

# Northumbria Research Link

Citation: Robson-Ansley, Paula, Cockburn, Emma, Walshe, Ian, Stevenson, Emma and Nimmo, Myra (2010) The effect of exercise on plasma soluble IL-6 receptor concentration: a dichotomous response. Exercise Immunology Review, 16. pp. 56-76. ISSN 1077-5552

Published by: Association for the Advancement of Sports Medicine

URL: <http://www.medizin.uni-tuebingen.de/transfusionsme...> <<http://www.medizin.uni-tuebingen.de/transfusionsmedizin/institut/eir/content/2010/56/article.pdf>>

This version was downloaded from Northumbria Research Link:  
<http://nrl.northumbria.ac.uk/1366/>

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: <http://nrl.northumbria.ac.uk/policies.html>

This document may differ from the final, published version of the research and has been made available online in accordance with publisher policies. To read and/or cite from the published version of the research, please visit the publisher's website (a subscription may be required.)

[www.northumbria.ac.uk/nrl](http://www.northumbria.ac.uk/nrl)



## ***The effect of exercise on plasma soluble IL-6 receptor concentration: a dichotomous response.***

**Paula Robson-Ansley, Emma Cockburn, Ian Walshe, Emma Stevenson, and Myra Nimmo\***

Department of Sports Sciences, Northumbria University, UK.

\*School of Sport, Exercise and Health Sciences, Loughborough University, UK.

### **ABSTRACT**

*The aim of this article is to review current literature on the response of soluble interleukin-6 receptor to exercise and identify a potential role for sIL-6R in skeletal muscle function. We also provide novel data on the impact of eccentric exercise on circulating levels. The aim of the research study was to investigate changes in plasma concentration of soluble interleukin-6 receptor (sIL-6R) and soluble glycoprotein 130 (sgp130) during recovery from exercise-induced muscle damage (EIMD) up to 72 h and their relationship with delayed onset muscle soreness (DOMS) and muscle function. 18 participants attended the laboratory on 4 consecutive days. On the first day, participants completed 6 sets of 10 repetitions of unilateral eccentric-concentric knee flexions at a test speed of 1.05 rad.s<sup>-1</sup> using a Cybex Isokentic dynamometer to induce muscle damage of the hamstrings. Prior to the eccentric exercise bout and each subsequent morning, following an overnight fast, participants had a venous blood sample taken which was centrifuged immediately and plasma frozen at -80°C until later analysis. Plasma IL-6 and sgp130 were unchanged at any time point during recovery but sIL-6R was significantly reduced at 48 h and 72 h post-exercise ( $p < 0.05$ ). Plasma sIL-6R was negatively correlated with DOMS at 48 h post EIMD ( $r = 0.45$ ,  $p < 0.05$ ) and peak muscle torque at 24 h and 48 h following EIMD ( $r = -.42$ ;  $p < 0.05$ ;  $r = -.57$ ;  $p < 0.01$  respectively). Our novel finding that sIL-6R concentrations are decreased 2 - 3 days following a single bout of EIMD which may reflect a regulatory mechanism controlling the influx of different leukocyte subpopulations into damaged tissue, although this needs to be confirmed by future studies. Our data suggests an association between sIL-6R, perception of pain and reduced peak muscle performance post-EIMD but further investigation is warranted to explore this relationship and implications for exercise performance.*

**Key Words:** Interleukin-6, soluble interleukin-6 receptor, glycoprotein 130, DOMS, eccentric exercise

---

*Address for Correspondence:*

Paula Robson-Ansley PhD, Department of Sport Sciences

Northumberland Building, Northumbria University

Newcastle Upon Tyne, NE1 8ST

Phone: 0191 227 7773, Fax: 0191 227 4713, Paula.ansley@unn.ac.uk

## INTRODUCTION

### *Background to sIL-6R*

Interleukin-6 (IL-6) is a multi-functional cytokine with a wide range of biological activities such as regulation of the immune system, generation of acute phase reactions (43) and also plays a key role in metabolism during exercise (63, 64). During physical exercise IL-6 is predominantly produced within the working skeletal muscles (37, 83) and this production can account for the exercise-induced increase in plasma IL-6 (85).

The effect of IL-6 on biological systems is dependent on the availability of IL-6 receptors (both membrane-bound and soluble forms). Initiation of IL-6 signalling occurs when IL-6 is bound to the IL-6 receptor and the ubiquitous signal transducing receptor glycoprotein 130 (gp130) (87) as illustrated in Figure 1. Express-

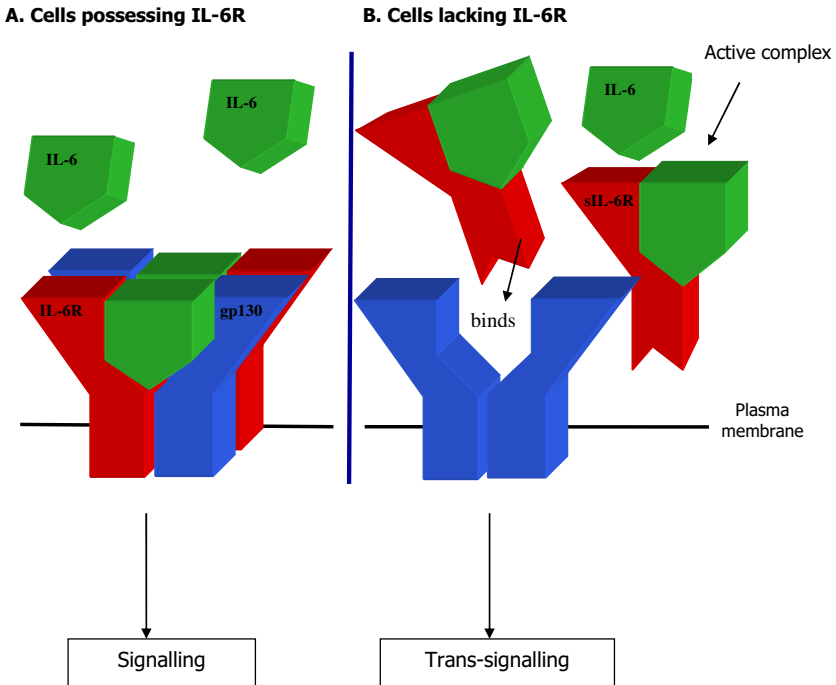


Figure 1. A schematic representation: **A.** The membrane-bound IL-6/IL-R/gp130 complex, consisting of 2 IL-6, 2 IL-6R and 2 gp130 molecules and **B.** The soluble IL-6/IL-6R complex in cells lacking the membrane-bound IL-6R. Adapted from (58).

sion of the membrane-bound form of the receptor is predominantly limited to hepatocytes and leucocytes with low expression in resting skeletal muscle (41). The circulating soluble IL-6 receptor (sIL-6R), in contrast to other soluble cytokine receptors such as the soluble tumour necrosis factor receptor, has an agonistic effect by further stimulating the biological activity of its ligand (35). sIL-6R

is a 50-55 kDa ligand binding protein derived from the extracellular part of the gp80 receptor by differential IL-6R mRNA splicing (DS-sIL-6R) or by proteolytic cleavage (shedding; PC-sIL-6R) of the cognate IL-6R. The two distinct isoforms control the overall properties of the soluble receptor, with evidence suggesting that in some tissues IL-6 signalling only occurs when the membrane form is lysed (91). The predominant sIL-6R isoform in plasma of healthy individuals is PC-sIL-6R (> 99%) but certain conditions, such as sleep, can influence the isoform ratios (15).

