Acute Interleukin-6 Administration Impairs Athletic Performance in Healthy, Trained Male Runners

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Abstract/Résumé

Fatigue is an inevitable consequence of physical activity; yet its biological cause remains uncertain. During exercise, a polypeptide messenger molecule interleukin-6 (IL-6) is actively produced. Previously, the administration of recombinant IL-6 (rhIL-6) induced a heightened sensation of fatigue in healthy humans at rest. In contrast, anti-IL-6 receptor antibodies reduced the symptoms of chronic fatigue. In the present study, athletic performance during an exercise challenge consisting of a 10-km running time trial was significantly impaired in trained male runners following the administration of a low dose of rhIL-6 compared to the placebo trial.

La fatigue est une conséquence incontournable de l'activité physique. Cependant, on en connaît moins bien les causes biologiques. Au cours d'un exercice, on remarque une grande production d'interleukine-6 qui agit comme messagère polypeptidique. L'administration du recombiné IL-6 (rhIL-6) provoque une plus grande sensation de fatigue chez des êtres humains en bonne santé et au repos. À l'opposé, les anticorps récepteurs anti-IL-6 atténuent les sensations de fatigue chronique. Dans cette étude, nous avons donné de faibles doses de rhIL-6 à des hommes entraînés à la course. Leur performance physique durant le défi sportif constitué d'une course contre la montre sur 10 km a été significativement réduite, comparativement au groupe placebo.

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Introduction

Exercise fatigue has been described as the inability to continue exercising at a prescribed work rate (Hagberg, 1981; Hawley and Reilly, 1997) in the presence of an increased subjective perception of effort (Enoka and Stuart, 1992). Most popular theories consider that fatigue develops as a result of specific biochemical changes in the active muscles (Fitts, 1994), although the importance of central neural command is also acknowledged (Gandevia, 2001).

During prolonged exercise, the polypeptide messenger molecule interleukin-6 (IL-6) is actively produced within contracting skeletal muscles (Jonsdottir et al., 2000), peritendinous tissue (Langberg et al., 2002), and the central nervous system (CNS) (Nybo et al., 2002). Plasma concentrations consistently increase during prolonged exercise lasting more than 2 hours (reviewed by Suzuki et al., 2002), with more than 60-fold increases reported during a 42-km marathon race (Ostrowski et al., 1998). Biological actions already ascribed to IL-6 include activation of the acute phase response as well as regulation of both erythropoiesis and immune system responses (reviewed by Kishimoto, 1989). Furthermore, recent research has led to the proposal that IL-6 may also act as an exercise-induced glucose-regulating hormone (Steensberg et al., 2000). In addition, IL-6 may cross the blood-brain barrier or act at the level of circumventricular organ outside the blood-brain barrier (Banks et al., 1994). IL-6 receptors exist at numerous sites in the brain such that there could be a signaling pathway between muscle and brain.

Several recent findings led to the postulate that IL-6 may contribute to sensations of fatigue during exercise. In response to recombinant human IL-6 (rhIL-6) administration, healthy resting individuals report increased sensations of fatigue, depressed mood states, and reduced ability to concentrate (Spath-Schwalbe et al., 1998). In contrast, patients with elevated IL-6 concentrations reported an immediate disappearance of previously debilitating fatigue when they received an IL-6 receptor antibody that blocks IL-6 signal transduction (Nishimoto et al., 2000). The effects of exogenously provided rhIL-6 on athletic performance and the sensations of fatigue during exercise in healthy trained male runners have yet to be reported. Thus the purpose of this study was to determine whether a low dose of rhIL-6 administered (with the intention of inducing plasma levels of IL-6 similar to those measured following a prolonged bout of exercise lasting over 2 hours) to trained male runners impaired athletic performance during a prescribed, repeatable exercise challenge.

