



©Journal of Sports Science and Medicine (2002) 2, 31-41
http://www.jssm.org

Review article

BIOCHEMICAL AND IMMUNOLOGICAL MARKERS OF OVER-TRAINING

Michael Gleeson ✉

School of Sport and Exercise Sciences, University of Birmingham, Edgbaston, Birmingham, England.

Received: 06 March 2002 / Accepted: 22 March 2002 / Published (online): 01 June 2002

ABSTRACT

Athletes fail to perform to the best of their ability if they become infected, stale, sore or malnourished. Excessive training with insufficient recovery can lead to a debilitating syndrome in which performance and well being can be affected for months. Eliminating or minimizing these problems by providing advice and guidelines on training loads, recovery times, nutrition or pharmacological intervention and regular monitoring of athletes using an appropriate battery of markers can help prevent the development of an overtraining syndrome in athletes. The potential usefulness of objective physiological, biochemical and immunological markers of overtraining has received much attention in recent years. Practical markers would be ones that could be measured routinely in the laboratory and offered to athletes as part of their sports science and medical support. The identification of common factors among overtrained athletes in comparison with well-trained athletes not suffering from underperformance could permit appropriate intervention to prevent athletes from progressing to a more serious stage of the overtraining syndrome. To date, no single reliable objective marker of impending overtraining has been identified. Some lines of research do, however, show promise and are based on findings that overtrained athletes appear to exhibit an altered hormonal response to stress. For example, in response to a standardized bout (or repeated bouts) of high intensity exercise, overtrained athletes show a lower heart rate, blood lactate and plasma cortisol response. Several immune measures that can be obtained from a resting blood sample (e.g. the expression of specific cell surface proteins such as CD45RO+ on T-lymphocytes) also seem to offer some hope of identifying impending overtraining. If an athlete is suspected of suffering from overtraining syndrome, other measures will also be required, if only to exclude other possible causes of underperformance including post-viral fatigue, glandular fever, clinical depression, poor diet, anaemia, asthma, allergies, thyroid disorders, myocarditis and other medical problems interfering with recovery.

KEY WORDS: Training, over-reaching, immune, metabolism, hormones

AŞIRI ANTRENMANIN BİYOKİMYASAL VE İMMUNOLOJİK BELİRLEYİCİLERİ

ÖZET

Hasta veya olmak üzere olan, aşırı antrenman yapan veya yetersiz beslenen atletler yeteneklerini en üst düzeyde gösteremezler. Yeterli toparlanma süresi bırakılmadan yapılan yoğun antrenmanlar kişisel performans ve iyi hissetme halini aylarca etkileyebilen halsizlik sendromuna yol açabilir. Bu tip sorunları ortadan kaldırmak veya en aza indirmek için antrenmanların şiddeti, aradaki toparlanma süreleri, beslenme veya farmakolojik uygulamalar üzerine tavsiyeler ve uygulama kılavuzları sağlamak ve atletleri düzenli izlemek için uygun bir "kurallar dizisi" hazırlayıp uygulamak aşırı antrenman sendromunun oluşmasını önleyebilir. Son yıllarda aşırı antrenmanın nesnel fizyolojik, biyokimyasal ve immunolojik belirteçlerinin potansiyel yararlılığı giderek ilgi çekmeye başlamıştır. Sözkonusu belirteçlerin uygulanabilir olması için laboratuvarlarda rutin olarak ölçülebilmeleri ve atletlere sunulan bilimsel sportif ve tıbbi desteğin bir parçası olması gerekmektedir. Yeterli performans gösteren iyi antrenmanlı atletlerle karşılaştırılarak fazla antrenmanlı atletlerde ki ortak unsurların saptanması aşırı antrenman sendromunun daha da ciddileşmesini durdurmak için uygun önlemlerin alınmasına fırsat verebilir. Bugüne kadar aşırı antrenman sendromuna ait güvenilir ve nesnel bir belirteç tespit edilememiştir. Son zamanlarda bazı araştırmalar aşırı antrenmanlı atletlerin "stress"e cevabında değişiklikler olduğunu gösteren sonuçlar bildirerek umut vermektedir. Örneğin; standartize (veya tekrarlayan) yüksek şiddetteki egzersize yanıt



