Psychological and immunological correlates of acute overtraining

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Five men undertook two intensive interval training sessions per day for 10 days, followed by 5 days of active recovery. Subjects supplied a venous blood sample and completed a mood-state questionnaire on days 1, 6, 11 and 16 of the study. Performance capabilities were assessed on days 1, 11 and 16 using a timed treadmill test to exhaustion at 18 km h⁻¹ and 1% grade. These individuals became acutely overtrained as indicated by significant reductions in running performance from day 1 to day 11. The overtrained state was accompanied by severe fatigue, immune system deficits, mood disturbance, physical complaints, sleep difficulties, and reduced appetite. Mood states moved toward baseline during recovery, but feelings of fatigue and immune system deficits persisted throughout the study.

Keywords: Overtraining, mood, immunity, interleukin-2, psychoneuroendocrinology

It has been suggested that there is an underlying physiological basis for the negative psychological changes that occur in athletes exposed to exercise stress¹⁻³. In particular, derangements in immune system function can induce lethargy and depression as well as other psychological states similar to those observed in people suffering from chronic fatigue and the overtraining syndrome⁴⁻¹³. Similarly, overtraining has been shown to affect the blood concentration of glutamine, dopamine and 5-hydroxytryptamine which are important neurotransmitters, and alterations in these substances have been shown to induce psychological changes, including feelings of chronic fatigue¹. It is also established that excessive exercise stress can cause imbalanced central nervous system control of certain physiological processes reflected in hypothyroidism, hypothalamic-pituitary dysfunction and deranged immunity¹⁴⁻¹⁶. Evidence is accumulating that this in turn accounts for many of the symptoms of excessive training and overtraining in athletes including amenorrhoea, increased suscepti-

bility to infection, and increased susceptibility to stress fractures³.

We have previously presented evidence that intensive training can cause perturbations in physiological processes involving the immune system and central nervous system¹⁶. In this article, we present additional findings on immune system functioning and discuss psychological reactions to acute overtraining. Physiological processes that may be associated with these psychological changes are described, and a rationale for using both physiological and psychological measures to screen for the onset of overtraining is established. Recognition of overtraining in its initial stages may allow intervention and prevent athletes from progressing to a more serious stage of the overtraining syndrome³.

Subjects and methods

Five well-trained men (mean(s.d.) age 31.6(3.5) years) who were familiar with treadmill running gave their written consent to participate in this study. The men were members of the Special Air Services (SAS) Regiment of the Australian Army, which is a regiment of elite troops specializing in tactical response operations. SAS personnel are required to maintain a high level of fitness, and they regularly engage in both endurance and interval (lactic anaerobic) training. The subjects were selected because of their familiarity with intensive physical training and because the nature of their employment allowed them to rest from other physical activity for the duration of the study.

Experimental protocol

Subjects underwent 10 days of intensive twice-daily interval training sessions followed by a 5-day active recovery period. An abbreviated Profile of Mood States questionnaire developed by Grove and Prapavassis¹⁷ was administered at 06.00 hours on the first morning of the 10-day training period (day 1), on the sixth day of the training period (day 6), on the day after the completion of the training (day 11), and on the day following the 5-day active recovery period (day 16). A battery of physiological tests and a performance test were also conducted on days 1, 11 and 16.

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With the exception of data on interleukin-2, the results of the physiological tests have been published elsewhere¹⁶. These data are, however, important for this report, and a brief description of the procedures employed is also important. At 06.00 hours on days 1, 6, 11, 12, 13, 14, 15 and 16, 25-ml blood samples were drawn from an antecubital vein before the subjects undertook any exercise. These samples were then assayed for a range of biochemical, haematological and immunological parameters. Mood states were assessed using an abbreviated form of the Profile of Mood States¹⁷ on days 1, 6, 11 and 16. On days 11 and 16 subjects were also questioned about their general welfare using a free-response format. Subjects were educated as to the effects of carbohydrate and fluid depletion, and they were instructed to drink large quantities of water to an extent beyond which their thirst demanded. They were also instructed to consume a high carbohydrate diet over the full course of the experiment.

