sIL-6R Is Related to Weekly Training Mileage and Psychological Well-being in Athletes

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ABSTRACT

CULLEN, T., A. W. THOMAS, R. WEBB, T. PHILLIPS, and M. G. HUGHES. sIL-6R Is Related to Weekly Training Mileage and Psychological Well-being in Athletes. *Med. Sci. Sports Exerc.*, Vol. 49, No. 6, pp. 1176–1183, 2017. **Introduction**: Interleukin 6 (IL-6) has been ascribed both positive and negative roles in the context of exercise and training. The dichotomous nature of IL-6 signaling seems 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 well-being, and upper respiratory symptoms in highly trained endurance athletes. **Methods**: Twenty-nine athletes provided resting blood samples and completed well-being and illness monitoring questionnaires on a weekly basis for a period of 18 wk during a winter training block. **Results**: Upper respiratory symptoms were not correlated to concentrations of sIL-6R or cortisol, but there was a nonsignificant 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 with the least ill (23.7 ± 4.3 vs 20.1 ± 3.8 ng·mL^{-1}). 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 subgroup 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. **Key Words:** sIL-6R, ATHLETES, FATIGUE, OVERREACHING

Interleukin 6 (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 etiology such as type 2 diabetes, rheumatoid arthritis (40), and clinical depression (8).

The dichotomous nature of IL-6 signaling seems 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 signaling pathways termed "classical" and "trans-signaling," respectively (17). Classical signaling is limited to cells and organs that possess IL-6R such as hepatocytes and leukocytes (17), brain (37), and skeletal muscle (19). By 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-signaling (i.e., sIL-6R interacting with the ubiquitously expressed cell-surface receptor gp130 to trigger intracellular responses within target cells [16]). Trans-signaling through sIL-6R is predominantly pro-inflammatory and seems 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

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undertaking high volumes of training. Although 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. Although 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 signaling 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 after 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 seems that the circulating concentration of sIL-6R is significantly reduced after a period of prolonged exercise training (1,44), and a recent study reported a significant reduction in sIL-6R after only 2 wk of high-intensity training (20). To our knowledge, no study has yet investigated changes in sIL-6R throughout prolonged training programs, but given the apparent importance of sIL-6R signaling 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 the sIL-6R, the subjective measures of well-being, and the reported rate of URS during an 18-wk winter training period.

METHODS

Participants

Twenty-nine (16 males and 13 females) endurance-trained athletes volunteered to participate in the study and provided informed consent before 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 organization responsible for sports science support services to elite athletes in Wales). All athletes within the study were typically training for approximately 20 $h \cdot wk^{-1}$ 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-wk winter training period (October 2013–February 2014), which took place after a period of relative rest after the end of the previous competitive season. Throughout the study, athletes conducted 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 nonfasted state and completed an illness and well-being questionnaire on a weekly basis. Data were 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 Fig. 1 for a schematic illustration of the study design).

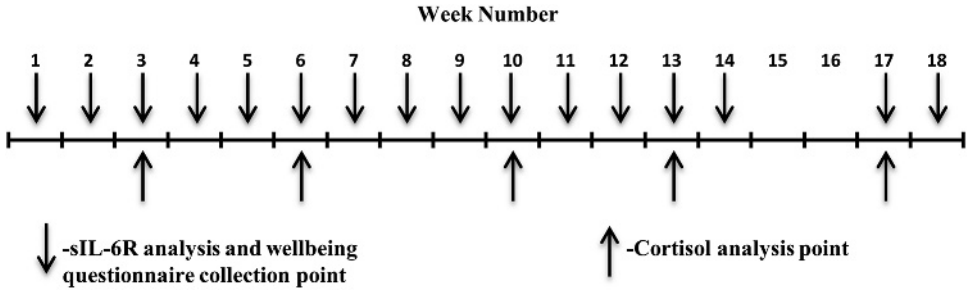

FIGURE 1—Schematic of the sampling procedure and study design.

To avoid the acute effects of individual exercise bouts, these measurements were obtained a minimum of 24 h after the most recent training session. For each squad, data collection took place at the same time of day before training on the same day of the week (individual squads were tested on different days of the week to comply with their normal training routine). The triathletes and swimmers B provided blood samples before their morning training sessions at 0600 h, whereas the rowers and swimmers A provided samples in the afternoon between 1400 and 1600 h. Although athletes from four separate squads were used, the entire study was conducted during 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 well-being. The concentration of sIL-6R was measured every week, whereas cortisol was measured every month. In a subgroup 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 to ascertain how these physiological variables responded to training on a weekly basis. Similar to the methods described by Purge et al. (30), 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.

