-6R and cortisol concentrations measured during the following week in order to ascertain how these physiological variables responded to training on a weekly basis. Similar to the methods described by Purge, Jürimäe and Jürimäe (2006), weekly training volumes were calculated

based on the sum of the distance prescribed by the coaching staff in each training session, and also athletes' individual daily training diaries (30).

Xxx Insert Figure 1 Here xxx

Illness and wellbeing questionnaire

The illness and wellbeing questionnaire employed throughout the present study was a 9-point questionnaire used internally at Sport Wales to monitor self-reported aspects of wellbeing and illness. The questionnaire is a modified version of the wellbeing questionnaire utilised in the study of McLean et al., 2010 (24); similar questionnaires have good reliability and validity, and have been shown to be sensitive to fatigue in Professional Rugby League (6). Questionnaire data was analysed alongside self-reported measures of training load; such data are routinely used with elite athletes and have been repeatedly shown to be sensitive to training overload in a number of sports (22, 25).

Athletes were asked to rate on a 5-point scale the following categories: fatigue, muscle soreness, stress, mood, motivation to train and quality of sleep. The questionnaire also required athletes to indicate the average number of hours of sleep per night in the last week. Prior to completing the questionnaire, athletes were provided with a full explanation of each question. With regards to illness, athletes were asked to indicate the number of days in the past week where they had suffered symptoms of upper respiratory illness, the severity of these symptoms and to what degree this had affected their training. This method was chosen in light of recent evidence suggesting that a greater percentage of URI are reported when using a self-reported questionnaire than when athletes are required to report their symptoms

to affiliated medical staff (5). However, it should be noted that this method does not allow for the clinical determination of pathogenic cause of any illness symptoms, and the physical presence of an infection could not be verified; as a result the term 'upper respiratory tract symptoms' (URS) rather than URTI or URI was used throughout this study. Athletes were not vaccinated against influenza as part of the study.

sIL-6R and cortisol

Capillary blood samples were collected from the fingertip in 200 µl heparinized microvette capillary blood collection tubes (Sarstedt, Germany) as previously described in more detail (4). Blood samples were fractionated by centrifugation (10 min; 3,000 x G), and the resulting plasma was aliquoted and stored at -80 °C until analysis. Circulating sIL-6R and cortisol concentrations were measured using enzyme-linked immunosorbent assays (ELISA) (R&D Systems Ltd., Abingdon, UK). All additional materials and chemical reagents were purchased from R&D systems, and the assays were carried out in accordance with the manufacturer's instructions. Plasma samples were diluted at 1:100 with a commercially available diluent (DY997, R&D Systems Ltd) prior to analysis of sIL-6R, and 1:20 prior to analysis of cortisol, in order to produce concentrations that were within the dynamic range of the assay. Both assays had been previously validated for use with plasma samples using standard spike recovery and linearity procedures (data not shown). The sIL-R assay had an intra-assay CV of 1.5 ± 0.7% across a range of 1.56-100 ng/ml. The cortisol assay had an intra-assay CV of 7.3 ± 3.9% across a range of 0.156-10 ng/ml. The intra-assay CVs were calculated from the duplicate readings obtained during each experiment. Protein concentrations were determined in relation to a four-parameter standard curve (GraphPad Prism, San Diego California, USA).

Statistical Analysis

A one-way ANOVA was used to analyse differences in mean sIL-6R and cortisol values between each squad of athletes. A Bonferroni *post hoc* was conducted to analyse where differences existed. Pearson product moment correlation was used to investigate the relationship between sIL-6R or cortisol, and the volume of training performed in the preceding week. Correlations were conducted on pooled data from all individuals within a subset of the total cohort (a single squad of 10 swimmers) for each training week.