Performance assessment and physiological measures

Subjects underwent a three-stage test on a treadmill to determine work efficiency and time to exhaustion at 18 km h⁻¹ and 1% grade. The first stage consisted of a 4-min run at 12 km h⁻¹ with the treadmill set at 1% grade and then a 3-min rest period. The second stage was a 4-min run at 15 km h⁻¹ (1% grade), followed by a 3-min rest period. The final stage was a run to volitional exhaustion with the treadmill set at 18 km h⁻¹, 1% grade. In order to ensure that subjects had reached $\dot{V}O_{2\max}$ they were required to achieve a respiratory exchange ratio of 1.15 in this test. Three of eight subjects failed to reach this criterion and were excluded from the experiment. The five remaining subjects achieved this criterion on each of their three performance tests (days 1, 11 and 16).

Heart rates were obtained using a Sportstester 3000 heart rate monitor (Polar electro, Kempele, Finland) and values recorded were those in the final 10 s of each work period. Blood samples for subsequent lactate (La) and glucose analysis were obtained from an earlobe previously treated with Finalgon ointment (Boehringer Ingelheim, Melbourne, Australia) to increase earlobe blood perfusion. Samples were collected into 100- μ l capillary tubes (Analox instruments, London, UK) and obtained within 30 s of the completion of each work period as well as 7 min after the final work period. Each of the immediate postworkload samples were analysed for La and the samples obtained after the final work period were analysed for blood glucose (GLUC). Assays for GLUC and La were conducted on an Analox analyser (Analox instruments) using recommended procedures.

Physiological tests were conducted in an indoor laboratory, and the test of each subject commenced at 07:00 hours. The subjects were requested not to eat or drink fluids except water on the morning of each blood test and to abstain from strenuous exercise for 36 h before the first test. Gas analysis was conducted throughout the test using a metabolic trolley consisting of an oxygen analyser (Applied Electrochemistry, Sunnyvale, California, USA), carbon dioxide analyser

(Applied Electrochemistry) and ventilometer (Morgan, Gillingham, UK). Peak and submaximal values for oxygen consumption ($\dot{V}O_2$) and ventilation ($\dot{V}_E$) were determined.

Morning and afternoon training sessions

The exercise intervention used in this study was designed to place a heavy demand on multiple energy systems and produce a state of acute overtraining. At 06.00 hours on days 2–10, subjects underwent intensive interval-training sessions. When the subjects first entered the laboratory for the morning sessions, their resting heart rates and body mass were measured. They then undertook a 3-min warm-up run at 12 km h⁻¹ followed by 3 min of stretching and 3 min of running at 15 km h⁻¹. After a 1-min recovery period, subjects began their training sessions.

The morning sessions consisted of 15 $\times$ 1-min work periods separated by 2-min recovery periods during which subjects were permitted to walk briskly within the laboratory. The work intervals consisted of running at a speed determined from their maximal time achieved on the treadmill during the 18-km h⁻¹ workload of the run to exhaustion on day 1. If the subjects' time to completion was between 1 min and 2 min 59 s at 18 km h⁻¹, they trained at 18 km h⁻¹; between 3 min and 4 min 59 s they trained at 19 km h⁻¹; between 5 min and 6 min 59 s they trained at 20 km h⁻¹; and between 7 min and 8 min 59 s they trained at 21 km h⁻¹. All training sessions were conducted with the treadmill grade set at 1%. Subjects were requested not to engage in any strenuous physical activity between training sessions and were reminded of this daily.

At 04.00 hours on days 1–10, subjects completed 10 $\times$ 1-min efforts at the same workload as in the morning sessions. During the afternoon sessions, however, work periods were separated by 1-min recovery periods rather than 2-min recovery periods.

Recovery days

On days 12–15, subjects reported to the laboratory at 06.00 hours and, after providing a blood sample, had their resting heart rate and body mass recorded. Subjects then undertook 10 min of unsupervised light running at a time of their choice during the day. They were instructed to run at just above walking pace and to walk if walking was more comfortable.

Questionnaires

An abbreviated version of the Profile of Mood States (POMS)¹⁸ was administered on days 1, 6, 11 and 16. This scale contained 40 items that assessed the usual POMS subscales of tension, depression, anger, vigour, fatigue, and confusion, as well as an additional subscale for esteem-related affect. The esteem subscale was added in order to tap a positive dimension of mood not normally assessed by the POMS. The instrument has sound internal consistency and discriminant validity, with the mean alpha coefficient for the seven subscales being 0.80. The

items associated with each of the seven subscales are described in detail by Grove and Prapavessis¹⁷. Since short-term mood effects were the focus of this study, we employed the 'how are you feeling today' response set.

