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The effect of repeated endurance exercise on IL-6 and sIL-6R and their relationship with sensations of fatigue at rest

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ABSTRACT

Strenuous, prolonged exercise increases interleukin-6 (IL-6) release. The effect of IL-6 is dependent on the availability of IL-6 receptors. Few studies have addressed the impact of exercise on IL-6 receptor levels or procalcitonin (PCT), an indicator of systemic inflammation. Changes in these molecules may give insight into cytokine-related mechanisms underlying exercise-related fatigue. Thirteen trained male subjects partook in the study. They cycled a total distance of 468 km over 6 days. Blood samples were obtained prior to and immediately following Day 1 of the study and then each morning prior to exercise. Blood samples were analysed for plasma IL-6, soluble IL-6 receptor (sIL-6R), C-reactive protein (CRP), PCT, creatine kinase (CK) and cortisol concentrations. Subjects also completed mood state questionnaires each day prior to exercise. IL-6 was elevated immediately post-exercise on Day 1 but was unchanged at rest for the duration of the event. In contrast, sIL-6R, CRP, PCT and CK concentrations were unchanged immediately post-exercise on Day 1 but were significantly elevated at rest over the duration of the event compared with pre-event baseline. sIL-6R was highly correlated to CRP. Cortisol concentrations remained unchanged at all time points. In conclusion, strenuous, prolonged exercise stimulated an acute phase response which was maintained throughout the 6-day event. sIL-6R increase is associated with CRP and may affect subjective sensations of post-exercise fatigue at rest.

1. Introduction

Strenuous, prolonged exercise has consistently been shown to increase concentrations of cytokines [1,2]; in particular interleukin-6 (IL-6), which is produced in greater amounts than any other cytokine [3,4]. IL-6 is a pleiotropic cytokine with a wide range of biological activities, such as support of erythropoiesis, regulation of immune system responses and generation of acute phase reactions such as C-reactive protein (CRP) [5] as well as having a metabolic role inducing counter-regulatory hormones e.g. cortisol, hepatic glucose output and lipolysis [6,7]. During physical exercise IL-6 is predominantly produced within the working skeletal muscles [8,9] and this production can account for the exercise-induced increase in plasma IL-6 [10]. This journal has also recently reported that prolonged exercise training results in an adaptive IL-6 response that attenuates abrupt changes in IL-6 concentrations [11].

The effect of IL-6 on biological systems is dependent on the availability of IL-6 receptors, both membrane bound and soluble (sIL-6R) forms [12]. Initiation of IL-6 signalling occurs when IL-6 is bound to the IL-6 receptor and the signal transducing receptor gp130 [13]. Elevated sIL-6R levels can increase sensitivity to IL-6; when IL-6 is bound to sIL-6R, the IL-6/sIL-6R complex acts as an agonist that is capable of stimulating cell types that, alone, are not innately responsive to IL-6. Increased levels of sIL-6R have also been demonstrated to increase the responsivity of the brain to IL-6 [14–16] and cause a greater suppression of locomotor activity and general sickness behaviour above that of raised IL-6 levels alone [12]. When the sIL-6R receptor is blocked in patients with previously high circulating levels of IL-6 by an anti-IL-6 receptor antibody, fatigue and fever are attenuated [17].

To date, few studies have addressed the impact of exercise on sIL-6R levels and findings are conflicting [18–20]. Gray et al. [18] demonstrated elevated sIL-6R levels in sedentary middle-aged men following a submaximal bout of cycling to volitional exhaustion however, other studies found acute exercise had no effect on sIL-6R levels [19,20]. Physiological concentrations of elevated CRP can increase sIL-6R concentrations by inducing receptor shedding from neutrophils [21,22] whereas this process is unaffected by exogenous administration of IL-6 [20,23] or reduced muscle

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glycogen levels [24]. The main impact of sIL-6R may be to upregulate the effects of IL-6 over a more prolonged period [12]. To date, the impact of prolonged repeated endurance exercise on sIL-6R levels and its relationship with other inflammatory markers has not been investigated. Given the relationship between sIL-6R and central fatigue its exploration may provide insight into cytokine-related mechanisms underlying exercise-related fatigue [25] and in particular, fatigue which is present following strenuous exercise.