A repeated-measures stepwise regression model was used to assess the relationships between physiological measures (i.e. sIL-6R and cortisol levels), self-reported wellbeing, and illness measures over the entire study using data from all groups, with analyses being conducted on pooled data from all individuals for each training week. In studies of this type there is potential for data dropout due to logistical reasons or personal circumstance, and so previous researchers have recommended regression modelling when studying URI risk in athletes, as it has been reported to be robust when data are missing (12). Athletes were categorised into most and least illness prone (upper and lower quartile for illness index), and a Mann Whitney U-test was used to investigate differences between the most and least illness-prone athletes. For the purpose of regression analysis, cortisol concentrations were expressed as an individual's relative cortisol concentration, defined here as the percentage difference compared to the average value for the individual, this allows for a fair comparison of the relative stress for the individual (26). All analysis was conducted in SPSS version 20.0. Statistical significance was set at $P \leq 0.05$.

Results

Physiological responses

Over the entirety of the study a total of 293 capillary blood samples were analysed with a

mean sIL-6R of 21.0 ± 4.6 ng/ml. There was no significant difference in mean sIL-6R between squads of athletes (Triathletes= 18.7 ± 4.6 ng/ml, Rowers= 22.3 ± 4.5 ng/ml, Swimmers A= 22.3 ± 3.3 ng/ml, Swimmers B= 20.4 ± 5.2 ng/ml Figure 2A). A significant difference was found for resting cortisol concentration between groups ($P<0.001$). Triathletes had significantly higher resting cortisol concentrations than Swimmers A and Rowers (mean difference =43.4 and 44.4 pg/ml) as did Swimmers B (mean difference =53.9 and 55.2 pg/ml) (Figure. 2B). In a subgroup of 10 swimmers (Swimmers B), prescribed training mileage was 64.6 ± 21.1km/wk (range=19.6-89km/wk), and sIL-6R levels on any given week were negatively correlated with the volume of training prescribed in the previous week ($r=-0.74$, $P<0.005$) (Figures 3A and 3B), while cortisol levels (as analysed on the same basis) showed a positive correlation with training volume ($r=0.89$, $P=0.045$) (Figures 4A and 4B). Finally, sIL-6R was negatively correlated with cortisol concentration ($r=-0.17$, $P=0.04$).

Xxx Insert Figure 2 Here xxx

Xxx Insert Figure 3 Here xxx

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Illness and wellbeing monitoring

All athletes reported experiencing URS at some point throughout the study. Athletes reported URS for an average of 17.4 ± 8.5 days (range 1-40) throughout the entire study, while the number of athletes reporting URS per week ranged from 5-12 (mean ± SD = 8.4 ± 2.6). Based upon regression analysis across the entire cohort, neither the absolute or relative concentration of sIL-6R nor the relative cortisol concentration was related to the number of days with illness symptoms or the severity of these symptoms. However, there was a non-

significant trend (P=0.08) for a higher average sIL-6R concentration in the most ill quartile compared to the least ill quartile of athletes (23.7 ± 4.3 Vs 20.1 ± 3.8 ng/ml).

With regard to subjective measures of wellbeing, circulating concentrations of sIL-6R were positively correlated to perceived stress (r=0.64, P=0.004) and worse mood (r=0.49, P=0.02) but negatively correlated to worse sleep quality (r=-0.43, P=0.05). Finally, the number of days with illness symptoms was positively correlated to subjective measures of fatigue (r=0.48, P=0.02), worse sleep quality (r=0.61, P=0.007), and the degree to which training was affected by illness (r=0.78, P<0.0001). No significant correlations were observed between measures of wellbeing and resting cortisol concentrations.

Discussion

The novel findings of this study were that changes in resting sIL-6R concentration were observed during a prolonged period of exercise training, and specifically that resting sIL-6R levels in any given week appear to be inversely related to the volume of training performed in the previous week. Moreover, resting sIL-6R levels were related to several self-reported measures of wellbeing, supporting previous suggestions of the central effects of IL-6 signalling and further highlighting the potential role of 'trans-signalling' via sIL-6R in these processes. These findings suggest the important prospect that differences in the concentration of sIL-6R could be linked to increases in perceived stress, decreased mood, and impaired quality of sleep in athletes. When taken together it is possible that sIL-6R could represent a marker of training stress that is sensitive of changes on weekly basis.