The mechanisms regulating plasma sIL-6R concentrations are unclear. sIL-6R is generated by direct production of an sIL-6R form through translation of alternatively spliced mRNAs or by proteolytic cleavage of membrane molecules. The predominant isoform of sIL-6R in plasma in healthy individuals is the membrane anchored proteolytically cleaved form (34). A decrease in plasma sIL-6R might be a consequence of several mechanisms. Firstly, an increased consumption of circulating sIL-6R owing to the formation of IL-6/sIL-6R complex in the presence of IL-6 and subsequent binding and internalization of the complex by effector cells expressing the gp130 where the complex is degraded may have occurred (97). Secondly, protein kinase C (PKC) appears to play a role in sIL-6R shedding and inhibition of PKC appears to have a down-regulating effect on sIL-6R production (50). Finally, increased renal clearance of sIL-6R cannot be dismissed. Very small amounts of sIL-6R in comparison to circulating levels are found in the urine of healthy individuals at rest (20).

#### *Effect of acute exercise on sIL-6R*

In contrast to the abundance of recent research investigating the effect of exercise on IL-6, relatively little research has been conducted on sIL-6R and exercise; hence, there are conflicting data on circulating sIL-6R concentrations immediately after and during recovery from exercise (Table 1).

After acute endurance exercise sIL-6R has been shown not to change (39, 41, 61, 66); although in these studies subject number has been small –around 6. Alternatively increases have been noted and peak levels occur from immediately after exercise to beyond 6 h after exercise (25, 69) Leggate *et al* unpublished data, Loughborough University, UK). The differing peak time points are likely due to differing exercise regimens and whether the data has been corrected for plasma volume. A novel finding in our own study (69) was that these levels were significantly correlated with fatigue and exercise load.

To date no research has been conducted on the influence of exercise on the sIL-6R isoform ratio but unpublished data from our laboratories suggest that the ratio is unaffected by prolonged endurance-type exercise (Walshe *et al*, Northumbria University, UK) and that during intermittent high intensity exercise the DS-sIL-6R component increases significantly (Nimmo, Loughborough University, UK).

The formation of the active binary complex (IL-6/sIL-6R) substantially extends the plasma half life of IL-6 (65) and mathematical modelling would suggest that 70% of the circulating IL-6 is in this form at rest (21) and that the complex

Table 1. Effect of exercise on plasma sIL-6R concentration and gene expression

<i>Duration and mode of exercise</i>	<i>Subject description</i>	<i>Sample time points</i>	<i>Effect on sIL-6R</i>	<i>Correction for changes in plasma volume</i>	<i>Analytical method</i>	<i>Reference</i>
<i>Exercise training</i>						
3 h knee extensor exercise at 55-60% individual Wmax before and after 10 week training programme (1 h x 5 x per week)	Sedentary males ( n = 7)	Pre-ex, post-ex @ 2 h	No effect on plasma sIL-6R but ↑sIL-6RmRNA expression at rest after training (not after exercise)	No	R&D systems, hs sIL-6R ELISA.	(39)
12 week 30 min (5 x per week) at 60-80% HR max or control (no exercise)	24 CHF patients (male & females)	Pre and post-training or detraining	↓plasma sIL-6R after training. Detraining group sig greater sIL-6R after 12 wks.	-	R&D systems, hs sIL-6R ELISA.	(1)
6 months 45-60min walking 3d/wk (50-75% HRR)	46 obese sedentary females	Pre and post-training	↓plasma sIL-6R after training	-	R&D systems, hs sIL-6R ELISA.	(78)
6 months 45-60min walking 3d/wk (50-75% HRR)	17 obese sedentary females	Pre and post-training	↓plasma sIL-6R after training	-	R&D systems, hs sIL-6R ELISA.	(96)
12 weeks community walking intervention. ↑steps by 3000 5 days per week	24 healthy inactive males and females	Pre and post-intervention	No effect	No	M5 & M182 IL-6R paired antibodies	(24)
>60% VO <sub>2</sub> max cycle ergometer/treadmill OR resistance ex (variable machines/dumbbells)	37 obese sedentary females	Pre and post-training	No effect	-	R&D systems, sIL-6R ELISA.	(96)

*Prolonged exercise  
(> 90 mins)*

~6.5h mountain bike cycling (race)	Trained male endurance cyclists (n = 13)	Pre-ex, post-ex @ 0 h, 24 h	↑sIL-6R @ 24 h post-ex	No	R&D systems, hs sIL-6R ELISA.	(69)
3 h cycling exercise at 60% individual $W_{\max}$	Sedentary males (n = 6 exercise group vs n = 5 non-exercise group)	Pre-ex, post-ex @ 0 h, 3 h, 4.5h, 6 h, 9 h, 24 h	No effect on plasma sIL-6R. ↑sIL-6RmRNA expression @ 6h and 9 h in exercise group	No	R&D systems, hs sIL-6R ELISA.	(41)
2 h run @ 65% $VO_{2\max}$ + 5 km time trial (intervention: CHO ingestion: DBPC)	Trained male endurance runners (n = 10)	Pre-ex, post-ex @ 0 h, 1 h and 24 h	↑plasma sIL-6R @ immediately, 1 h post-ex. CHO ingestion had no effect on sIL-6R	No	R&D systems, hs sIL-6R ELISA.	(92)
3 h knee extensor exercise at 55-60% individual $W_{\max}$ (intervention: low intramuscular glycogen)	Sedentary males (n = 6)		↑ sIL-6RmRNA @ 0h and 2 h post-ex. Low glycogen status had no effect on plasma sIL-6R or sIL-6RmRNA.	No	R&D systems, hs sIL-6R ELISA.	(39)

*Exercise (<90 mins)*

10 km time trial (intervention: rhIL-6 administration: DBPC)	Trained male endurance runners (n=7)	Pre-ex and post-ex @ 0h	No effect on sIL-6R post-ex under placebo or rhIL-6 conditions	No	R&D systems, hs sIL-6R ELISA.	(68)
1 h cycling at 90% lactate threshold	Recreationally trained males (n = 9)	Pre-ex, post-ex @ 0 h 1.5 h	↑ plasma sIL-6R @ immediately post-ex	Yes	M5 & M182 IL-6R paired antibodies	(25)
1 h cycling at 70% $VO_{2\max}$ (at 0°C and 20°C)	Recreationally trained males (n = 8)	Pre-ex, 30 min, post-ex @ 0 h, 1 h	No effect on sIL-6R at 0°C and 20°C	No	M5 & M182 IL-6R paired antibodies	(61)

Cycling at 96% lactate threshold to exhaustion (56 min)	Sedentary males (n = 12)	Pre-ex, post-ex	↑ plasma sIL-6R @ immediately post-ex	No	M5 & M182 IL-6R paired antibodies	(27)
Cycling at 96% lactate threshold to exhaustion (56 min)	Chronic fatigue syndrome patients (n = 6) vs healthy controls (n = 6)	Pre-ex, post-ex @ 0 h, 24 h	No effect on sIL-6R	No	M5 & M182 IL-6R paired antibodies	(66)
<i>Eccentric exercise</i>						
3 sets x 15 repetitions of maximal eccentric contractions of elbow flexors	Males and females (training status not indicated) (n = 25)	Pre-ex, post-ex @ 4 h, 8 h and 12 h	↑plasma sIL-6R @ 4 h and 8 h	No	R&D systems, hs sIL-6R ELISA.	(14)
3 x 15 min downhill running 75% VO <sub>2max</sub>	Healthy active young (n = 16) and elderly males (n = 16)	Pre-ex, post-ex @ 6 h 24 h	↑ plasma sIL-6R @ 6 h and 24 h in young compared to elderly	No	R&D systems, hs sIL-6R ELISA	(73)
6 sets x 10 repetitions of maximal	Recreationally trained males (n = 18)	Pre-ex, post-ex @ 24h, 48h and 72h	↓plasma sIL-6R @ 48h and 72 h	No	R&D systems, hs sIL-6R ELISA.	Current study

*Key*

DBPC: double-blinded, placebo-controlled, cross-over design study

hs: high sensitivity

increases two fold after endurance exercise (25). However, to prevent an uncontrolled generalised stimulation by the IL-6/sIL-6R complex, research indicates that the soluble form of glycoprotein 130 (sgp130) acts as a natural inhibitor of the agonistic complex (38).