IL-6 may also play a role in the release of procalcitonin (PCT), a sensitive indicator of systemic inflammation although its biological role remains unclear [26]. PCT is the pre-hormone of calcitonin and is mainly released during severe systemic inflammation caused by bacterial infection; but non-infectious stimuli such as proinflammatory cytokines [27] and tissue trauma [28] can also raise PCT levels. The liver [29] and peripheral blood leucocytes [26] are believed to be mainly responsible for the elevations in PCT during inflammatory episodes although research suggests a ubiquitous synthesis in almost every cell [30]. To date only one study has investigated the effect of exercise on PCT and determined that PCT was not elevated above baseline following a single bout of ultra-endurance running [31]. The results of that study suggest that the exercise-induced inflammatory response does not affect the mechanism for PCT production but this has not been investigated during multi-day repeated exercise bouts.

The aim of this study is to investigate changes in IL-6, sIL-6R and CRP over a prolonged endurance exercise event. We hypothesise that strenuous, prolonged exercise will transiently elevate systemic levels of IL-6 which will stimulate the hepatic release of CRP and PCT and that elevated CRP will be associated with increased plasma sIL-6R concentrations. Furthermore, sIL-6R levels will be persistently increased at rest and will relate to indices of fatigue.

2. Methods

2.1. Subjects

Thirteen endurance trained male mountain bike cyclists were volunteered to be subjects in the study (mean (±SD) age 35 (4.2) years, mass 76 (5.9) kg). None received medication or been unwell in the preceding two weeks. Subjects were informed of experimental procedures and gave written informed consent. Local ethics committee approval was obtained and all procedures were conducted according to the Declaration of Helsinki.

2.2. Experimental protocol

All subjects completed a mountain bike event that took place over a 6-day period with each subject covering a total distance of 468 km (14.6 km ascent). Eight of the subjects wore heart rate monitors so that average percentage of maximum heart rate for each stage could be calculated. Maximal heart rate was determined as the highest heart rate achieved during the 6-day event. Subjects consumed fluid *ad libitum* during the race. Dietary consumption was similar for all subjects during the cycle event as all food was provided by the race organisers. Pre-event baseline and post-event baseline blood samples were collected the morning of the first race-day and the morning after the final stage of the race, respectively, between 06:00 and 07:00 before subjects had broken their overnight fast. During the 6-day race event early morning venous blood samples were obtained daily from subjects following an overnight fast and prior to exercise between 06:00 and 07:00. On the first day of the race blood samples were also collected immediately after exercise; due to the nature of the terrain over which the race was conducted it was not logistically possible to collect samples at a similar time point for subsequent days.

2.3. Sample handling and analysis

Venous blood was collected into appropriate vacutainer tubes (Becton Dickinson, Swindon, UK) and centrifuged at 1500g for 10 min. The supernatant was aspirated and collected into Eppendorf tubes and immediately frozen at −80 °C until later analysis.

Plasma IL-6 and sIL-6 concentrations were analysed from K_3EDTA treated venous blood using an enzyme linked immunosorbent assay (R&D systems, Minneapolis, USA; Diaclone, Bolton, UK, respectively). Intra- and inter-assay coefficient of variations for IL-6 were less than 2% and 6%, respectively, and for sIL-6R were 7% and 6%, respectively. High sensitivity plasma CRP analysis was performed on the Siemens Medical solutions Advia 2400, UK with intra- and inter-assay coefficient of variations of less than 4% and 5%, respectively. Further aliquots of plasma were analysed to determine the concentrations of cortisol (IMMULITE®, DPC, Gwynedd, UK) with intra- and inter-assay coefficient of variations of less than 7% and 6%, respectively, and plasma procalcitonin (Brahms KRYPTOR sensitive assay BRAHMS AG, Berlin, Germany) with intra- and inter-assay coefficient of variations of less than 5% and 10%, respectively. Serum creatine kinase activity was also analysed (Randox Olympus AU 400, Antrium, UK). CK intra-assay coefficient of variation was 6.5%.

Minimum detectable plasma concentrations were 0.8 pg mL^{-1} for IL-6, 5 pg mL^{-1} for sIL-6R, 0.1 mg L^{-1} for CRP, 5.5 nmol L^{-1} for cortisol and 0.02 μg L^{-1} for PCT.