olarak aşırı antrenmanlı atletler daha düşük kalp hızı, kan laktatı ve plazma kortizol artışı cevabı göstermişlerdir. Dinlenimdeki kan örneklerinden sağlanan bazı immün ölçümler (örn, CD45RO gibi T lenfositlerdeki özgün hücre yüzeyi proteinlerini işaret edenler) de aşırı antrenman sendromunu belirlemede umut vermektedir. Aşırı antrenman sendromuna maruz kaldığından şüphelenilen bir atlette, yetersiz performansın diğer olası sebeplerini -viral hastalık sonrası yorgunluk, infeksiyöz mononükleaz, klinik depresyon, zayıf beslenme, anemi, astma, çeşitli alerjiler, tiroid bozuklukları, miyokardit ve diğer toparlanmayı geciktiren tıbbi sorunlar gibi- ayırt etmek için diğer ölçümlere de başvurmak gerekecektir.

ANAHTAR KELİMELER: Antrenman, aşırıya varmak, immün sistem, metabolizma, hormonlar

INTRODUCTION

Overtraining is defined as excessive training, characterized by long-lasting fatigue and worsening of competitive performance with further attempts to improve physical condition. Overtraining may also be described as staleness, overwork, over-reaching, burnout and chronic fatigue. Although improvements in athletic performance hinge on increasing the training load or “over-reaching”, overtraining - a vicious circle of more training producing lower performance and chronic fatigue - seems to be a stress response to training too hard too often, with insufficient recovery time between exercise bouts. In some cases, the term overtraining may not be appropriate, as other stressors (e.g. psychological, lifestyle, malnutrition, infection) may be responsible for underperformance (Budgett, 1990). Perhaps a better terminology is the description of this syndrome as “unexplained underperformance, confirmed by the athlete and coach, that is not resolved following at least two weeks rest”. This definition recognises that the cause of the underperformance and chronic fatigue is not necessarily solely related to the training load.

Overtraining and infections are two reasons why some athletes fail to perform to expectations. Athletes engaged in heavy training programmes, particularly those involved in endurance events, appear to be more susceptible to infection. For example, sore throats and flu-like symptoms are more common in such athletes than in the general population, and once infected, colds may last for longer in athletes who are training hard (Nieman, 1994; Pedersen and Bruunsgaard, 1995). There is some evidence that this increased susceptibility to infection arises due to a depression of immune system function. The causes of this and the reasons for the common association of recurrent infections with heavy training are the subject of current research worldwide. Upper respiratory tract infections (URTI) often seem to be cited as reasons for athletes having to pull out of competitive events; certainly an athlete suffering from a sore throat, runny nose or stuffy, tickly chest is unlikely to perform to the best of his/her ability and attempting

to compete whilst suffering from a viral illness could be dangerous. The links between heavy training and immunosuppression may provide a means of identifying athletes at risk of overtraining. This short review will discuss this possibility and assess the value of this approach and the use of other potential physiological and biochemical markers of impending overtraining.

OVERTRAINING AND OVER-REACHING

It is important to distinguish between overtraining - in which there is a chronic decrement in performance and which can take weeks or months to recover from - and over-reaching in which a relatively short-term decrement in performance may be experienced, but which is followed within a few days by a full recovery or improvement in performance (supercompensation). One of the problems is that it is often difficult to distinguish between over-reaching and the early stages of overtraining. Indeed, training can be viewed as a continuum from undertraining to optimal training, to over-reaching, to overtraining. The difference between over-reaching and overtraining is that the athlete recovers within days from the former, whereas overtraining results in sustained reductions in performance and is often (though not always) accompanied by other biochemical, physiological and psychological changes.