The role of a recombinant isoform of gp130 as a pharmacological therapy to reduce IL-6-driven inflammation in rheumatoid arthritis is under investigation and results indicate that the use of the sgp130 isoform is an effective antagonist of IL-6 signalling pathways. To date studies report that exercise (eccentric or prolonged) has little (27) or no impact on circulating levels of sgp130 (14, 39, 41) in males (age range 18 – 40 years) although recently elevations in the sgp130 were reported in sedentary, middle aged men (age  $50.6 \pm 7.8$  years) following a single bout of fatiguing acute exercise (27) as well as in older men (age  $71 \pm 2.0$  years) versus younger men both at rest and 6 h after a bout of downhill running (73).

#### *Effect of endurance training on sIL-6R*

Endurance training has been shown to decrease resting sIL-6R in patients with chronic heart failure (1) and in obese subjects who reduce body fat (78, 96). This reduction in sIL-6R is contrary to the increase occurring in the expression of IL-6R mRNA in skeletal muscle with a consequent decrease in the response to acute exercise (39). The possibility may exist that the membrane-bound receptor and the soluble receptor may work reciprocally (i.e. when the membrane form increases the circulating levels are able to decrease). This could have significant implications for the way in which exercise interventions relate to chronic low grade inflammation.

#### *Metabolic aspects of sIL-6R*

A reported metabolic effect of IL-6 signalling is to stimulate lipolysis and fat oxidation (90) in humans at rest and to exert an effect on glucose turnover during exercise (19). When muscle glycogen is low mRNA expression of IL-6 in skeletal muscle is enhanced (10, 40, 84) hence, the release of IL-6 from the working muscle is greater and subsequently plasma IL-6 concentrations rise (40, 84). Glucose ingestion, therefore offsets this increase in IL-6 (18) however IL-6R expression in skeletal muscle was independent of glycogen availability (39); and we have shown that although glucose ingestion during a 90-minute time trial run reduced elevations in IL-6, these changes did not affect performance (70).

The majority of glucose turnover studies have concentrated on the effects of IL-6 without considering IL-6R. Both whole body infusion studies and *in vitro* studies have shown contradictory results. The rhIL-6 infusion studies are discussed fully by Pedersen et al (62). Analysis of the *in vitro* studies reveals that in cultured skeletal muscle cells (L6 myotubes) when exposed to IL-6 enhanced basal and insulin-stimulated glucose uptake at  $1 \text{ ng.ml}^{-1}$  and  $100 \text{ ng.ml}^{-1}$  respectively, (9) whereas in rodent skeletal muscle, IL-6 ( $120 \text{ ng ml}^{-1}$ ) has been demonstrated to increase basal glucose uptake only in a muscle predominantly composed of Type 2 fibres unless insulin is present (22). In human biopsy, Glund et al (23) were able to demonstrate that at the same concentration of IL-6 ( $120 \text{ ng ml}^{-1}$ ) the action of insulin was not enhanced, but basal glucose transport was increased. These facts



taken together with the evidence that glucose turnover has been shown to increase in response to rhIL-6 infusion during exercise (19) the expression of IL-6R (41) and sIL-6R increases with exercise (25, 27, 69) suggests that there may be a significant role for the receptor. In a recent study therefore (26) we investigated the combined effects of IL-6 and sIL-6R, at physiological and supraphysiological concentrations on glucose transport in mouse soleus muscle (previously shown by Geiger *et al.* (22)) to be unresponsive to IL-6 incubations in the absence of insulin). In this study, we were able to demonstrate that the combined administration of IL-6 and sIL-6R increases basal glucose transport whilst having no effect on the action of insulin stimulated glucose uptake.

In agreement with other studies on IL-6 signalling (2, 42), the elevation in glucose transport at supraphysiological doses ( $120 \text{ ng.ml}^{-1}$ ) was also associated with an increase in phosphorylation of AMPK which, amongst its various roles, can stimulate glucose transport (47, 51). Whilst the increase in glucose transport at supra-physiological concentrations of IL-6 may have resulted from an increase in phosphorylation of AMPK, glucose transport was, however, increased by 125% whereas AMPK phosphorylation was increased by 90%, suggesting that another signalling pathway may be involved. In further support of this assertion, at physiological concentrations of IL-6 and sIL-6R glucose transport was increased with no appreciable changes in total or phosphorylated AMPK (26).

An alternative pathway which has been shown previously to be activated by IL-6 and its receptor system is the phosphorylation of PKB/Akt (93). Protein kinase B/Akt is well known for its role in insulin but not contraction-mediated stimulation of skeletal muscle glucose transport through glucose transporter isoform 4 (GLUT4) translocation (6). Neither Glund *et al.* (23) nor Gray *et al.* (26) were able to identify any changes in the phosphorylation of PKB/Akt. Using the L6 myotube model Carey *et al.* (9) demonstrated that signalling through the IL-6/IL-6R/gp130 receptor, resulted in STAT3 phosphorylation after 10 minutes whilst SOCS3 increased after 60 minutes. Insulin affected neither of these proteins. Alongside the well-established link between IL-6 and AMPK, this supports the notion that the role of IL-6 in mediating glucose transport is independent of insulin. (Figure 2).

#### *sIL-6R and fatigue in disease and exercise*

IL-6 can cross the blood-brain barrier (3) and IL-6 receptors exist at numerous sites in the brain (75) raising the intriguing possibility that IL-6 released at the muscle can initiate signalling in the brain. Increased levels of sIL-6R have also been demonstrated to increase the responsiveness of the brain to IL-6 (76) and cause a greater suppression of locomotor activity and general sickness behaviour above that of raised IL-6 levels alone (74).

Clinical research indicates that IL-6 levels are positively related to the degree of fatigue experienced in certain conditions, for example, Castleman's disease (55), primary biliary cirrhosis (94) and terminal cancer (33). Patients reporting excessive fatigue had significantly higher levels of IL-6 than those who were non-fatigued (32, 94). Exogenous peripheral administration of IL-6 has been associat-

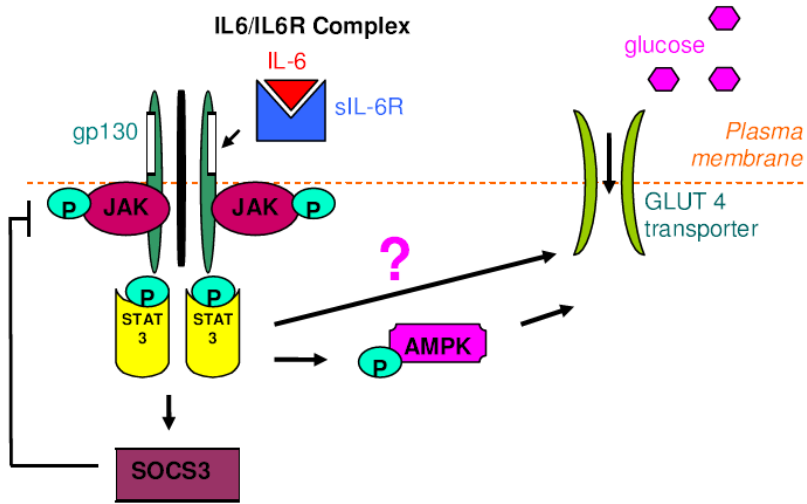


Figure 2. Schematic diagram of IL-6/IL-6R/gp130 signalling mechanism

ed with increased feelings of fatigue and reduced ability to concentrate (82) as well as impaired athletic performance in trained athletes (72) suggesting that systemically elevated IL-6 can induce a centrally-mediated effect.