2.4. Questionnaire

Prior to the event, all subjects were familiarised with an abbreviated Profile of Mood State (POMS) questionnaire [32]. The POMS questionnaire [33] has been used extensively as a daily questionnaire which can be scored by the athlete during training to monitor stress [34–37]. POMS was completed prior to the event and each morning prior to exercise. The individual score for fatigue mood state was used as a measure of fatigue. In addition, by scoring individual mood states of fatigue, anger, depression, confusion, tension and vigour a score for total mood disturbance (TMD) was calculated; sum the five negative scores (fatigue, anger, depression, confusion, tension) add 100 and subtract the one positive mood score, vigour.

2.5. Data analysis

Data in the figures and tables are presented as mean values and standard deviation. Statistical evaluation of the results was carried out using a one-way repeated measures analysis of variance (ANOVA) with post hoc Tukey tests where applicable. Relationships between sIL-6R and fatigue and other inflammatory blood markers were analysed using multiple regression analysis. All statistical calculations were performed on InStat™ (Graphpad®). The accepted level of significance was $P \leqslant 0.05$.

3. Results

All 13 subjects completed a total of 468 km during the 6-day mountain bike event at an average intensity of 72% maximal heart rate (Table 1). Average daily temperature during the event was 17 °C with a maximum reached of 20 °C.

3.1. Changes in biochemical and hormonal variables compared with pre-event values

Plasma IL-6 was significantly elevated immediately following exercise on Day 1 (0.60 ± 0.85 pg mL^{-1} vs. 6.32 ± 2.49 pg mL^{-1};

Table 1
Characteristics of the 6-day mountain bike event.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Distance (km)	85	49	82	75	80	97
Ascent (m)	2600	1110	2580	3000	2330	3010
Mean time to complete stage (min)	375 (35)	160 (43)	277 (59)	378 (56)	308 (36)	391 (30)
Mean percentage of maximum heart rate during exercise ($n = 8$)	74 (5)	75 (2)	73 (4)	68 (4)	71 (3)	69 (5)

$P < 0.0001$) but returned to baseline by the following morning (1.17 ± 1.07 pg mL^{-1}) and for all subsequent mornings prior to exercise (Fig. 1). Plasma sIL-6R (Fig. 2), CRP (Fig. 3) and CK concentrations were unchanged immediately post-exercise on Day 1 (sIL-6R: 50.77 ± 6.03 pg mL^{-1}vs. 56.00 ± 12.33 pg mL^{-1}; CRP: 0.25 ± 0.15 mg L^{-1} vs. 0.32 ± 0.20 mg L^{-1}; CK: 199.68 ± 88.11 IU L^{-1} vs. 363.56 ± 149.85 IU L^{-1}) but were all elevated from baseline on subsequent mornings, pre-exercise (Fig. 4) ($P < 0.0001$). Pre-exercise PCT was not different from post-exercise concentrations (0.04 ± 0.02 μg L^{-1} vs. 0.06 ± 0.03 μg L^{-1}) but was elevated in the mornings on Days 2, 3, 4 and 5 (Fig. 5) ($P < 0.0001$) before returning to baseline levels on Days 6 and 7. sIL-6R was strongly correlated with CRP and CK over the 6 days of exercise, and to a lesser (but still significant) degree with IL-6; but not with PCT or cortisol (Table 2). Plasma cortisol concentrations did not change immediately post-exercise on Day 1 (602.53 ± 75.45 nmol L^{-1} vs. 465.80 ± 159.73 nmol L^{-1}) and remained unchanged each subsequent morning prior to exercise (Table 3).

3.2. Changes in TMD and fatigue compared with pre-event values

Completed POMS questionnaires were collected from 12 of the subjects. Total mood state significantly worsened from Day 4 to Day 6 compared to pre-event baseline ($P < 0.0001$) (Table 3). Subjects reported heightened sensations of fatigue the morning following exercise on Days 4, 5, 6 and post-event compared with pre-event baseline ($P < 0.0001$). The changes in fatigue were significantly correlated with sIL-6R levels ($r = 0.40$; $P = 0.02$) but displayed no such significant relationship with CRP ($r = 0.08$; $P = 0.78$), IL-6 ($r = 0.17$; $P = 0.27$), CK ($r = 0.16$; $P = 0.78$) or cortisol ($r = 0.08$; $P = 0.91$).