Methods

SUBJECTS

Seven healthy, trained male runners (mean ± *SD* age 25 ± 2 years, mass 74 ± 8 kg, height 179.4 ± 4.2 cm) volunteered for the study. Approval of this study was obtained from the Research and Ethics Committee of the Faculty of Health Sciences, University of Cape Town. Prior to participation in the trial, all subjects completed an informed consent form. They had no history of autoimmune, cardiovascular, endocrine, or hematopoietic diseases and were accepted into the trial on the basis of having a personal best performance for a 10-km run of under 40 minutes in the

past 6 months in a recognized 10-km race. All subjects completed an exercise challenge which consisted of a 10-km time trial on a motorized treadmill on two occasions under the different treatment conditions, each separated by one week.

TRIAL PROTOCOL

All subjects had experience with treadmill running and, in the week prior to the trials, they all completed a familiarization 10-km time trial. On each trial occasion they reported to the laboratory at precisely the same time of day following an overnight fast. To standardize procedures, subjects were instructed to keep an accurate diary of their exercise training and dietary intake in the week prior to the first exercise trial so that they could replicate these factors in the week prior to the second exercise trial.

Subjects were asked to refrain from heavy exercise in the 72-hr period and any exercise in the 24-hr period preceding each exercise trial. Following pretrial measurements, they were given either a placebo (saline) or rhIL-6 subcutaneous injection at a dose of 0.05μg per kg body mass to induce plasma Il-6 concentrations similar to those reported following a bout of prolonged exercise lasting longer than 2 hours. The true identity of the injection was not disclosed to either the athletes or the persons supervising the trials. After a 2-hr rest period, subjects completed a 10-km time trial on a motorized treadmill during which they controlled the speed of the treadmill with a hand-held remote controller. The only verbal feedback they received during the trial was distance run (every 1 km). The reliability of this form of testing has a coefficient of variation of 0.89 (personal communication, M. Kirkman, Dept. of Biology, Univ. of Cape Town, Nov. 2000). Treatment conditions were randomly assigned in a double-blind manner and codes were not broken until all sample analyses had been completed.

Preparation of Treatments. Lyophilized recombinant human interleukin-6 (PeproTech Inc., Rocky Hill, NJ) was reconstituted in 10 mM acetic acid to give a final concentration of 0.5 $\mu g \cdot \mu l^{-1}$, agitated gently for 10 minutes and stored at –20 °C until use. Prior to injection, 0.5 μg per kg body mass of reconstituted IL-6 was added to phosphate-buffered saline to give a final volume of 400 μl.

Measurements. Venous blood samples were drawn from the antecubital vein. An abbreviated Profile of Mood State questionnaire (Grove and Prappavessis, 1992) was completed prior to the treatment as well as before and after the 10-km time trials. The calculation for total mood disturbance involves adding the five negative scores (fatigue, anger, depression, confusion, tension), adding 100, and subtracting the one positive mood score, vigor (Grove and Prappavessis, 1992). Heart rate and rectal measurements of core temperature were taken every kilometer during each 10-km time trial.

Sample Handling and Analysis. Venous blood was collected into appropriate vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 1500 × g for 10 min in a refrigerated centrifuge at 4 °C. The supernatant was transferred into eppendorf tubes and immediately frozen at –80 °C until later analysis. Plasma IL-6 concentrations were analysed from tripotassium ethylene diamine tetraacetic acid-treated blood using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Plasma cortisol and prolactin concentrations were