Illness and well-being questionnaire. The illness and well-being questionnaire used throughout the present study was a nine-point questionnaire used internally at Sport Wales to monitor self-reported aspects of well-being and illness. The questionnaire is a modified version of the well-being questionnaire used in the study of McLean et al. (24); similar questionnaires have good reliability and validity and have been shown to be sensitive to fatigue in Professional Rugby League (6). Questionnaire data were analyzed 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 several sports (22,25).

Athletes were asked to rate the following categories on a five-point scale: 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. Before completing the questionnaire, athletes were provided with a full explanation of each question. With regard 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 is reported when using a self-reported questionnaire compared with 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 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, 3000*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 (R&D Systems Ltd., Abingdon, UK). All additional materials and chemical reagents were purchased from R&D systems, and the assays were conducted in accordance with the manufacturer's instructions. Plasma samples were diluted at 1:100 with a commercially available diluent (DY997, R&D Systems Ltd.) before analysis of sIL-6R, and 1:20 before analysis of cortisol, 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^{-1}. The cortisol assay had an intra-assay CV of 7.3% ± 3.9% across a range of 0.156–10 ng·mL^{-1}. 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).

Statistical Analysis

A one-way ANOVA was used to analyze differences in mean sIL-6R and cortisol values between each squad of athletes. A Bonferroni *post hoc* test was conducted to analyze 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 well-being, and illness measures during 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 because of logistical reasons or personal circumstance, and so previous researchers have recommended regression modeling when studying URI risk in athletes, as it has been reported to be robust when data are missing (12). Athletes were categorized 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 regression analysis, cortisol concentrations were expressed

as an individual's relative cortisol concentration, defined here as the percentage difference compared with 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. During the entirety of the study, a total of 293 capillary blood samples were analyzed with a mean sIL-6R of 21.0 ± 4.6 ng·mL^{-1}. There was no significant difference in mean sIL-6R between squads of athletes (triathletes = 18.7 ± 4.6 ng·mL^{-1}, rowers = 22.3 ± 4.5 ng·mL^{-1}, swimmers A = 22.3 ± 3.3 ng·mL^{-1}, swimmers B = 20.4 ± 5.2 ng·mL^{-1} Fig. 2A). A significant difference was found for resting cortisol concentration between groups ($P < 0.001$). Triathletes had significantly higher resting cortisol concentrations than rowers and swimmers A (mean difference = 43.4 and 44.4 pg·mL^{-1}) as did swimmers B (mean difference = 53.9 and 55.2 pg·mL^{-1}) (Fig. 2B). In a subgroup of 10 swimmers (swimmers B), prescribed training mileage was 64.6 ± 21.1 km·wk^{-1} (range = 19.6–89 km·wk^{-1}), and sIL-6R levels on any given week were negatively correlated with the volume of training prescribed in the previous week ($r = -0.74$, $P < 0.005$) (Figs. 3A and 3B), whereas cortisol levels (as analyzed on the same basis) showed a positive correlation with training volume ($r = 0.89$, $P = 0.045$) (Figs. 4A and 4B). Finally, sIL-6R was negatively correlated with cortisol concentration ($r = -0.17$, $P = 0.04$).

Illness and well-being monitoring. All athletes reported experiencing URS at some point throughout the study. Athletes reported URS for an average of 17.4 ± 8.5 d (range 1–40) throughout the study, whereas the number of athletes reporting URS per week ranged from 5 to 12 (mean ± SD = 8.4 ± 2.6). On the basis of 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 nonsignificant trend ($P = 0.08$) for a higher average sIL-6R concentration in the most ill quartile compared with the least ill quartile of athletes (23.7 ± 4.3 vs 20.1 ± 3.8 ng·mL^{-1}).

With regard to subjective measures of well-being, 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 degree to which training was affected by illness ($r = 0.78$, $P < 0.0001$). No significant correlations were observed between measures of well-being and resting cortisol concentrations.