4. Discussion

The main finding of this study is that plasma sIL-6R levels were persistently elevated at rest during a 6-day bout of repeated prolonged strenuous exercise and related to general sensations of fatigue. Furthermore, elevations in resting sIL-6R levels occurred in the presence of normal serum IL-6 levels supporting our hypothesis that whilst IL-6 is transiently elevated post-endurance exercise, sIL-6R remains chronically elevated and may play a role in mediating fatigue at rest following strenuous exercise.

A key objective of the study was to determine if there was a relationship between fatigue, mood state and changes in IL-6 and sIL-6R. IL-6 is well known to contribute to general sickness behaviour in mice [38] and furthermore, Robson-Ansley [39] have previously demonstrated that exogenous administration of IL-6 prior to a treadmill time-trial impairs performance and heightens subjective sensations of fatigue. In the current study cyclists reported increasing mood disturbance and an increased sensation of fatigue as the event progressed, the latter of which correlated to morning levels of sIL-6R but notably not to plasma IL-6 concentrations. This

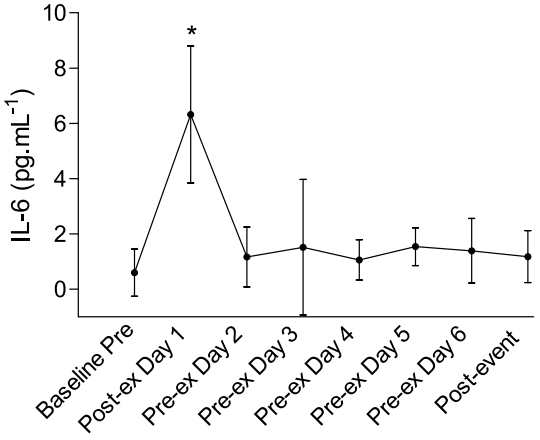

Fig. 1. The effect of a 6-day multi-day endurance mountain bike event on plasma IL-6 concentrations. * ($P < 0.0001$) significantly greater than baseline pre-event.

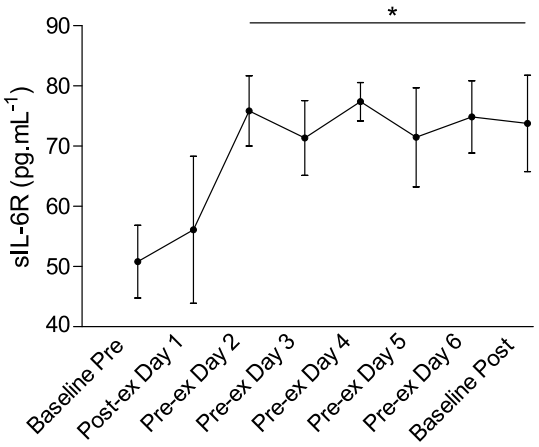

Fig. 2. The effect of a 6-day multi-day endurance mountain bike event on plasma sIL-6R concentrations. * ($P < 0.0001$) significantly greater than baseline pre-event.

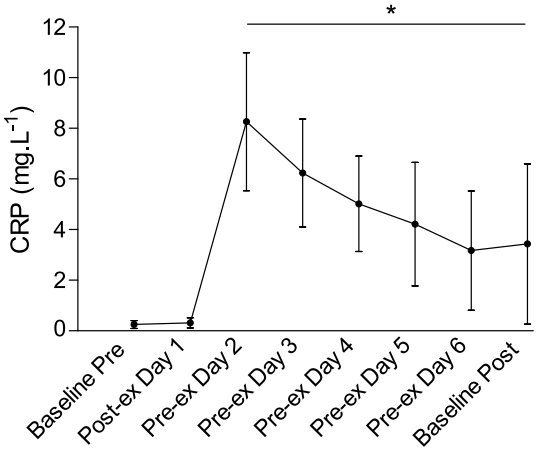

Fig. 3. The effect of a 6-day multi-day endurance mountain bike event on plasma CRP concentrations. * ($P < 0.0001$) significantly greater than baseline pre-event.