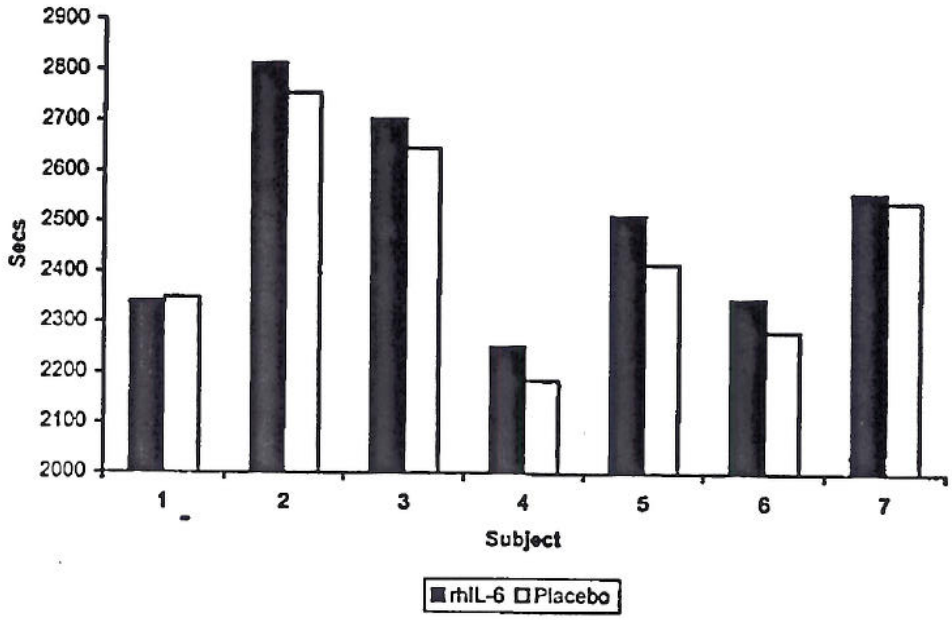

Figure 1. Effect of rhIL-6 and placebo administration on time taken to complete 10-km time trial run (individual subject data).

analysed using a competitive immunoassay by direct chemiluminescence (Chiron Diagnostics, Suffolk, UK; Bayer Corp., New York, respectively) and plasma ACTH by a sequential immunoassay (Diagnostic Products Corp., Los Angeles). Plasma glucose and lactate were analysed on a Beckman Glucose Analyzer 2 (Beckman Instruments Inc., Fullerton, CA) and using a commercial spectrophotometric assay (Bio Merieux, Marcy-L Etiole, France).

Data Analysis. Statistical evaluation of the results was carried out using either Student's paired *t*-tests (comparison of time taken to complete 10-km time trials) or repeated-measures analysis of variance with post-hoc Tukey tests for all other comparisons (Statistica 6, Statsoft Inc., Tulsa, OK). The accepted level of significance was $p < 0.05$.

Results

Recombinant human IL-6 administration significantly impaired 10-km time trial running performance during the exercise challenge compared to the placebo 10-km time trial (2,504 ± 205 s and 2,453 ± 203 s, respectively, $p < 0.01$) (Figure 1) with 6 of the 7 subjects having completed the rhIL-6 time trial in a slower time than during the placebo time trial (Figure 1). RhIL-6 administration significantly elevated the plasma IL-6, cortisol, adrenocorticotrophic hormone (ACTH), and prolactin concentrations compared to the placebo trial, $p < 0.01$ (Table 1). There were no significant differences in plasma lactate or glucose concentrations (Table 1) between trials, nor core temperature, $p > 0.05$ (Figure 2) or heart rate data (Figure 2). Following the rhIL-6 exercise challenge, subjects reported a heightened

Table 1 Effect of 10-km Time Trial Run Under rhIL-6 and Placebo Treatments on Six Variables (N = 7)