Interleukin-2 assay system

The interleukin-2 (IL-2) assay was adapted from that described by Gearing, Johnstone and Thorpe¹⁹. In brief, 50 µl of CTLL cells at 2×10^5 cells per ml were seeded into the wells of a 96-well culture plate (Linbro, Horsham, Pennsylvania, USA) and a standard recombinant IL-2 preparation was serially diluted over the cultures. Samples of serum and culture media from peripheral blood leucocytes stimulated with concanavalin A were serially diluted over a similar set of cultures. After 24 h culture at 37°C in a humidified atmosphere of 5% CO₂, 0.5 µCi of 3H-6-thymidine (5 Ci mmol⁻¹, Amersham, UK) were added to all wells, and 18 h later the cultures were harvested on to glass fibre filter (Whatman, Maidstone, UK) using a Skatron cell harvester (Skatron, Lieberbyen, Norway) and the radioactivity incorporated into DNA determined by liquid scintillation. The results were expressed as the number of IL-2 units present in the preparations in comparison with the standard curve.

Results

Statistical analyses

One-way analysis of variance with repeated measures was used with a Scheffe *post hoc* comparison being applied to determine significant mean differences. Stringent alpha levels ($P < 0.01$) and a conservative *post hoc* comparison procedure were selected so as to reduce the chance of type one errors. These precautions were deemed necessary because many parameters were measured in a small sample, and the data had possible clinical implications. Results are presented as mean(s.e.m.) throughout.

Running performance and subjective responses

The mean(s.e.m.) time taken to reach exhaustion at 18 km h⁻¹, 1% grade was 369(33) s on day 1 and was significantly depressed by 29.3% on day 11 (mean(s.e.m.) 261(27) s). On day 16 performance time had returned toward baseline (mean(s.e.m.) 349(34) s) and was not significantly different from the pretraining level. These changes indicate that the exercise intervention did indeed produce an overtrained state among the subjects. Additional evidence for the existence of an overtrained state comes from the comments made by the subjects on the questionnaire distributed at the conclusion of the training phase. These comments indicated that the subjects were experiencing a variety of physical and psychological difficulties as a consequence of the training regimen. More specifically, they reported a variety of symptoms related to general fatigue, emotionality, concentration, physical complaints, sleep and appetite. (See Table 1.)

Table 1. Symptoms reported during acute overtraining (c.f. Fry et al. 1992)¹⁶

Category	Symptom*
General fatigue	Feel lethargic (all) Exhausted during the day (2) Ordinary tasks are an effort (2) Tired in the afternoon (3) Just walking around is an effort (5) Getting out of bed is hard (5) No interest in everyday tasks (5)
Emotionality	A bit quick-tempered (1) Emotionally unstable (2) Shorter fuse than normal (2) Snappiness at work (4) Personality change (5)
Concentration difficulties	Spasmodic concentration (1) Failure to remember things (2) Unable to narrow concentration (3) Difficult to focus/hold concentration (4) Concentration 'out the window' (5)
Physical complaints	Sore muscles (all) Dehydrated (1) Heavy feeling in upper legs (2) Stomach complaints/nausea (3) Loose bowels (3) Diarrhoea (4)
Sleep problems	Restless sleeping patterns (1) Sometimes difficult to get to sleep (2) Have trouble getting to sleep (4)
Appetite changes	Loss of appetite (1, 2) Not able to eat well (4)

*These are paraphrased versions of actual responses. Numbers in parentheses indicate which subject(s) made each response

Physiological responses

The data also demonstrate that subjects were under significant physiological stress as a result of the training regimen. Table 2 shows that glucose levels after exercise were suppressed following maximal exercise at the conclusion of the training period (day 11) when compared with values obtained before training (day 1). This effect occurred for glucose values obtained at 1 and 7 min after exercise, with both measures returning to before-exercise levels after the active recovery period (day 16). Ferritin levels fell progressively over the course of the study. They were depressed, on average, by 11.8% at the conclusion of the 10-day training period and fell significantly below before-training values by the end of the recovery period. There was an increase in the level of 'activation' of peripheral blood lymphocytes as indicated by significant elevations in the expression of CD25⁺ and HLA-DR⁺ surface antigens as well as a significant depression of the CD3⁺:CD25⁺ ratio. These changes were evident on day 6 and persisted at the conclusion of the recovery period.