Previous theories have suggested that elevated IL-6 levels may play a role in the debilitating fatigue reported by some athletes undergoing excessive exercise loading (67, 79) but to date research has not supported this conjecture (28, 59, 69, 71). However, Robson postulated that whilst athletes with excessive fatigue may not exhibit IL-6 values above the norm they may exhibit an increased sensitivity to IL-6 which could possibly occur as a consequence of elevated levels of the sIL-6R. Indeed, in the clinical setting, a positive relationship between sIL-6R and fatigue has been reported: breast cancer survivors with persistent fatigue had significantly elevated sIL-6R levels compared to non fatigued survivors but IL-6 levels were not different between groups (12). A similar finding for IL-6 was reported in individuals with chronic fatigue syndrome compared to healthy matched controls although in this study there was no difference in IL-6R (60). Interestingly, when the sIL-6R receptor signalling is blocked by anti-IL-6 receptor antibody therapy in conditions such as Castleman's disease, which is characterised by an overproduction of IL-6 and debilitating fatigue, fatigue was significantly attenuated (55). A similar finding has also been reported in rheumatoid arthritis patients following such treatment (54).

In the exercise setting, a positive relationship has also been reported between sIL-6R and fatigue: we demonstrated that sIL-6R concentrations were significantly elevated in individuals completing a 6-day endurance mountain bike event (468 km in 6 days) where sIL-6R concentrations were positively correlated with daily fatigue scores (69). To date, no studies have reported any interventions that can reduce the sIL-6R response to exercise. Whilst carbohydrate ingestion can signif-

icantly attenuate the rise in circulating IL-6 concentrations during prolonged exercise (5, 52, 70), we have found that carbohydrate ingestion during prolonged exercise (>2 h) had no effect on plasma sIL-6R up to 24 h post-endurance exercise (92).

### *The effect of exercise induced muscle damage on sIL-6R*

To date, little is known about the effect of eccentric exercise on plasma concentrations of sIL-6R or sgp130 or their relationship with DOMS and muscle function. Activities involving unaccustomed eccentric muscle actions result in exercise-induced muscle damage (EIMD) (8, 77, 88). EIMD is associated with a variety of consequential effects such as structural damage of the sarcomeres, protein leakage from damaged myofibres (81), loss of muscle force (8, 29, 88), delayed onset of muscle soreness (DOMS) (46, 56, 77), and an acute but low grade inflammatory response (46, 48). Indeed, elevations in circulating, IL-6, have been correlated with magnitude of DOMS and rises in circulating myosin heavy chain fragments (a marker of disruption within the muscle fibre contractile unit) following eccentric exercise suggesting a relationship between soreness, damage and inflammation (46).

One study has investigated the effect of maximal eccentric contractions (using elbow flexors) on plasma sIL-6R concentration and reported an increase at 4 h and 8 h post-exercise but levels returned to baseline levels by 12 h (14). Following a bout of downhill running in a healthy young and older men, sIL-6R concentrations were unaltered by exercise (at 6 h and 24 h post-exercise) although levels were significantly higher in the older participants (73). Neither study reported relationships between inflammatory mediators and other indicators of EIMD. Considering there is substantial evidence that both IL-6 and sIL-6R play a role in fatigue (27, 69, 72), nociception and inflammatory hyperalgesia (reviewed elsewhere (13, 80)), it is surprising that only a few studies have investigated the relationship between IL-6 and indices of EIMD. To date, no studies have investigated changes in sIL-6R or sgp130 beyond 24 h into recovery nor their relationship with DOMS or muscle function.

The purpose of this study was to examine the relationship between sIL-6R, sgp130 and recovery from eccentric exercise up to 72 h. It is our intention that the findings from this study will help to further our understanding of the relationship between inflammatory mediators and indicators of exercise-induced muscle damage.

## **MATERIALS AND METHODS**

### **Participants**

Eighteen healthy recreationally trained males (age  $21 \pm 3$  y; height  $182 \pm 7.4$  cm; mass  $78.8 \pm 10.3$  kg) volunteered to take part in the study. None had been unwell or taken medication in the preceding two weeks. All subjects were informed of experimental procedures and gave their written informed consent to participate in the study. Institutional ethical approval was obtained and all procedures were con-

ducted according to the Declaration of Helsinki. Subjects were instructed to arrive at the laboratory in a fasted state, having avoided physical activity for at least 48 h and not taken any nutritional supplements, caffeine, alcohol or anti-inflammatory drugs. All subjects were tested at approximately the same time of day to minimise diurnal variation.

## **Procedures**

### *Design*

All subjects were required to attend the laboratory on 4 consecutive days. Day 1 baseline blood samples were collected prior to any exercise testing and before subjects had broken their overnight fast. On all subsequent days early morning venous blood samples were obtained from subjects following an overnight fast. Following this, subjects rated their level of muscle soreness on a visual analogue scale, completed a standardised warm-up and carried out isokinetic muscle performance measures. On day 1, after completion of pre-testing baseline measurements, all participants completed a bout of exercise designed to induce acute muscle damage.

### *Muscle-damaging exercise*

Muscle damage was induced in the hamstrings. Participants completed 6 sets of 10 repetitions of unilateral eccentric/concentric actions of the knee flexors at a test speed of 1.05 rad/s using a Cybex Isokinetic Dynamometer (Cybex Norm, Cybex International, New York). Participants were instructed to provide a maximal effort during the eccentric phase of each leg flexion and return their leg to the starting position during the concentric phase. This protocol has previously been shown to induce muscle damage (11).

### *DOMS measurement*

The degree of DOMS experienced was measured on a visual analogue scale. Participants were required to rate the level of soreness, combined for both legs, that they perceived to have in their hamstrings when standing from 0 (no pain/soreness) up to 10 (pain/soreness as bad as it could be).

### *Measures of isokinetic muscle performance*

Peak torque of the best attempt in a repetition of a set of 6 concentric maximal-effort knee flexion actions was measured on the dominant leg at a test speed of 1.05 rad/s using a Cybex Isokinetic Dynamometer (Cybex Norm, Cybex International, New York).

### *Blood sample collection and analysis*

Venous blood was collected into appropriate vacutainer tubes (Becton Dickinson, Swindon, UK) and centrifuged at 1500 g for 10 min. The supernatant was aspirated and immediately frozen at  $-80^{\circ}\text{C}$  until later analysis.

Plasma IL-6, sIL-6R, sgp130 concentrations were analysed from  $\text{K}_3\text{EDTA}$  treated venous blood using an enzyme linked immunosorbent assay (R&D Systems, Minneapolis, USA). Intra- and inter-assay coefficient of variations for IL-6 were less than 2% and 6%, respectively; sIL-6R were 7% and 6%, respectively and sgp130

were 5% and 6%, respectively. High sensitivity plasma C-reactive protein (CRP) analysis and creatine kinase (CK) activity was performed on the Siemens Medical solutions Advia 2400, UK with intra- and inter-assay coefficient of variations of less than 5.8% and 6.6%, respectively and 1.1% and 2.2%, respectively.

### Data Analysis

Data in the figures and tables are presented as mean values and standard error of the mean. Statistical evaluation of the results was carried out using repeated measures analysis of variance with post-hoc Tukey tests where applicable. Relationships between variables were analyzed using Pearson's product moment correlations. The accepted level of significance was  $p \leq 0.05$ .

## RESULTS

### *Evidence of Muscle Damage*

For all subjects the protocol was deemed to have caused EIMD in both legs. This was evident from reductions in isokinetic muscle performance and increases in CK and DOMS over the 3 days post-eccentric exercise.