	Pretreatment		Preexercise		Postexercise	
	M	*SD*	*M*	*SD*	*M*	*SD*
IL-6 ($pg \cdot ml^{-1}$)						
rhIL-6	2.7	±2.8	21.7	±15.4*	41.3	±18.3*
Placebo	2.4	±2.0	2.14	±2.7	6.9	±2.5
Prolactin ($pmol \cdot L^{-1}$)						
rhIL-6	8.9	±2.7	6.0	±1.8	28.9	±11.3*
Placebo	9.4	±2.5	5.8	±2.9	23.6	±9.0
ACTH ($nmol \cdot L^{-1}$)						
rhIL-6	7.8	±2.8	4.5	±1.2	65.8	±7.0*
Placebo	6.5	±2.5	4.2	±1.4	38.0	±15.0
Cortisol($nmol \cdot L^{-1}$)						
rhIL-6	694	±153	520	±178	1001	±203*
Placebo	693	±81	447	±133	767	±244
Glucose ($mmol \cdot L^{-1}$)†						
rhIL-6	4.1	±0.3	4.2	±0.3	7.1	±1.6
Placebo	4.1	±0.3	4.3	±0.2	7.0	±1.9
Lactate ($mmol \cdot L^{-1}$)†						
rhIL-6	1.5	±0.4	1.6	±1.0	7.7	±2.4
Placebo	1.3	±0.6	1.0	±0.7	6.9	±2.4

*rhIL-6 trial significantly greater than placebo trial $p < 0.01$; †main effect of time, $p < 0.01$

sense of fatigue and global mood disturbance, $p < 0.05$ (Table 2). On completion of the study, each subject correctly identified the trial in which he had received rhIL-6, reporting increased sensations of "heaviness in the legs" and generalized fatigue during the rhIL-6 exercise challenge.

Discussion

This was the first study to directly identify fatigue-inducing properties of a circulating peptide produced by muscle, brain, and peritendinous tissue during prolonged exercise which is a blood-borne "fatigueogen." In accordance with the aim of the study, prior to the exercise challenge we induced plasma IL-6 concentrations similar to those reported following prolonged bouts of exercise (Suzuki et al., 2002). The exact mechanism explaining the impaired performance during the exercise challenge following rhIL-6 administration is not clear, but the results suggest that serotonergic pathways may be activated.