The presence of natural killer cells (CD56⁺ cells) in the peripheral circulation was decreased following the training and recovery periods, suggesting that there may have been a redistribution of these cells to tissues damaged by exercise. Mean mitogen responses, *in vitro*, were also decreased (14.2% after day 11 and 30.4% after day 16), but these changes were not statistically significant. In addition, serum IL-2 levels were significantly elevated following the

Table 2. Changes in physiological and performance measures during acute overtraining (days 1, 6 and 11) and recovery (day 16)

Parameter	Day 1	Day 6	Day 11	Day 16
GLUC 1 min (mmol l ⁻¹)*	8.0(0.3)	NA	6.9(0.4)*	8.5(0.4)
GLUC 7 min (mmol l ⁻¹)*	9.0(0.6)	NA	7.3(0.4)*	9.2(0.4)
FERR (µg l ⁻¹)*	110(24)	109(24)	97(22)	83(20)*
CD25 ⁺ (10 ⁹ cells l ⁻¹)*	0.18(0.02)	0.44(0.06)*	0.52(0.06)*	0.48(0.05)*
HLA-DR ⁺ (10 ⁹ cells l ⁻¹)*	0.26(0.02)	0.34(0.04)	0.48(0.07)*	0.42(0.04)*
CD3 ⁺ /CD25 ⁺ (ratio)*	10.1(0.4)	3.3(0.4)*	3.0(0.4)*	3.2(0.3)*
CD56 ⁺ (10 ⁹ cells l ⁻¹)*	0.60(0.06)	0.32(0.04)*	0.36(0.05)*	0.30(0.03)*
Serum IL-2 (U ml ⁻¹)*	0.06(0.01)	NA	0.17(0.09)*	1.00(0.30)*
Supernatant IL-2 (U ml ⁻¹)*	0.64(0.20)	NA	5.00(1.23)*	5.18(1.30)*

Values are mean(s.e.m.); FERR, ferritin; GLUC, blood glucose; NA, not available. *Significantly different, $P < 0.01$; †significantly different, $P < 0.001$; ‡indicates significant mean differences from pre-training (day 1) values

training period (day 11) and the recovery period (day 16) in both the serum and mitogen-stimulated cell culture supernatants. Plasma glutamine concentrations were significantly depressed on day 6 and day 11 but had begun to return to before-exercise levels by day 16 of the study. Subjects had increasing difficulty completing the prescribed training as the overload training period progressed, and over the last few days they reported feeling nauseous in the final stages of the training sessions (see Table 1).

Mood state responses

The abbreviated POMS revealed a significant increase in the subscale measuring fatigue after the first 5 days of training. Fatigue scores were elevated even further at the conclusion of the training period (day 11) and then moved toward the baseline during the recovery phase (day 16). The fatigue ratings after recovery were, however, still significantly greater than baseline levels. A significant decrease in vigour and a significant increase in total mood disturbance (TMD) were also evident at the conclusion of training (day 11), with a return toward baseline following the active recovery period (day 16). Confusion scores tended to increase during the training phase ($P < 0.05$), but this change was not significant according to the criterion used in this study. (See Table 3.)

Discussion

The overtraining and chronic fatigue syndromes are well documented in the medical literature²⁰, and they appear to have similar pathogenesis and symptoms^{3,21,22}. By examining changes that occur during acute overtraining, we may gain insight into the aetiology of these conditions as well as the interrelationships among the immune system, the central nervous system and the neuroendocrine axis. It is known that increased levels of stress can induce a deranged immunological and neuroendocrine status, and that such imbalances are sometimes associated with psychopathology^{6,7,9,12,20,22-25}. The results of this study indicate that acute overtraining can also cause imbalances in the immune system and, at the same time, produce negative psychological consequences such as mood disturbance, fatigue/lethargy, and (perhaps) an inability to concentrate. Similar symptoms are known to occur in certain psychopathological states^{12,25}.

The subjects in this study were clearly overtrained as indicated by significantly depressed performance capacity at the end of the 10-day training period. After 5 days of recovery, running performance moved toward baseline but was still depressed by more than 2%. Changes in the psychological variables supported these performance findings and were consistent with findings from other studies of

Table 3. Abbreviated Profile of Mood States measures during acute overtraining (days 1, 6 and 11) and recovery (day 16)

Subscale	Before training Day 1	Midtraining Day 6	After training Day 11	After recovery Day 16
Fatigue*	0.4(0.2)	9.2(2.4)*	15.2(1.4)*	8.0(3.3)*
Vigour*	12.4(1.0)	8.0(1.1)	4.0(1.9)*	7.6(3.2)
Confusion†	0.2(0.2)	1.2(0.7)	2.2(1.8)	1.4(0.7)
Tension	4.4(1.2)	1.8(0.8)	2.8(1.2)	2.6(1.2)
Depression	0.2(0.1)	0.4(0.4)	1.6(0.7)	1.0(0.8)
Anger	0.2(0.2)	0.4(0.2)	1.0(0.8)	1.2(0.7)
Esteem	19.6(0.9)	20.0(1.2)	18.8(1.8)	19.2(1.5)
TMD*	75.8(3.4)	85.0(2.9)	100.0(5.5)*	87.4(7.6)