In accordance with these findings, two previous studies have reported significant reductions in the circulating concentration of sIL-6R following a prolonged exercise training programme

in post-menopausal women (24.5 ± 5.2 to 22.4 ± 5.1 ng/ml) (44) and chronic heart failure patients (34.0 ± 3.0 to 29.2 ± 3.0 ng/ml) (1). The average concentration of sIL-6R in the present study (21.0 ± 4.6 ng/ml) appears lower than that reported in the two aforementioned studies; however, given that these studies reported significant reductions in sIL-6R following chronic training it is perhaps unsurprising that athletes display a relatively lower concentration than their non-athletic counterparts. With regard to resting cortisol concentrations, we observed a significant relationship with training volume, an observation that has been observed in previous longitudinal studies of highly trained endurance athletes (30). As such the physiological responses of the athletes in this study appear normal in the context of other literature. Also in agreement with previous literature (43), it was observed that the two squads providing samples early in the morning (Triathletes and Swimmers B) displayed significantly higher concentrations of cortisol than those from squads where samples were obtained in the afternoon (Rowers and Swimmers A) (Figure 2B). Interestingly this apparent diurnal effect was not evident in sIL-6R, the concentrations of which were negatively associated with perceived sleep quality. These results are in agreement with recent research suggesting that sleep increases the concentration of sIL-6R (7). While the limitations of subjective measurement of sleep should be acknowledged, this is an interesting finding and represents another example of the complex differences between IL-6 and sIL-6R. Specifically, sleep disturbance is reported to be associated with increased IL-6 (23) but reduced sIL-6R (7) which in the case of sIL-6R demonstrates a different relationship to those observed for other measures of wellbeing. We suggest that this is an area that warrants comprehensive further investigation in a more controlled environment.

It should be stressed that this is the first study to longitudinally monitor the concentration of sIL-6R in highly trained athletes during a prolonged period of training, and a novel finding

was that sIL-6R concentrations were negatively correlated to the volume of training performed in the previous week ($r=-0.74$, $P<0.005$) (Figure 3B). Another novel finding in this study was that higher levels of sIL-6R were associated with higher reported levels of stress ($r=0.64$, $P=0.004$) and worse mood ($r=0.49$, $P=0.02$). These data are in accordance with the apparent consensus that clinical depression can have an inflammatory aetiology (8), and that psychological mood state can be negatively affected by an up regulation in pro-inflammatory cytokines (22, 23). Given that self-reported measures of stress and mood are routinely reported as worse in overtrained athletes (15), and the fact that sIL-6R was not only related to these measures but also to weekly training distance, it appears plausible that high levels of sIL-6R could predispose athletes to some of the regular symptoms associated with overtraining and that sIL-6R may be sensitive measure of the relative stress of training performed on a weekly basis.

In the current study, athletes reported illness symptoms for an average of 17.4 ± 8.5 days throughout the study (18 weeks) with an average of approximately eight athletes (or ~30% of the entire cohort) reporting symptoms during each week. There are discrepancies among previous similar longitudinal studies with some reporting fewer illnesses (26) and others a greater number (9) than the current study. While this fact makes it difficult to make comparisons to other studies, reported illness rates in the present study appear similar to those of a study employing a similar method of subjective reporting of illness symptoms (5). In the current study, the number of days with illness symptoms was significantly correlated to subjective ratings of fatigue at rest, perceived sleep quality, and the degree to which training had been affected by illness. These data indicate that athletes were negatively impacted by URS, and therefore support the notion that illness could negatively impact upon either training performance or the ability to maintain a high training volume.

It has previously been suggested that the increased rates of URS experienced by some athletes could be due to an excessive pro-inflammatory response as indicated by a higher IL-6 response to exercise (3). As such it was hypothesised that a higher sIL-6R might predispose athletes to a greater frequency of URS. In the current study sIL-6R tended to be higher for the most illness-prone compared to the least illness-prone athletes (upper Vs lower quartile of days with illness symptoms), although the difference was not significant (P=0.08). It is possible that with a larger sample size or a more prolonged period of monitoring a significant difference may have been found. However, a further complicating factor is that sIL-6R is likely only of relevance when URS are inflammatory in origin; current research estimates that this is the case in approximately 30-40% of incidents where URS are reported (2). Therefore, while differences in the concentration of sIL-6R may play a part in the higher rate of URS experienced by some athletes, it should be noted that sIL-6R is unlikely to be the sole predictor of URS.