### *Isokinetic muscle performance*

Peak torque of the dominant leg was significantly reduced by 48 h post-exercise but returned to baseline levels by 72 h (Table 2).

Table 2. The effect of muscle damaging exercise on mechanical and biochemical parameters.

	Baseline pre-exercise	24 h post- exercise	48 h post- exercise	72 h post- exercise
<i>Delayed onset of muscle soreness</i>	0.3 ± 0.1	3.1 ± 0.5*	4.7 ± 0.5*	3.2 ± 0.5*
<i>Peak Torque (Nm)</i>	137 ± 8	124 ± 8	120 ± 8*	122 ± 8
<i>Creatine Kinase activity (U.l<sup>-1</sup>)</i>	319 ± 192	1485 ± 772*	7966 ± 3116*	15143 ± 5504*
<i>C-reactive protein (mg.ml<sup>-1</sup>)</i>	2.7 ± 0.4	2.6 ± 0.4	2.7 ± 0.4	3.8 ± 0.6

\*significantly different from baseline ( $p < 0.05$ )

### *DOMS*

Ratings of perceived muscle soreness were significantly higher than baseline at 24 h and 48 h post-eccentric exercise ( $p < 0.05$ ). DOMS was inversely correlated with peak muscle torque at 48 h post-exercise ( $r = -0.50$ ;  $p < 0.05$ ) (Table 2).

### *Blood-borne markers*

Plasma CK activity was significantly elevated during recovery at all post-exercise time points ( $p < 0.05$ ) (Table 2). CRP, IL-6 and sgp130 concentrations remained unaltered for the duration of the trial whereas sIL-6R was significantly reduced at

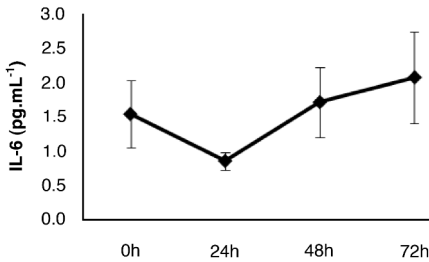


Figure 3. The effect of muscle damaging exercise on plasma IL-6 concentration (n=18).

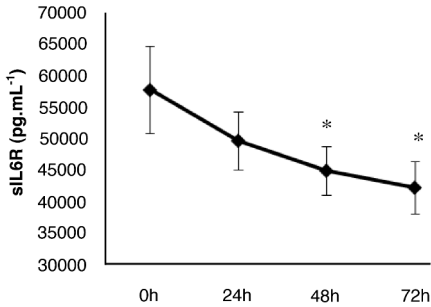


Figure 4. The effect of muscle damaging exercise on plasma sIL-6R concentration (n=18).

\*significantly lower than baseline ( $p < 0.05$ ).

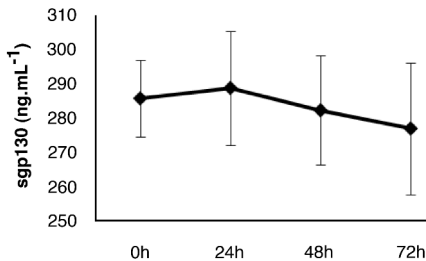


Figure 5. The effect of muscle damaging exercise on plasma sgp130 concentration (n=18).

48 h and 72 h post-exercise ( $p < 0.05$ ) compared to baseline measures (Figures 3-5). Peak torque and sIL-6R at 24 h and 48 h were negatively correlated ( $r = -0.42$ ,  $p < 0.05$ ;  $r = -0.57$ ;  $p < 0.01$ , respectively). DOMS and sIL-6R at 48 h were positively correlated at 48 h ( $r = 0.45$ ;  $p < 0.05$ ).

## DISCUSSION

The novel finding from our study was that sIL-6R concentrations were reduced for 72 h following a single bout of eccentric exercise which induced muscle injury. Furthermore, significant relationships were found between sIL-6R, peak muscle torque and DOMS during the recovery period.

In contrast to these findings, we have previously demonstrated that sIL-6R concentrations are elevated the morning (~16 h post-exercise) after prolonged cycling exercise which we postulated may have occurred as a consequence of increased CRP and IL-6 concentrations (69). *In vitro* elevations in CRP within physiological ranges can induce shedding of sIL-6R from neutrophils (36) and hepatocyte CRP production is increased in the presence of IL-6 (44). However, we found no significant increase in IL-6 or CRP during recovery from EIMD. Although eccentric exercise causes greater local muscle damage

than prolonged endurance exercise, alterations in plasma cytokine levels and CRP appear to be smaller (7, 69, 86). During endurance exercise a host of factors aside from muscle damage which are known inflammatory stimuli such as energy crisis, oxidative stress, endotoxaemia due to gut ischemia, metabolic and hormonal alterations may contribute to the increase in inflammatory mediators. A single bout of eccentric exercise takes a relatively short period of time, involves activation of isolated small muscle groups compared to cycling or running and is unlikely to induce an energy crisis or endotoxaemia (although some oxidative

damage may occur as a consequence of neutrophil infiltration (4)). It is, therefore, perhaps unsurprising that a lower or insignificant IL-6 response is triggered following the current protocol.

Elevated sIL-6R concentrations are also reported during infectious episodes and in a variety of inflammatory conditions such as rheumatoid arthritis (53), asthma (95) and Castleman's disease (55). However, there are a few clinical conditions where sIL-6R levels are reduced. In stroke patients (16) and following a myocardial infarction (89) sIL-6R is lowered during recovery. Both of these conditions generally involve a single inflammatory event and the downregulation of sIL-6R may be part of a regulatory response attenuating an acute inflammatory response (17).

During an acute inflammatory event such as EIMD, leukocyte recruitment is characterised by an initial infiltration of neutrophils and then replaced by a more sustained influx of monocytes approximately 24 – 48 h post-injury (30, 45). Hurst *et al* (31) have demonstrated that IL-6 and sIL-6R play a role in controlling the pattern of leukocyte recruitment during an acute inflammatory episode. They determined that infiltrating neutrophils shed sIL-6R from their cell surface which regulate neutrophil-activating chemokines (Modur *et al* (49) suggest that the limiting factor for IL-6 signalling from neutrophils to endothelial cells is sIL-6R, not the cytokine IL-6). This in turn contributes to the suppression of neutrophil recruitment and the concurrent attraction of monocytes. Thus sIL-6R plays an active role in the trafficking of leukocytes.

During short-term recovery from eccentric exercise, studies have reported elevations in sIL-6R levels at 4 and 8 h post-exercise with levels returning to baseline at 12 h (14) and remaining at baseline at 24 h (73). In this study we found no change in sIL-6R levels at 24 h (in agreement with Sacheck *et al* (73)) but that levels were significantly reduced during longer term recovery at 48 h and 72 h post-exercise. We propose that this biphasic pattern in sIL-6R concentration post-EIMD reflects changes in leukocyte sub-population migration into affected tissue. When sIL-6R levels are elevated in the initial period following EIMD this reflects signalling suppressing the neutrophil infiltration into the damaged area and increasing monocyte/macrophage infiltration. When sIL-6R levels are lowered during the later stage of recovery from EIMD, this may indicate the regenerative phase when macrophage infiltration into the affected tissue is suppressed and tissue repair and remodelling occurs.

Although we report a mean reduction in sIL-6R concentrations during recovery from EIMD at 48 h and 72 h, a relationship was found between sIL-6R, peak muscle torque and DOMS. Our data suggests that higher levels of sIL-6R may partly contribute to the concurrent reduction in peak muscle strength and increase in perceived muscle soreness (48 h only). Some of the reduction in peak muscle torque may be due to soreness in the recruited muscles inhibiting the production of a maximal effort by subjects as a negative relationship was also found between soreness scores and peak torque. The positive relationship between DOMS soreness score and sIL-6R suggests the soluble receptor may be involved in perception of pain following EIMD. There is considerable evidence that IL-6 plays a



role in nociception and inflammatory hyperalgesia (comprehensively reviewed elsewhere (13, 80)) and that IL-6 in combination with sIL-6R may have a greater sensitising effect than IL-6 alone (57). Indeed, neutralizing the IL-6 signalling pathway can abolish the sIL-6R-mediated sensitising effect and reduce the perception of pain following heat injury in rats (57), this has yet to be demonstrated in humans following EIMD.