SYMPTOMS AND POTENTIAL MARKERS OF OVERTRAINING

The consequences of overtraining range from altered muscle function to motivation. The pathophysiology of overtraining can include muscle soreness and weakness, cytokine actions, hormonal and haematological changes, mood swings and psychological depression and nutritional problems such as loss of appetite and diarrhoea (Eichner, 1994). The number of symptoms that have been reported in overtrained athletes is large: Fry et al. (1991) have listed over 200. However, there are few if any, reliable markers of impending overtraining. The more commonly reported physiological and psychological symptoms associated with clinically



confirmed cases of overtraining are shown in Table 1. In some individuals the underlying cause could be a persistent viral infection, similar to glandular fever, or a type of postviral fatigue syndrome not unlike myalgic encephalomyelitis (ME). A marked fall in the number of circulating white blood cells (leukocytes) is often indicative of a chronic viral infection and low blood leukocyte counts are commonly found in athletes engaged in heavy training (Mackinnon, 1998a). Athletes suffering from overtraining syndrome are often reported to be immunosuppressed. Several indices of immune system function appear to be sensitive to both acute and chronic exercise stress. Hence, some of these and other biochemical changes that are commonly associated with heavy training have been proposed as potential markers of impending overtraining (Table 2). In other words this sensitivity of the immune system to changes in training load and other forms of stress could provide a means of assessing an athlete's ability to cope with intensified training and hence provide a way of identifying individuals who are verging on overtraining.

Table 1. Commonly reported physiological and psychological changes associated with overtraining.

- Underperformance
- Muscle weakness
- Chronic fatigue
- Sore muscles
- Increased perceived exertion during exercise
- Reduced motivation
- Sleep disturbance
- Increased early morning or sleeping heart rate
- Altered mood states (e.g. low scores for vigour; increased scores for fatigue and depression)
- Loss of appetite
- Gastrointestinal disturbance
- Recurrent infection

A major aim of recent research has been to try and identify suitable markers of immune system status in athletes. These markers together with performance testing, a diary of training and perceived reactions to it (e.g. fatigue, muscle soreness), monitoring of heart rate during sleep and psychological profiling may provide advance warning of impending overtraining. Some scientists believe that the best gauge of overtraining is how the athlete feels: as training advances, athletes tend to develop dose-related mood disturbances with low scores for vigour and rising scores for negative moods such as depression, tension, anger, fatigue and confusion (Morgan et al., 1987). These mood changes may reflect underlying biochemical or

immunological changes that are communicated to the brain via hormones and cytokines. With several weeks or more of heavy training, associated with repetitive large stress hormone responses (e.g. catecholamines, ACTH, cortisol, prolactin) it is likely that the body will respond by down-regulating specific hormone receptors in the target tissues, making the tissues less responsive to the effects of these hormones. Negative feedback responses, reduced sympathetic drive and down-regulation of anterior pituitary gland receptors for hypothalamic releasing factors (e.g. corticotrophin releasing factor) and/or inhibition of pituitary hormone pulse generators could result in a decreased pituitary hormone (e.g. adrenocorticotrophin, ACTH; growth hormone; follicle stimulating hormone, FSH; luteinising hormone, LH) response to stress. This and/or a down-regulation of receptors for ACTH on the cells of the adrenal cortex could result in a decreased release of cortisol in response to stress (Fry et al., 1991). There is good evidence from animal studies in which the adrenal cortex has been surgically removed and from human patients suffering from Addison's disease (who fail to secrete sufficient cortisol) that a glucocorticoid response to stress is essential to allow individuals to cope with a variety of stressors. There appear to be a number of hormonal abnormalities in athletes engaged in very heavy training and in those suffering from overtraining syndrome and it has been suggested that a disorder of regulation at the hypothalamus-pituitary may be the central disorder in overtraining syndrome (Lehmann et al., 1998). The fall in plasma levels of pituitary gonadotrophic hormones (FSH and LH) and gonadal sex steroids (e.g. oestrogen and testosterone) which causes a loss of normal menstrual function in females and a loss of libido in males may provide an early marker of this disorder (Foster and Lehmann, 1999).

There is also an increasing body of evidence to suggest that peripheral (and perhaps central) β -adrenergic receptors are down-regulated in overtraining syndrome. Although there appears to be an increased secretion of noradrenaline during exercise in overtrained athletes, the blunted heart rate and blood lactate responses (even with normal muscle glycogen) suggest that the heart and muscle (and possibly other tissues) are less responsive to the effects of catecholamines (Jeukendrup et al., 1992).