Given the pro-inflammatory role ascribed to sIL-6R and its association with a number of inflammatory diseases (36), it is plausible that exercise-induced reductions in sIL-6R could be partly responsible for certain exercise-induced health benefits, especially in people with inflammatory conditions. This contention is supported by studies reporting that blockade of IL-6 signalling, via tocilizumab, significantly reduces disease severity in inflammatory conditions such as rheumatoid arthritis and Castleman syndrome (27). More recent studies have identified selective blockade of sIL-6R as a potential therapeutic target for pharmacological intervention, and have shown that such a blockade reduces atherosclerotic plaque development in a mouse model of atherosclerosis (38). While the present study does not provide any mechanistic insight into the mechanisms of how sIL-6R concentration is

regulated, it does shed some light onto the pattern of regulation in the context of exercise training. Given this pattern of regulation, our study supports the notion that the anti-inflammatory effects of exercise are related to the volume of exercise performed. However, caution should be applied when interpreting the data from this study given that the results are from highly trained endurance athletes, and hence the responses seen here may not necessarily be representative of what might be seen in untrained or indeed diseased populations. Nevertheless, we recommend that future work investigating exercise related anti-inflammatory effects should include sIL-6R, and specifically should further examine exercise-induced changes in sIL-6R and their relationship to chronic inflammatory diseases.

In summary, the results of this study provide further evidence that sIL-6R is reduced by exercise training, and demonstrate for the first time that this response is related to the volume of training performed. Moreover, given that sIL-6R levels were related to psychological measures of stress and mood, and appeared to be higher in athletes reporting the most URS, this study provides evidence that IL-6 trans-signalling via sIL-6R may play a role in some aspects of overreaching and that its assessment for quantifying the effects of prior training should be considered, especially in athletes where high volumes are undertaken.

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References

1. Adamopoulos S, Parissis J, Karatzas D, et al. Physical training modulates proinflammatory cytokines and the soluble Fas/soluble Fas ligand system in patients with chronic heart failure. *J Am Coll Cardiol* 2002;39(4):653–63.
2. Cox AJ, Gleeson M, Pyne DB, Callister R, Hopkins WG, Fricker PA. Clinical and laboratory evaluation of upper respiratory symptoms in elite athletes. *Clin J Sport Med* 2008;18(5):438–45.
3. Cox AJ, Pyne DB, Saunders PU, Callister R, Gleeson M. Cytokine responses to treadmill running in healthy and illness-prone athletes. *Med Sci Sports Exerc* 2007;39(11):1918–26.
4. Cullen T, Thomas AW, Webb R, Hughes MG. The relationship between interleukin-6 in saliva, venous and capillary plasma, at rest and in response to exercise. *Cytokine* 2015;71(2):397–400.
5. Cunniffe B, Griffiths H, Proctor W, Davies B, Baker JS, Jones KP. Mucosal immunity and illness incidence in elite rugby union players across a season. *Med Sci Sports Exerc* 2011;43(3):388–97.
6. De Vries J, Michielsen HJ, Van Heck GL. Assessment of fatigue among working people: a comparison of six questionnaires. *Occup Environ Med* 2003;60 Suppl 1:i10–5.
7. Dimitrov S, Lange T, Benedict C, et al. Sleep enhances IL-6 trans-signaling in humans. *FASEB J* 2006;20(12):2174–6.
8. Dowlati Y, Herrmann N, Swardfager W, et al. A Meta-Analysis of Cytokines in Major Depression. *Biol Psychiatry* 2010;67(5):446–57.