Values are mean(s.e.m.); TMD, Total mood disturbance; *significantly different from day 1 ($P < 0.001$); †tendency toward difference from day 1 ($P < 0.05$); ‡indicates significant differences from before-training (day 1) values

overtraining in sport²⁶. The comments in Table 1 and the POMS responses (Table 3) show that subjects were experiencing negative psychological states as a consequence of the exercise intervention. All participants referred to concentration difficulties on the open-ended symptoms questionnaire, and the POMS confusion scores tended to increase between day 1 and day 11. In addition, TMD, vigour and fatigue were significantly altered by the training regimen, and fatigue scores remained elevated even after 5 days of active recovery. Blood glucose levels fell significantly between day 1 and day 11 but returned to baseline on day 16. Significant increases in the activation level of peripheral blood lymphocytes (CD25⁺, HLA-DR⁺, CD3⁺:CD25⁺ ratio) and significant decreases in natural killer cell activity were evident as early as day 6 and were still present after recovery. IL-2 levels also increased in response to the exercise intervention and remained elevated after recovery.

Nonspecific, general activation of the immune system (documented in this study by changes in the CD25⁺, HLA-DR⁺, CD3⁺:CD25⁺, serum IL-2, and supernatant IL-2 variables) is accompanied by a range of nonspecific symptoms of illness. These symptoms include loss of interest in usual activities, poor appetite, weight loss, sleep changes, decreased social investigation, loss of energy, fatigue, irritability and anorexia^{4, 10, 23, 27–32}. Altered immune status is also known to affect the hypothalamic–pituitary axis^{7, 9, 33–39} and may be responsible for the hypothalamic–pituitary dysfunction and hypothyroidism known to occur in overtrained athletes^{14, 15}. Imbalances in these control systems may cause feelings of chronic fatigue and may also be responsible for many of the overtraining symptoms previously reported including amenorrhoea, osteoporosis, and altered testosterone levels³. Hypothyroidism has been reported in both the chronic fatigue syndrome and overtraining syndrome^{10, 15, 40–43}.

We have shown elsewhere that acute overtraining resulted in depressed levels of plasma glutamine for the subjects in this study⁴⁴, and these findings are in agreement with those of Parry-Billings and colleagues¹ who found depressed plasma glutamine concentrations in overtrained athletes. Glutamine is an important neurotransmitter precursor and is also an essential nutrient for the cells of the immune system^{45–48}. Parry-Billings *et al.*¹ also found that levels of tryptophan (which is converted in the brain to the neurotransmitter 5-hydroxytryptamine) and dopamine (another neurotransmitter) are both altered under conditions of excessive exercise stress. It is possible that alterations in the concentrations of these neurotransmitters may be partly responsible for the psychological perturbations observed in this study. In particular, there is evidence that 5-hydroxytryptamine is the neurotransmitter responsible for causing a state of tiredness and sleep in both man and experimental animals^{2, 49}.

Plasma ferritin concentrations fell progressively over the course of the experiment to reach their lowest levels on day 16 (Table 2). Chronic hypoferraemia can have immunomodulatory effects and induce feelings of fatigue^{50–53}. There may also be a

feedback loop involved, because it is known that immune system perturbations can affect the production of ferritin. For example, tumour necrosis factor is a known enhancer of the gene controlling the production of ferritin⁵⁴. Decreased ferritin levels may affect central nervous system processes via an effect on the immune system and by the decreased oxygen delivery which occurs during hypoferraemia.

In summary, psychological and immunological effects from acute overtraining were obtained in this study, but the time course of these effects differed. Changes in immune system activity were apparent after 5 days of intensive training and were still present after 5 days of recovery. POMS fatigue scores followed a similar pattern, but POMS confusion and TMD demonstrated shorter-term changes. On the basis of these findings, we suggest that physiological mechanisms may mediate some of the mood changes associated with overtraining, and that psychological monitoring of overtraining can be useful if supported by physiological measurements. We encourage other researchers to examine psychological and immunological responses to acute and chronic overtraining, since the key to early diagnosis and treatment may lie in this area.

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