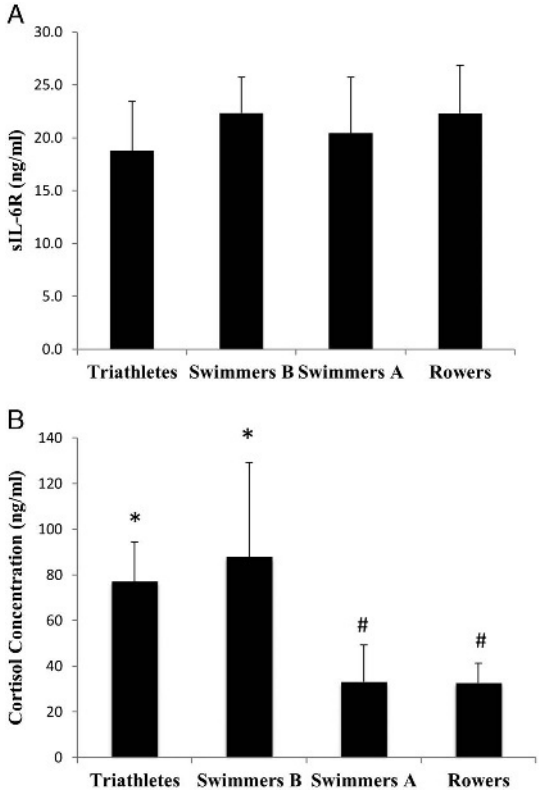

FIGURE 2—Mean sIL-6R (A) and cortisol (B) for each squad of athletes for the entire study. *Significantly different to rowers and swimmers A. #Significantly different to triathletes and swimmers B ($P < 0.05$).

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 seem 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 well-being, supporting previous suggestions of the central effects of IL-6 signaling and further highlighting the potential role of "trans-signaling" 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 after a prolonged exercise training program in postmenopausal women (24.5 ± 5.2 to 22.4 ± 5.1 ng·mL^{-1}) (44) and chronic heart failure patients (34.0 ± 3.0 to 29.2 ± 3.0 ng·mL^{-1}) (1). The average concentration of sIL-6R in the present study (21.0 ± 4.6 ng·mL^{-1}) seems lower than that reported in the two aforementioned studies; however, given that these studies reported significant reductions in sIL-6R after chronic training, it is perhaps unsurprising that athletes display a relatively lower concentration than their nonathletic

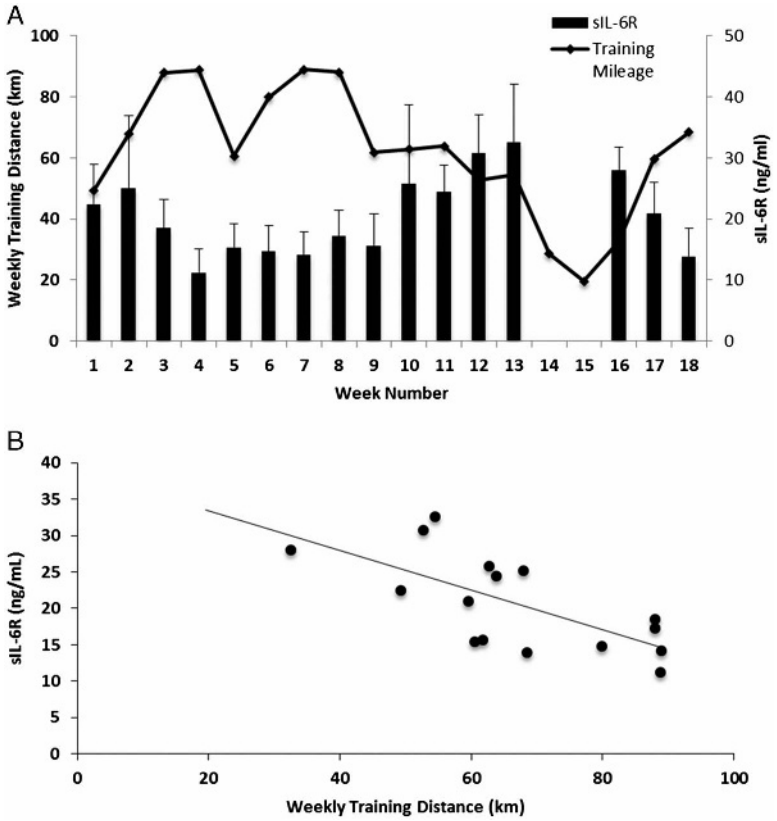

FIGURE 3—A, Prescribed weekly training distance and mean sIL-6R on a weekly basis for a single squad of 10 swimmers. B, The relationship between the prescribed weekly training distance and mean sIL-6R concentration in the following week for a squad of 10 swimmers ($r = -0.74$, $P = 0.005$).