9. Fahlman MM, Engels H-J. Mucosal IgA and URTI in American College Football Players: A Year Longitudinal Study. *Med Sci Sport Exerc* 2005;37(3):374–80.
10. Gleeson M. Immune function in sport and exercise [Internet]. *J Appl Physiol* 2007;103(2):693–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17303714
11. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease [Internet]. *Nat Rev Immunol* 2011;11(9):607–15. Available from: http://dx.doi.org/10.1038/nri3041
12. Gleeson M, McDonald WA, Pyne DB, et al. Salivary IgA levels and infection risk in elite swimmers. *Med Sci Sports Exerc* 1999;31(1):67–73.
13. Gray SR, Ratkevicius A, Wackerhage H, Coats P, Nimmo M a. The effect of interleukin-6 and the interleukin-6 receptor on glucose transport in mouse skeletal muscle. *Exp Physiol* 2009;94(2004):899–905.
14. Gray SR, Robinson M, Nimmo MA. Response of plasma IL-6 and its soluble receptors during submaximal exercise to fatigue in sedentary middle-aged men. *Cell Stress Chaperones* 2008;13(2):247–51.
15. Halson SL, Jeukendrup AE. Does overtraining exist? An analysis of overreaching and overtraining research. *Sport Med* 2004;34(14):967–81.
16. Halson SL, Lancaster GI, Jeukendrup AE, Gleeson M. Immunological responses to overreaching in cyclists. *Med Sci Sports Exerc* 2003;35(5):854–61.
17. Jones SA. The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *FASEB J* 2001;15(1):43–58.
18. Kallen KJ. The role of transsignalling via the agonistic soluble IL-6 receptor in human diseases. *Biochim Biophys Acta - Mol Cell Res* 2002;1592(3):323–43.
19. Keller C, Steensberg A, Hansen AK, et al. Effect of exercise, training, and glycogen

availability on IL-6 receptor expression in human skeletal muscle. [Internet]. *J Appl Physiol* 2005;99(6):2075–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16099893

20. Leggate M, Carter WG, Evans MJC, Vennard R a., Sribala-Sundaram S, Nimmo M a. Determination of inflammatory and prominent proteomic changes in plasma and adipose tissue after high-intensity intermittent training in overweight and obese males. *J Appl Physiol* 2012;112(8):1353–60.

21. Leggate M, Nowell MA, Jones SA, Nimmo MA. The response of interleukin-6 and soluble interleukin-6 receptor isoforms following intermittent high intensity and continuous moderate intensity cycling. *Cell Stress Chaperones* 2010;15(6):827–33.

22. Main LC, Dawson B, Grove JR, Landers GJ, Goodman C. Impact of training on changes in perceived stress and cytokine production. [Internet]. *Res Sports Med* 2009;17(2):121–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19479630

23. Main LC, Dawson B, Heel K, Grove JR, Landers GJ, Goodman C. Relationship between inflammatory cytokines and self-report measures of training overload. [Internet]. *Res Sport Med* 2010;18(2):127–39. Available from: http://hdl.handle.net/10536/DRO/DU:30031231%5Cnhttp://www.informaworld.com/smpp/title~content=t713926139%5Cnhttp://dx.doi.org/10.1080/15438621003627133%5Cnhttp://www.informaworld.com/

24. McLean BD, Coutts AJ, Kelly V, McGuigan MR, Cormack SJ. Neuromuscular, endocrine, and perceptual fatigue responses during different length between-match microcycles in professional rugby league players. *Int J Sports Physiol Perform* 2010;5(3):367–83.

25. Morgan WP, Brown DR, Raglin JS, O'Connor PJ, Ellickson KA. Psychological Monitoring of Overtraining and Staleness. *Br J Sports Med* 1987;21(3):107–14.