EFFECTS OF EXERCISE ON IMMUNE FUNCTION

The main component of the immune system comprises the leukocytes. The circulating numbers



and functional capacities of these cells may be decreased by repeated bouts of intense prolonged exercise (for a recent, comprehensive review see Mackinnon, 1998b). The reason is probably related to increased levels of stress hormones during exercise and entry into the circulation of less mature leukocytes from the bone marrow. Falls in the blood concentration of glutamine may also be implicated in causing the immunosuppression associated with heavy training (Parry Billings et al., 1992).

Table 2. Possible immunological and biochemical markers of impending overtraining.

-
- Leukocyte responses to antigens (e.g. lymphocyte proliferation, neutrophil degranulation, NK cytotoxic activity)
 - Salivary IgA
 - Neutrophil/Lymphocyte Ratio
 - T-cell CD4+/CD8+ Ratio
 - T-cell CD4+CD45RO+ expression
 - Plasma cortisol or cortisol/testosterone ratio
 - Urinary steroids or catecholamines
 - Plasma glutamine
 - Plasma urea
 - Plasma cytokines (e.g. IL-6)
 - Blood lactate response to incremental or high intensity exercise
 - Plasma or salivary cortisol response to high intensity exercise
-

During exercise exposure to airborne pathogens is increased due to the higher rate and depth of breathing. An increase in gut permeability may also allow increased entry of gut bacterial endotoxins into the circulation, particularly during prolonged exercise in the heat (Bosenberg et al., 1988). An acute bout of physical activity is accompanied by responses that are remarkably similar in many respects to those induced by infection: there is a substantial increase in the number of circulating leukocytes (mainly lymphocytes and neutrophils), the magnitude of which is related to both the intensity and duration of exercise. There are also increases in the plasma concentrations of various substances that are known to influence leukocyte functions, including inflammatory cytokines such as interferon- α , tumour necrosis factor, interleukins 1, 2 and 6, acute phase proteins like C-reactive protein and activated complement fragments (Mackinnon, 1998b). Hormonal changes also occur in response to exercise, including rises in the plasma concentration of several hormones (e.g. adrenaline, cortisol, growth hormone and prolactin) that are known to have immunomodulatory effects (Khansari et al.,

1990). Acute exercise temporarily increases natural killer (NK) cell lytic activity, but has been shown to diminish the proliferative response of lymphocytes to mitogens. Phagocytic neutrophils appear to be activated by an acute bout of exercise, but show a diminished responsiveness to stimulation by bacterial lipopolysaccharide (LPS) and reduced oxidative burst (killing capacity) after exercise which can last for many hours (Robson et al., 1999a). During recovery from exercise, NK cell numbers and activity fall below pre-exercise levels, and if the exercise bout was of high intensity or very prolonged, the number of circulating lymphocytes may be decreased below pre-exercise levels for several hours after exercise and the T-lymphocyte CD4+/CD8+ (helper/ suppressor) ratio is decreased. Following prolonged strenuous exercise the production of immunoglobulins (antibodies) by B-lymphocytes is inhibited. After prolonged exercise, the plasma concentration of glutamine has been reported to fall by about 20% and may remain depressed for some time (Parry Billings et al., 1992; Walsh et al., 1998a). Normal levels of plasma glutamine (about 600 μ M) appear to be required for optimal functioning of some leukocytes including lymphocytes and macrophages. These changes during early recovery from exercise would appear to weaken the potential immune response to pathogens and have been suggested to provide an "open window" for infection representing the most vulnerable time period for an athlete in terms of their susceptibility to contracting an infection (Pedersen and Bruunsgaard, 1995). Certainly, at this time, there does seem to be a temporary reduction in several aspects of immune function and athletes should be encouraged to adopt practices to minimise the risk of contracting an infection.