In conclusion, our finding that sIL-6R concentrations are decreased 2 - 3 days following a single bout of EIMD may reflect a regulatory mechanism controlling the influx of different leukocyte subpopulations into damaged tissue. Our data suggests an association between sIL-6R, perception of pain and reduced peak muscle performance post-EIMD but further investigation is warranted to explore this relationship.

#### *Summary and future directions*

In summary, we believe there is a significant case to consider the IL-6R in exercise studies. These studies could focus on the role of sIL-6R on human skeletal muscle at physiological levels, the relationship between muscle soreness and power production with sIL-6R, clarification of the signalling mechanism associated with short term and long term exposure to IL-6 and the role of receptor in insulin resistance.

## REFERENCES

1. Adamopoulos S, Parissis J, Karatzas D, Kroupis C, Georgiadis M, Karavolias G, Paraskevaidis J, Koniavitou K, Coats AJS and Kremastinos DTh. Physical training modulates proinflammatory cytokines and the soluble fas/soluble fas ligand system in patients with chronic heart failure. *Journal of the American College of Cardiology* 39: 653-663, 2002.
2. Al Khalili L, Bouzakri K, Glund S, Lonnqvist F, Koistinen HA and Krook A. Signaling specificity of interleukin-6 action on glucose and lipid metabolism in skeletal muscle. *Mol Endocrinol* 20: 3364-3375, 2006.
3. Banks WA, Kastin AJ and Gutierrez EG. Penetration of interleukin-6 across the murine blood-brain barrier. *Neurosci Lett* 179: 53-56, 1994.
4. Best TM, Fiebig R, Corr DT, Brickson S and Ji L. Free radical activity, antioxidant enzyme, and glutathione changes with muscle stretch injury in rabbits. *J Appl Physiol* 87: 74-82, 1999.
5. Bishop NC, Gleeson M, Nicholas CW and Ali A. Influence of carbohydrate supplementation on plasma cytokine and neutrophil degranulation responses to high intensity intermittent exercise. *Int J Sport Nutr Exerc Metab* 12: 145-156, 2002.
6. Brozinick JT, Jr., Hawkins ED, Strawbridge AB and Elmendorf JS. Disruption of cortical actin in skeletal muscle demonstrates an essential role of the cytoskeleton in glucose transporter 4 translocation in insulin-sensitive tissues. *J Biol Chem* 279: 40699-40706, 2004.
7. Bruunsgaard H, Galbo H, Halkjaer-Kristensen J, Johansen TL, MacLean DA and Pedersen BK. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *J Physiol* 499 ( Pt 3): 833-841, 1997.



8. Byrne C and Eston R. The effect of exercise-induced muscle damage on isometric and dynamic knee extensor strength and vertical jump performance. *J Sports Sci* 20: 417-425, 2002.
9. Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG, Ramm G, Prelovsek O, Hohnen-Behrens C, Watt MJ, James DE, Kemp BE, Pedersen BK and Febbraio MA. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes* 55: 2688-2697, 2006.
10. Chan MH, Carey AL, Watt MJ and Febbraio MA. Cytokine gene expression in human skeletal muscle during concentric contraction: evidence that IL-8, like IL-6, is influenced by glycogen availability. *Am J Physiol Regul Integr Comp Physiol* 287: R322-R327, 2004.
11. Cockburn E, Hayes PR, French DN, Stevenson E and St Clair GA. Acute milk-based protein-CHO supplementation attenuates exercise-induced muscle damage. *Appl Physiol Nutr Metab* 33: 775-783, 2008.
12. Collado-Hidalgo A, Bower JE, Ganz PA, Cole SW and Irwin MR. Inflammatory biomarkers for persistent fatigue in breast cancer survivors. *Clin Cancer Res* 12: 2759-2766, 2006.
13. De Jongh RF, Vissers KC, Meert TF, Booij LH, De Deyne CS and Heylen RJ. The role of interleukin-6 in nociception and pain. *Anesth Analg* 96: 1096-103, table, 2003.
14. Depner CM, Gordon LK, Kidd JR and Miles MP. IL-6 bio-availability varies with basal CRP concentration and is unaffected by eccentric exercise. *Med Sci Sports Exerc* 40: S433, 2008.
15. Dimitrov S, Lange T, Benedict C, Nowell MA, Jones SA, Scheller J, Rose-John S and Born J. Sleep enhances IL-6 trans-signaling in humans. *Faseb Journal* 20: 2174+, 2006.
16. Dziedzic T, Slowik A and Szczudlik A. Interleukin-6 and stroke: cerebral ischemia versus nonspecific factors influencing interleukin-6. *Stroke* 34: e229-e230, 2003.
17. Dziedzic T, Slowik A and Szczudlik A. Interleukin-6 and stroke: cerebral ischemia versus nonspecific factors influencing interleukin-6. *Stroke* 34: e229-e230, 2003.
18. Febbraio MA, Steensberg A, Keller C, Starkie RL, Nielsen HB, Krstrup P, Ott P, Secher NH and Pedersen BK. Glucose ingestion attenuates interleukin-6 release from contracting skeletal muscle in humans. *J Physiol* 549: 607-612, 2003.
19. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP and Pedersen BK. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes* 53: 1643-1648, 2004.
20. Frieling JM, van Deuren JW, van der Meer CC, Visser M and Sauerwein RW. Circulating interleukin-6 receptor in patients with sepsis syndrome. *J Infection* 171: 469-472, 1995.
21. Gaillard J, Pugniere M, Tresca J, Mani J, Klein B and Brochier J. Interleukin-6 receptor signaling. II. Bio-availability of interleukin-6 in serum. *Eur Cytokine Netw* 10: 337-344, 1999.
22. Geiger PC, Hancock C, Wright DC, Han DH and Holloszy JO. IL-6 increases muscle insulin sensitivity only at superphysiological levels. *Am J Physiol Endocrinol Metab* 292: E1842-E1846, 2007.
23. Glund S, Trebak JT, Long YC, Barres R, Viollet B, Wojtaszewski JF and Zierath JR. Role of adenosine 5'-monophosphate-activated protein kinase in interleukin-6 release from isolated mouse skeletal muscle. *Endocrinology* 150: 600-606, 2009.