26. Neville V, Gleeson M, Folland JP. Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. *Med Sci Sports Exerc* 2008;40(7):1228–36.
27. Nishimoto N, Sasai M, Shima Y, et al. Improvement in Castleman's disease by humanized anti-interleukin-6 receptor antibody therapy. *Blood* 2000;95(1):56–61.
28. Patterson S, Reid S, Gray S, Nimmo M. The response of plasma interleukin-6 and its soluble receptors to exercise in the cold in humans. *J Sports Sci* 2008;26(788992146):927–33.
29. Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. [Internet]. *J Appl Physiol* 2005;98(4):1154–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15772055
30. Purge P, Jürimäe J, Jürimäe T. Hormonal and psychological adaptation in elite male rowers during prolonged training. [Internet]. *J Sports Sci* 2006;24(10):1075–82. Available from: http://www.tandfonline.com/doi/abs/10.1080/02640410500432516
31. Robson-Ansley P, Barwood M, Canavan J, et al. The effect of repeated endurance exercise on IL-6 and sIL-6R and their relationship with sensations of fatigue at rest [Internet]. *Cytokine* 2009;45(2):111–6. Available from: http://dx.doi.org/10.1016/j.cyto.2008.11.006
32. Robson-Ansley P, Cockburn E, Walshe I, Stevenson E, Nimmo M. The effect of exercise on plasma soluble IL-6 receptor concentration: A dichotomous response. *Exerc Immunol Rev* 2010;16:56–76.
33. Robson-Ansley PJ, Blannin A, Gleeson M. Elevated plasma interleukin-6 levels in trained male triathletes following an acute period of intense interval training. *Eur J Appl Physiol* 2007;99(4):353–60.
34. Robson-Ansley PJ, de Milander L, Collins M, Noakes TD. Acute interleukin-6 administration impairs athletic performance in healthy, trained male runners. *Can J*

Appl Physiol 2004;29(4):411–8.

35. Robson PJ. Elucidating the unexplained underperformance syndrome in endurance athletes: The interleukin-6 hypothesis. *Sport Med* 2003;33(10):771–81.

36. Rose-John S. Il-6 trans-signaling via the soluble IL-6 receptor: Importance for the proinflammatory activities of IL-6. *Int J Biol Sci* 2012;8(9):1237–47.

37. Schöbitz B, Pezeshki G, Pohl T, et al. Soluble interleukin-6 (IL-6) receptor augments central effects of IL-6 in vivo. *FASEB J* 1995;9(8):659–64.

38. Schuett H, Oestreich R, Waetzig GH, et al. Transsignaling of interleukin-6 crucially contributes to atherosclerosis in mice. *Arterioscler Thromb Vasc Biol* 2012;32(2):281–90.

39. Steensberg A, Fischer CP, Keller C, Møller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *Am J Physiol Endocrinol Metab* 2003;285(2):E433–7.

40. Tanaka T, Narazaki M, Kishimoto T. Il-6 in inflammation, Immunity, And disease. *Cold Spring Harb Perspect Biol* 2014;6(10)

41. Walsh NP, Gleeson M, Shephard RJ, et al. Position statement part one: Immune function and exercise. *Exerc Immunol Rev* 2011;17:6–63.

42. Walshe I, Robson-Ansley P, St Clair Gibson A, Lawrence C, Thompson KG, Ansley L. The reliability of the IL-6, sIL-6R and sgp130 response to a preloaded time trial. *Eur J Appl Physiol* 2010;110(3):619–25.

43. Wittert G a, Livesey JH, Espiner E a, Donald R a. Adaptation of the hypothalamopituitary adrenal axis to chronic exercise stress in humans. *Med Sci Sports Exerc* 1996;28(8):1015–9.

44. You T, Berman DM, Ryan AS, Nicklas BJ. Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women. *J*

Clin Endocrinol Metab 2004;89(4):1739–46.

Figure Legends

Figure 1. Schematic of the sampling procedure and study design.

Figure 2: Mean sIL-6R (A) and cortisol (B) for each squad of athletes for the entire study. *= Significantly different to Swimmers A and Rowers; #= Significantly different to Triathletes and Swimmers B ($P<0.05$).

Figure 3: Prescribed weekly training distance and mean sIL-6R on a weekly basis for a single squad of 10 swimmers (A). The relationship between the prescribed weekly training distance and mean sIL-6R concentration in the following week for a squad of 10 swimmers, $r=-0.74$, $P=0.005$ (B).

Figure 4: Prescribed weekly training distance and mean cortisol on a monthly basis for a single squad of 10 swimmers (A). The relationship between prescribed weekly training distance and mean cortisol concentration in the following week for a squad of 10 swimmers, ($r=0.89$, $P=0.045$) (B).