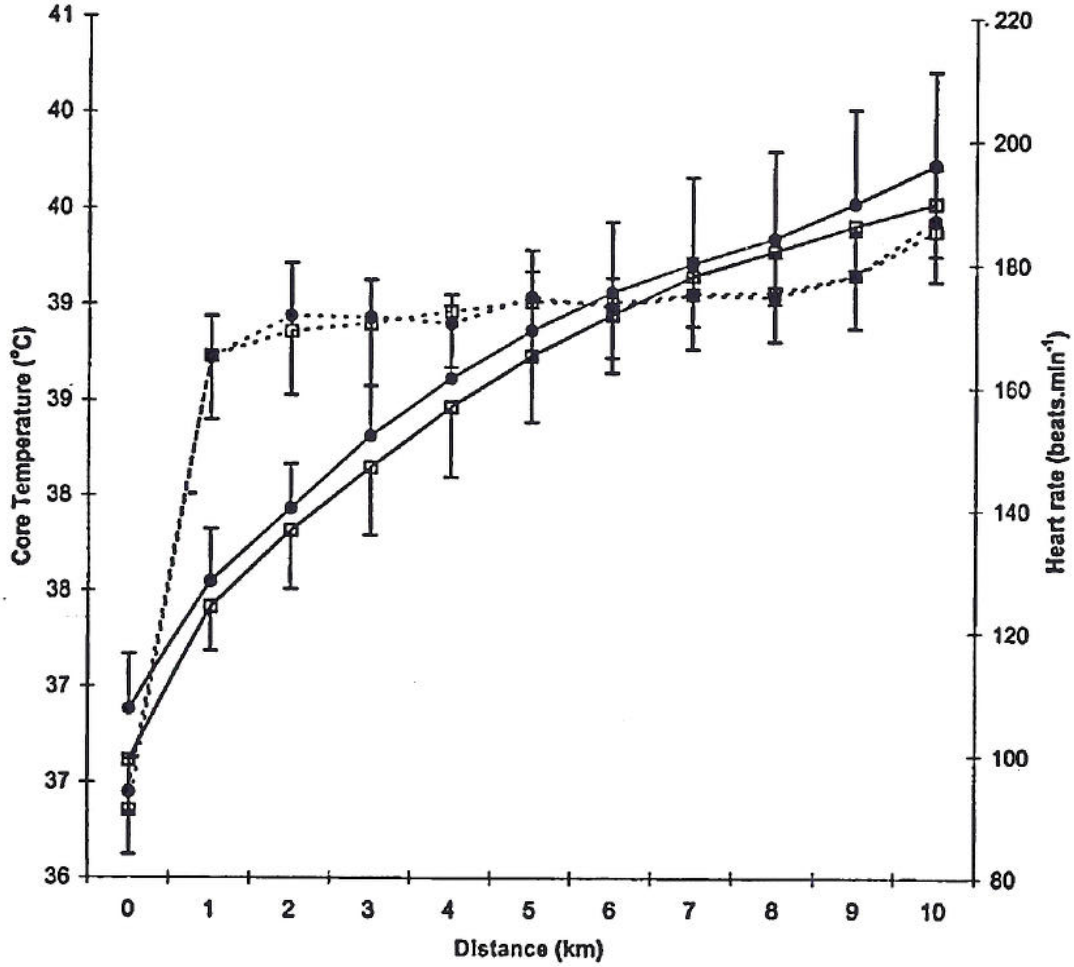

Figure 2. Effect of rhIL-6 (●) and placebo (□) administration on core temperature (solid lines) and heart rate (broken lines) during 10-km time trial. Main effect of time, $p < 0.01$. Data are mean ± *SD* ($n = 7$).

The combined stimulus of exercise and IL-6 activates the hypothalamic-pituitary-adrenal axis, evident by an elevated serum prolactin concentration in the present study (Table 1), which has previously been used as a neuroendocrine marker of 5-hydroxytryptamine (5-HT) activity (Cowen et al., 1990). Therefore, the potentiation by rhIL-6 of the natural rise of prolactin during exercise may reflect the stimulation of hypothalamic 5-HT receptors that control prolactin release. This is supported by Wang and Dunn (1998), who have shown in rat studies that IL-6 administration increases brain tryptophan and serotonin metabolism and suggested that this response contributes to the manifested effects of IL-6. Furthermore, the Central Fatigue Hypothesis implicates increased brain 5-HT concentrations in impaired CNS function which is observed during prolonged exercise (Newsholme and Blomstrand, 1995; Romanowski and Grabiec, 1974). It is therefore possible that the IL-6 released by the muscle during exercise could partly explain this impaired neural function.

Table 2 Effect of 10-km Time Trial Run Under rhIL-6 and Placebo Treatments on Fatigue and Total Mood Disturbance ($N = 7$)

	Pretreatment $M \pm SD$	Preexercise $M \pm SD$	Postexercise $M \pm SD$
Fatigue			
rhIL-6	3 ± 3	3 ± 2	14 ± 8*
Placebo	4 ± 4	4 ± 2	11 ± 7
Total Mood Disturbance			
rhIL-6	110 ± 6	108 ± 10	121 ± 4*
Placebo	107 ± 5	109 ± 13	111 ± 11

*rhIL-6 trial significantly greater than placebo trial, $p < 0.05$.

Our hypothesis, that the rhIL-6 impaired exercise performance and increased sensations of generalized fatigue by altering CNS serotonergic activity, is supported by the observation that fatigue could not be explained by differences in body temperature, heart rate, blood glucose, or lactate concentrations, which did not differ between trials.

Our finding that rhIL-6 at physiologically relevant concentrations influences the CNS, neuroendrocrine system, and fatigue, and impairs exercise performance, may be relevant not only to athletes but also to patients with fatigue related disorders such as chronic fatigue syndrome in which exercise tolerance is severely impaired and the symptoms of fatigue are exaggerated. The consequence of blocking IL-6 production and/or IL-6 signal transduction on the sensation of fatigue during prolonged exercise is unknown and awaits further research.

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