24. Gray SR, Baker G, Wright A, Fitzsimons CF, Mutrie N and Nimmo MA. The effect of a 12 week walking intervention on markers of insulin resistance and systemic inflammation. *Prev Med* 48: 39-44, 2009.
25. Gray SR, Clifford M, Lancaster R, Leggate M, Davies M and Nimmo MA. The response of circulating levels of the interleukin-6/interleukin-6 receptor complex to exercise in young men. *Cytokine* 47: 98-102, 2009.
26. Gray SR, Ratkevicius A, Wackerhage H, Coats P and Nimmo MA. The effect of interleukin-6 and the interleukin-6 receptor on glucose transport in mouse skeletal muscle. *Exp Physiol* 94: 899-905, 2009.
27. Gray SR, Robinson M and 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.
28. Halson SL, Lancaster GI, Jeukendrup AE and Gleeson M. Immunological responses to overreaching in cyclists. *Med Sci Sports Exerc* 35: 854-861, 2003.
29. Harrison AJ and Gaffney SD. Effects of muscle damage on stretch-shortening cycle function and muscle stiffness control. *J Strength Cond Res* 18: 771-776, 2004.
30. Hikida RS, Staron RS, Hagerman FC, Sherman WM and Costill DL. Muscle fiber necrosis associated with human marathon runners. *J Neurol Sci* 59: 185-203, 1983.
31. Hurst SM, Wilkinson TS, McLoughlin RM, Jones S, Horiuchi S, Yamamoto N, Rose-John S, Fuller GM, Topley N and Jones SA. IL-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. *Immunity* 14: 705-714, 2001.
32. Inagaki M, Isono M, Okuyama T, Sugawara Y, Akechi T, Akizuki N, Fujimori M, Mizuno M, Shima Y, Kinoshita H and Uchitomi Y. Plasma interleukin-6 and fatigue in terminally ill cancer patients. *J Pain Symptom Manage* 35: 153-161, 2008.
33. Inagaki M, Isono M, Okuyama T, Sugawara Y, Akechi T, Akizuki N, Fujimori M, Mizuno M, Shima Y, Kinoshita H and Uchitomi Y. Plasma interleukin-6 and fatigue in terminally ill cancer patients. *J Pain Symptom Manage* 35: 153-161, 2008.
34. Jones SA, Horiuchi S, Novick D, Yamamoto N and Fuller GM. Shedding of the soluble IL-6 receptor is triggered by Ca<sup>2+</sup> mobilization, while basal release is predominantly the product of differential mRNA splicing in THP-1 cells. *Eur J Immunol* 28: 3514-3522, 1998.
35. Jones SA, Horiuchi S, Topley N, Yamamoto N and Fuller GM. The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *FASEB J* 15: 43-58, 2001.
36. Jones SA, Novick D, Horiuchi S, Yamamoto N, Szalai AJ and Fuller GM. C-reactive protein: a physiological activator of interleukin 6 receptor shedding. *J Exp Med* 189: 599-604, 1999.
37. Jonsdottir IH, Schjerling P, Ostrowski K, Asp S, Richter EA and Pedersen BK. Muscle contractions induce interleukin-6 mRNA production in rat skeletal muscles. *J Physiol* 528 Pt 1: 157-163, 2000.
38. Jostock T, Mullberg J, Ozbek S, Atreya R, Blinn G, Voltz N, Fischer M, Neurath MF and Rose-John S. Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. *Eur J Biochem* 268: 160-167, 2001.
39. Keller C, Steensberg A, Hansen AK, Fischer CP, Plomgaard P and Pedersen BK. Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle. *J Appl Physiol* 99: 2075-2079, 2005.

40. Keller C, Steensberg A, Pilegaard H, Osada T, Saltin B, Pedersen BK and Neuffer PD. Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *FASEB J* 15: 2748-2750, 2001.
41. Keller P, Penkowa M, Keller C, Steensberg A, Fischer CP, Giralt M, Hidalgo J and Pedersen BK. Interleukin-6 receptor expression in contracting human skeletal muscle: regulating role of IL-6. *FASEB J* 19: 1181-1183, 2005.
42. Kelly M, Keller C, Avilucea PR, Keller P, Luo Z, Xiang X, Giralt M, Hidalgo J, Saha AK, Pedersen BK and Ruderman NB. AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. *Biochem Biophys Res Commun* 320: 449-454, 2004.
43. Kishimoto T. The biology of interleukin-6. *Blood* 74: 1-10, 1989.
44. Kordula T, Rokita H, Koj A, Fiers W, Gauldie J and Baumann H. Effects of interleukin-6 and leukemia inhibitory factor on the acute phase response and DNA synthesis in cultured rat hepatocytes. *Lymphokine Cytokine Res* 10: 23-26, 1991.
45. Lowe DA, Warren GL, Ingalls CP, Boorstein DB and Armstrong RB. Muscle function and protein metabolism after initiation of eccentric contraction-induced injury. *J Appl Physiol* 79: 1260-1270, 1995.
46. MacIntyre DL, Sorichter S, Mair J, Berg A and McKenzie DC. Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. *Eur J Appl Physiol* 84: 180-186, 2001.
47. Merrill GF, Kurth EJ, Hardie DG and Winder WW. AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *Am J Physiol* 273: E1107-E1112, 1997.
48. Miles MP, Andring JM, Pearson SD, Gordon LK, Kasper C, Depner CM and Kidd JR. Diurnal variation, response to eccentric exercise, and association of inflammatory mediators with muscle damage variables. *J Appl Physiol* 104: 451-458, 2008.
49. Modur V, Li Y, Zimmerman GA, Prescott SM and McIntyre TM. Retrograde inflammatory signaling from neutrophils to endothelial cells by soluble interleukin-6 receptor alpha. *J Clin Invest* 100: 2752-2756, 1997.
50. Mullberg J, Schooltink H, Stoyan T, Heinrich PC and Rose-John S. Protein kinase C activity is rate limiting for shedding of the interleukin-6 receptor. *Biochem Biophys Res Commun* 189: 794-800, 1992.
51. Musi N and Goodyear LJ. AMP-activated protein kinase and muscle glucose uptake. *Acta Physiol Scand* 178: 337-345, 2003.
52. Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Henson DA, Utter A, Davis JM, Williams F and Butterworth DE. Influence of mode and carbohydrate on the cytokine response to heavy exertion. *Med Sci Sports Exerc* 30: 671-678, 1998.
53. Nishimoto N, Kishimoto T and Yoshizaki K. Anti-interleukin 6 receptor antibody treatment in rheumatic disease. *Ann Rheum Dis* 59 Suppl 1: i21-i27, 2000.
54. Nishimoto N, Kishimoto T and Yoshizaki K. Anti-interleukin 6 receptor antibody treatment in rheumatic disease. *Ann Rheum Dis* 59 Suppl 1: i21-i27, 2000.
55. Nishimoto N, Sasai M, Shima Y, Nakagawa M, Matsumoto T, Shirai T, Kishimoto T and Yoshizaki K. Improvement in Castleman's disease by humanized anti-interleukin-6 receptor antibody therapy. *Blood* 95: 56-61, 2000.
56. Nosaka K, Newton M and Sacco P. Delayed-onset muscle soreness does not reflect the magnitude of eccentric exercise-induced muscle damage. *Scand J Med Sci Sports* 12: 337-346, 2002.