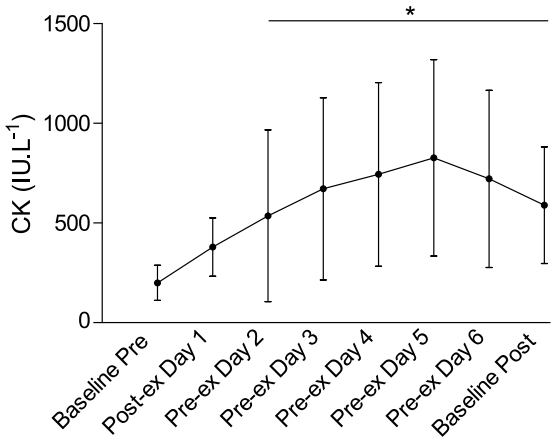

Fig. 4. The effect of a 6-day multi-day endurance mountain bike event on plasma CK concentrations. * ($P < 0.0001$) significantly greater than baseline pre-event.

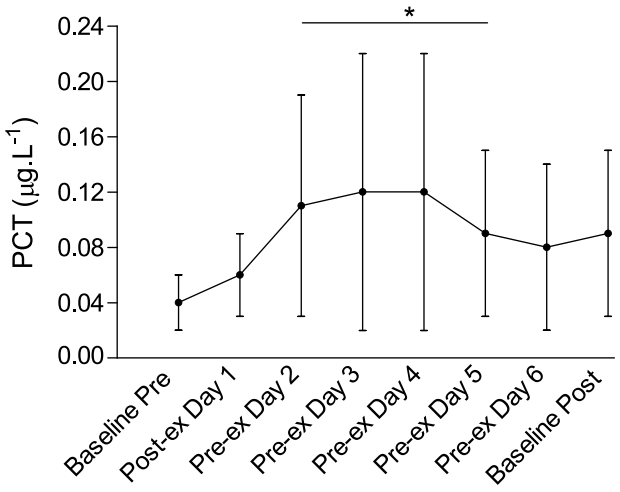

Fig. 5. The effect of a 6-day multi-day endurance mountain bike event on plasma PCT concentrations. * ($P < 0.0001$) significantly greater than baseline pre-event.

Table 2
Multiple regression analysis for variance in sIL-6R.

	R
CRP	0.54 ($P < 0.0001$)
CK	0.39 ($P = 0.0001$)
IL-6	−0.34 ($P = 0.019$)
PCT	0.24 ($P = 0.13$)
Cortisol	0.27 ($P = 0.17$)

relationship is supported by clinical studies that have found sIL-6R to be highly diagnostic of fatigue in breast cancer survivors [40]. Further studies utilising a control group are needed to investigate this relationship in the setting of a period of controlled intensified training or overreaching where external factors are minimised [41,42].

Keller et al. [19] previously reported no change in sIL-6R up to 24 h following 3 h of cycling exercise at a moderate intensity; the contrasting findings be explained by the higher intensity and longer exercise duration in the current study. More prolonged exercise elicits a greater inflammatory response and subsequent CRP release than shorter bouts (reviewed elsewhere [2]), which would have contributed to the higher sIL-6R levels reported in our study. Surprisingly, Gray et al. [18] reported a slight but significant rise in immediately post-exercise sIL-6R concentrations following a submaximal bout of cycling to exhaustion lasting approximately 1 h whereas we found no change in sIL-6R at this time point nor did Keller et al. [19]. The mechanism for the release of sIL-6R in Gray et al.'s study is not clear as CRP would not be elevated at this time [43,44], although the authors did report that they did not account for changes in plasma volume during the exercise period, which although minimal, may have accounted for some of the change in sIL-6R.

In contrast with sIL-6R, plasma IL-6 (although elevated immediately post-exercise) and cortisol remained unchanged the morning following the exercise bouts. The observation that IL-6 levels were transiently elevated immediately following a bout of exercise (Day 1) but had returned to baseline by the following morning supports previously documented kinetics of IL-6 to prolonged exercise (reviewed by Fischer [2]). Our finding that daily pre-exercise IL-6 levels remained unchanged each morning following a day of excessive exercise is in accordance with Halson et al. [41] who reported no change in IL-6 at this time point despite intensified cycle training.

Cycle ergometer exercise is predominantly composed of concentric muscle contractions and elevations in CK that occur following prolonged exercise would be as a consequence of muscle membrane peroxidation, which leaks CK from the muscle into the circulation [45]. However, Ortega et al. [46] have reported increased biochemical markers of muscle cell damage following a mountain bike race indicating an eccentric component to muscle loading during such events. Robson-Ansley et al. [42] have previously suggested that mechanical injury to the musculoskeletal system as a result of eccentric muscle action (indicated by CK) may induce either a chronic low level inflammatory response [47] or incomplete muscle glycogen replenishment [48] resulting in an associated elevation IL-6 levels. In this study the degree of muscle damage was significantly less than that reported by Robson-Ansley et al., which may explain why we did not see the same increase in IL-6 as they observed in their study.