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 seem 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) (Fig. 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). Although 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 well-being. 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 ($r = -0.74$, $P < 0.005$) (Fig. 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 etiology (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 seems 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 d throughout the study (18 wk) 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. Although this fact makes it difficult to make comparisons to other

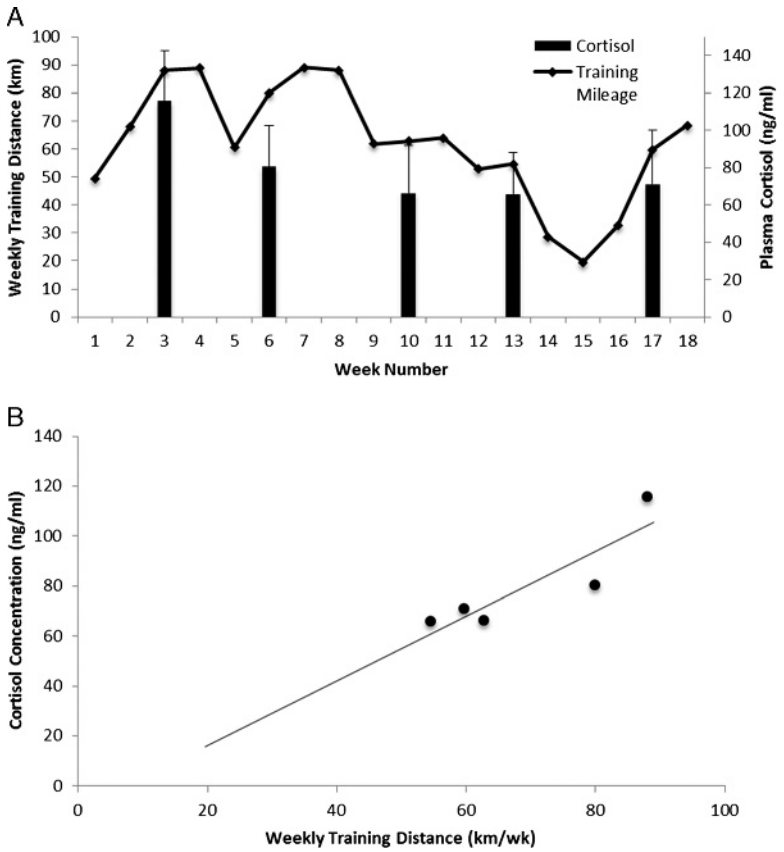

FIGURE 4—A, Prescribed weekly training distance and mean cortisol on a monthly basis for a single squad of 10 swimmers. B, The relationship between prescribed weekly training distance and mean cortisol concentration in the following week for a squad of 10 swimmers ($r = 0.89$, $P = 0.045$).

studies, reported illness rates in the present study seem similar to those of a study using 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 the subjective ratings of fatigue at rest, the perceived sleep quality, and the degree to which training had been affected by illness. These data indicate that athletes were negatively affected by URS and, therefore, support the notion that illness could negatively affect either training performance or 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 hypothesized 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 with 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, although 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 several 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 signaling, 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). Although 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 seemed to be higher in athletes reporting the most URS, this study provides evidence that IL-6 trans-signaling 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.

The authors wholeheartedly thank the athletes, coaches, and support staff that facilitated the study. The authors declare that they have no conflicts of interest or sources of income relating to the research. The data presented within are presented clearly, honestly, and without fabrication, falsification, or inappropriate manipulation. The results of the study do not constitute endorsement by the American College of Sports Medicine.

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(1 Suppl):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 HJ. Mucosal IgA and URTI in American college football players: a year longitudinal study. *Med Sci Sports Exerc*. 2005;37(3):374–80.
10. Gleeson M. Immune function in sport and exercise. *J Appl Physiol (1985)*. 2007;103(2):693–9.
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. *Nat Rev Immunol*. 2011;11(9):607–15.
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 MA. The effect of interleukin-6 and the interleukin-6 receptor on glucose transport in mouse skeletal muscle. *Exp Physiol*. 2009;94(8):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. *Sports 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, Horiuchi S, Topley N, Yamamoto N, Fuller GM. 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*. 2002;1592(3):323–43.
19. Keller C, Steensberg A, Hansen AK, Fischer CP, Plomgaard P, Pedersen BK. Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle. *J Appl Physiol (1985)*. 2005;99(6):2075–9.
20. Leggate M, Carter WG, Evans MJ, Vennard RA, Sribala-Sundaram S, Nimmo MA. 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 (1985)*. 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. *Res Sports Med*. 2009;17(2):121–32.
23. Main LC, Dawson B, Heel K, Grove JR, Landers GJ, Goodman C. Relationship between inflammatory cytokines and self-report measures of training overload. *Res Sports Med*. 2010;18(2):127–39.
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(9):927–33.

29. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. *J Appl Physiol (1985)*. 2005;98(4):1154–62.
30. Purge P, Jürimäe J, Jürimäe T. Hormonal and psychological adaptation in elite male rowers during prolonged training. *J Sports Sci*. 2006;24(10):1075–82.
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.
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. *Sports Med*. 2003;33(10):771–81.
36. Rose-John S. Il-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory 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):a016295.
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 GA, Livesey JH, Espiner EA, Donald RA. 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.