57. Obreja O, Schmelz M, Poole S and Kress M. Interleukin-6 in combination with its soluble IL-6 receptor sensitises rat skin nociceptors to heat, in vivo. *Pain* 96: 57-62, 2002.
58. Paonessa G, Graziani R, De SA, Savino R, Ciapponi L, Lahm A, Salvati AL, Toniatti C and Ciliberto G. Two distinct and independent sites on IL-6 trigger gp 130 dimer formation and signalling. *EMBO J* 14: 1942-1951, 1995.
59. Parry-Billings M, Budgett R, Koutedakis Y, Blomstrand E, Brooks S, Williams C, Calder PC, Pilling S, Baigrie R and Newsholme EA. Plasma amino acid concentrations in the overtraining syndrome: possible effects on the immune system. *Med Sci Sports Exerc* 24: 1353-1358, 1992.
60. Patarca M, Garcia M, Walters M, Dombroski D, Pons H and Fletcher M. Dysregulated expression of soluble immune mediator receptors in a subset of patients with Chronic Fatigue syndrome. *Journal of Chronic Fatigue syndrome* 1: 81-96, 1994.
61. Patterson S, Reid S, Gray S and Nimmo M. The response of plasma interleukin-6 and its soluble receptors to exercise in the cold in humans. *Journal of Sports Science* 26: 927-933, 2008.
62. Pedersen BK. Edward F. Adolph Distinguished Lecture: Muscle as an Endocrine Organ: IL-6 and other myokines. *J Appl Physiol* 2009.
63. Pedersen BK, Bruunsgaard H, Klokke M, Kappel M, MacLean DA, Nielsen HB, Rohde T, Ullum H and Zacho M. Exercise-induced immunomodulation--possible roles of neuroendocrine and metabolic factors. *Int J Sports Med* 18 Suppl 1: S2-S7, 1997.
64. Pedersen BK, Steensberg A and Schjerling P. Muscle-derived interleukin-6: possible biological effects. *J Physiol* 536: 329-337, 2001.
65. Peters M, Jacobs S, Ehlers M, Vollmer P, Mullberg J, Wolf E, Brem G, Meyer zum Buschenfelde KH and Rose-John S. The function of the soluble interleukin 6 (IL-6) receptor in vivo: sensitization of human soluble IL-6 receptor transgenic mice towards IL-6 and prolongation of the plasma half-life of IL-6. *J Exp Med* 183: 1399-1406, 1996.
66. Robinson M, Gray SR, Watson MS, Kennedy G, Hill A, Belch JJ and Nimmo MA. Plasma IL-6, its soluble receptors and F-isoprostanes at rest and during exercise in chronic fatigue syndrome. *Scand J Med Sci Sports* 2009.
67. Robson P. Elucidating the unexplained underperformance syndrome in endurance athletes : the interleukin-6 hypothesis. *Sports Med* 33: 771-781, 2003.
68. Robson-Ansley P, de Milander L, Collins M and Noakes TD. Acute interleukin-6 administration does not alter plasma soluble interleukin-6 receptor concentration following an acute bout of exercise. 11th Annual Congress of the European College of Sport Science P33, 2006.
69. Robson-Ansley P, Barwood M, Canavan J, Hack S, Eglin C, Davey S, Hewitt J, Hull J and Ansley L. The effect of repeated endurance exercise on IL-6 and sIL-6R and their relationship with sensations of fatigue at rest. *Cytokine* 45: 111-116, 2009.
70. Robson-Ansley P, Barwood M, Eglin C and Ansley L. The effect of carbohydrate ingestion on the interleukin-6 response to a 90-minute run time trial. *Int J Sports Physiol Perform* 4: 186-194, 2009.
71. Robson-Ansley PJ, Blannin A and Gleeson M. Elevated plasma interleukin-6 levels in trained male triathletes following an acute period of intense interval training. *Eur J Appl Physiol* 99: 353-360, 2007.

72. Robson-Ansley PJ, de Milander L, Collins M and Noakes TD. Acute interleukin-6 administration impairs athletic performance in healthy, trained male runners. *Can J Appl Physiol* 29: 411-418, 2004.
73. Satchek JM, Cannon JG, Hamada K, Vannier E, Blumberg JB and Roubenoff R. Age-related loss of associations between acute exercise-induced IL-6 and oxidative stress. *Am J Physiol Endocrinol Metab* 291: E340-E349, 2006.
74. Schobitz B, Pezeshki G, Pohl T, Hemmann U, Heinrich PC, Holsboer F and Reul JM. Soluble interleukin-6 (IL-6) receptor augments central effects of IL-6 in vivo. *FASEB J* 9: 659-664, 1995.
75. Schobitz B, Voorhuis DA and de Kloet ER. Localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. *Neurosci Lett* 136: 189-192, 1992.
76. Schobitz B, Voorhuis DA and de Kloet ER. Localization of interleukin 6 mRNA and interleukin 6 receptor mRNA in rat brain. *Neurosci Lett* 136: 189-192, 1992.
77. Semark A, Noakes TD, St Clair GA and Lambert MI. The effect of a prophylactic dose of flurbiprofen on muscle soreness and sprinting performance in trained subjects. *J Sports Sci* 17: 197-203, 1999.
78. Silverman NE, Nicklas BJ and Ryan AS. Addition of aerobic exercise to a weight loss program increases BMD, with an associated reduction in inflammation in overweight postmenopausal women. *Calcif Tissue Int* 84: 257-265, 2009.
79. Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med Sci Sports Exerc* 32: 317-331, 2000.
80. Sommer C and Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neurosci Lett* 361: 184-187, 2004.
81. Sorichter S, Puschendorf B and Mair J. Skeletal muscle injury induced by eccentric muscle action: muscle proteins as markers of muscle fiber injury. *Exerc Immunol Rev* 5: 5-21, 1999.
82. Spath-Schwalbe E, Hansen K, Schmidt F, Schrezenmeier H, Marshall L, Burger K, Fehm HL and Born J. Acute effects of recombinant human interleukin-6 on endocrine and central nervous sleep functions in healthy men. *J Clin Endocrinol Metab* 83: 1573-1579, 1998.
83. Starkie RL, Arkinstall MJ, Koukoulas I, Hawley JA and Febbraio MA. Carbohydrate ingestion attenuates the increase in plasma interleukin-6, but not skeletal muscle interleukin-6 mRNA, during exercise in humans. *J Physiol* 533: 585-591, 2001.
84. Steensberg A, Febbraio MA, Osada T, Schjerling P, van Hall G, Saltin B and Pedersen BK. Il-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *J Physiol* 537: 633-639, 2001.
85. Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B and Klarlund PB. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol* 529 Pt 1: 237-242, 2000.
86. Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K and Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. *Exerc Immunol Rev* 8: 6-48, 2002.
87. Taga T and Kishimoto T. Gp130 and the interleukin-6 family of cytokines. *Annu Rev Immunol* 15: 797-819, 1997.
88. Twist C and Eston R. The effects of exercise-induced muscle damage on maximal intensity intermittent exercise performance. *Eur J Appl Physiol* 94: 652-658, 2005.

89. Ueda K, Takahashi M, Ozawa K and Kinoshita M. Decreased soluble interleukin-6 receptor in patients with acute myocardial infarction. *Am Heart J* 138: 908-915, 1999.
90. van Hall G, Sacchetti M, Radegran G and Saltin B. Human skeletal muscle fatty acid and glycerol metabolism during rest, exercise and recovery. *Journal of Physiology* 543.3: 1047-1058, 2002.
91. Vermes C, Jacobs JJ, Zhang J, Firneisz G, Roebuck KA and Glant TT. Shedding of the interleukin-6 (IL-6) receptor (gp80) determines the ability of IL-6 to induce gp130 phosphorylation in human osteoblasts. *J Biol Chem* 277: 16879-16887, 2002.
92. Walshe I, Ansley L and Robson-Ansley P. Carbohydrate supplementation does not alter the plasma soluble gp130 concentration in response to prolonged running. 9th Symposium of the International Society of Exercise and Immunology 103, 2009.
93. Weigert C, Hennige AM, Brodbeck K, Haring HU and Schleicher ED. Interleukin-6 acts as insulin sensitizer on glycogen synthesis in human skeletal muscle cells by phosphorylation of Ser473 of Akt. *Am J Physiol Endocrinol Metab* 289: E251-E257, 2005.
94. Yamashiki M, Kosaka Y, Nishimura A, Watanabe S, Nomoto M and Ichida F. Analysis of serum cytokine levels in primary biliary cirrhosis patients and healthy adults. *J Clin Lab Anal* 12: 77-82, 1998.
95. Yokoyama A, Kohno N, Sakai K, Kondo K, Hirasawa Y and Hiwada K. Circulating levels of soluble interleukin-6 receptor in patients with bronchial asthma. *Am J Respir Crit Care Med* 156: 1688-1691, 1997.
96. You T, Berman DM, Ryan AS and Nicklas BJ. Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women. *Journal of clinical endocrinology and metabolism* 89: 1793-1746, 2004.
97. Zohlhofer D, Graeve L, Rose-John S, Schooltink H, Dittrich E and Heinrich PC. The hepatic interleukin-6 receptor. Down-regulation of the interleukin-6 binding subunit (gp80) by its ligand. *FEBS Lett* 306: 219-222, 1992.