Prolonged exercise at a moderate intensity has also been associated with elevations in cortisol concentration [49] but this was not observed in the current study. The relatively small increase in IL-6 and unchanged cortisol levels immediately post-exercise may be due to the ingestion of sports drinks, which were consumed *ad libitum* during each exercise by subjects. Previous studies have consistently shown that carbohydrate ingestion during prolonged exercise maintains glycaemic homeostasis resulting in a significant attenuation of exercise-induced IL-6 production as well as plasma cortisol release [3,9,50]. Furthermore, cortisol exhibits a strong circadian rhythm with levels peaking in the morning and reducing towards the evening [51], hence, any potential rises in cortisol levels immediately post-exercise may have been masked

Table 3
Mean (SD) biochemical and psychological parameters during the mountain bike event.

	Baseline pre-event	Post-ex day 1	Pre-ex day 2	Pre-ex day 3	Pre-ex day 4	Pre-ex day 5	Pre-ex day 6	Baseline post-event
Plasma cortisol (nmol L^{-1})	603 (75)	468 (160)	696 (72)	648 (128)	595 (120)	689 (166)	647 (122)	670 (121)
Total mood disturbance	94 (10)	—	93 (8)	102 (11)	108 (13)*	111 (19)*	108 (12)*	106 (12)
Fatigue	2 (2)	—	4 (3)	5 (4)	7 (3)*	9 (5)*	9 (5)*	9 (4)*

* Significantly greater than baseline pre-event; $P < 0.0001$.

by this rhythm as subjects started cycling at 08:00 h and finished in the mid-afternoon.

The mechanism underlying sIL-6R increases in this setting currently remain unclear and require further exploration. The relationship with CRP suggests this inflammatory mediator may play an important role. *In vitro* elevations in CRP within physiological ranges have been shown to induce shedding of sIL-6R from neutrophils [21] and our study supports this finding *in vivo*. Hepatocyte CRP production is increased by IL-6 exposure [52] and whilst we were only able to measure IL-6 immediately post-exercise on Day 1, it seems reasonable to assume that plasma IL-6 levels would have been elevated immediately following each day of exercise. The elevated IL-6 concentrations would have repeatedly triggered hepatic release of CRP thus acting indirectly to maintain the chronic elevation in plasma sIL-6R levels, which were observed for the duration of the event.

PCT has been identified as a sensitive marker of systemic inflammation and there exists significant literature underlining the importance of this peptide in disease (e.g. sepsis) [26]. Following endotoxin injection in healthy subjects plasma IL-6 and tumour necrosis factor-α peaked before the appearance of PCT which lead to the suggestion that cytokines may have a role in inducing the release of PCT [27]. The timeline for IL-6 and PCT kinetics following a bout of exercise in this study supports the possible role of IL-6 in mediating the release of PCT. Interestingly, pre-exercise PCT levels were only significantly elevated for days 2, 3, 4 and 5 of the event, which suggests a down regulation of PCT as the event progressed. However, the normal plasma concentration of PCT ranges from 0.0 to 0.5 $\mu g\ L^{-1}$; levels in excess of 2 $\mu g\ L^{-1}$ are suggestive of bacterial infection while levels between 0.5 and 2 $\mu g\ L^{-1}$ are observed in other non-infectious conditions [53]. Therefore, although there was an increase in plasma PCT during the event, it did not reach clinically significant levels. The extent and time course of the changes in our results support those recently reported following a 21-km running race [54]; the only other study we are aware of that has described the changes in PCT occurring with exercise. Further research is required to evaluate the source and significance of this marker in exercise.

In summary, we found that a strenuous, prolonged exercise stimulated an acute phase response which was evidenced by augmented levels of sIL-6R and CRP throughout the 6-day event. Elevations in sIL-6R related to post-exercise fatigue at rest and suggest further work is needed to explore the role of sIL-6R in the development of chronic fatigue and long term sickness behaviour experienced at rest. We further conclude that PCT secretion is stimulated by prolonged cycling exercise, probably due to cytokine kinetics, although whether there is any clinical relevance has yet to be elucidated.

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