Exercise training also modifies immune function, with most changes on balance suggesting an overall decrease in immune system function, particularly when training loads are high. Circulating numbers of leukocytes are generally lower in athletes at rest compared with sedentary people (Blannin et al., 1996; Mackinnon, 1998a; 1998b). A low blood leukocyte count may arise from the haemodilution (expansion of the plasma volume) associated with training, or may represent altered leukocyte kinetics including a diminished release from the bone marrow. Indeed, the large neutrophilia that accompanies a bout of prolonged exercise could, over periods of months of heavy training, deplete the bone marrow reserve of these important cells. Certainly, the blood population of these cells seems to be less mature than those found in sedentary individuals (Keen et al., 1995) and the phagocytic activity of blood neutrophils has been reported to be markedly lower in well-trained



cyclists compared with age and weight-matched sedentary controls (Blannin et al., 1996). Levels of secretory immunoglobulins such as salivary IgA are lower in well-trained subjects (Mackinnon, 1996) as are T-lymphocyte CD4+/CD8+ ratios and *in vitro* mitogen-stimulated lymphocyte proliferation responses (Mackinnon, 1998b).

There are several possible causes of the diminution of immune function associated with heavy training. One mechanism may simply be the cumulative effects of repeated bouts of intense exercise with the consequent elevation of stress hormones, particularly glucocorticoids, causing temporary immunosuppression (Khansari et al., 1990). When exercise is repeated frequently there may not be sufficient time for the immune system to recover fully. Furthermore, plasma glutamine levels can change substantially after exercise and may become chronically depressed after repeated bouts of prolonged strenuous training (Parry Billings et al., 1992; Walsh et al., 1998a). Complement activation also occurs during exercise and a diminution of the serum complement concentration with repeated bouts of exercise, particularly when muscle damage is incurred, could also contribute to decreased non-specific immunity in athletes; well-trained individuals have a lower serum complement concentration compared with sedentary controls (Mackinnon, 1998b).

ATTEMPTING TO IDENTIFY ATHLETES AT RISK OF IMPENDING OVERTRAINING BY SCREENING PROCEDURES

Reliable techniques for the detection of the *onset* of overtraining have not yet been established. Possible markers are being studied, including blood levels of stress hormones, antibodies, cytokines and glutamine as well as the ability of leukocytes to respond to stimulation by antigens. Sleeping heart rate and psychological profiling are other approaches that have been tried and may be of some use. Measures of these potential markers made in athletes undertaking their normal training and in others whose training loads have been markedly increased, as well as in athletes who are diagnosed to be currently suffering from overtraining syndrome may enable sports scientists to screen athletes for the onset of overtraining. While no single marker can be taken as an indicator of impending overtraining, the regular monitoring of a combination of performance, physiological, biochemical, immunological and psychological variables would seem to be the best strategy to identify athletes who are failing to cope with the stress of training. Some of the approaches which

show some promise are outlined below. Again it is important to emphasise the need to distinguish overtraining from over-reaching and other potential causes of temporary underperformance such as anaemia, acute infection and insufficient carbohydrate intake.

There are several criteria that a reliable marker for the onset of overtraining must fulfil: the marker should be sensitive to the training load and ideally, be unaffected by other factors (e.g. diet). Changes in the marker should occur prior to the establishment of the overtrained state and changes in response to acute exercise should be distinguishable from chronic changes. Ideally, the marker should be relatively easy to measure and not too expensive.

BIOCHEMICAL MARKERS

Plasma glutamine

The concentration of plasma glutamine has been suggested as a possible indicator of excessive training stress (Rowbottom et al., 1996). Abnormally low levels of plasma glutamine are commonly reported in overtrained athletes, although not all studies have found a fall during periods of increased training and overtraining (for a recent review see Walsh et al., 1998a). The plasma glutamine concentration falls after an acute bout of prolonged exercise, but not after short-term high intensity exercise. Falls in glutamine can also occur after physical trauma, burns, inflammation and infection. The plasma glutamine concentration increases temporarily after consumption of a meal containing protein but falls by about 25% after several days on a low carbohydrate diet (Greenhaff et al., 1988). Thus, if glutamine is to be used as a marker of impending overtraining, as some authors have suggested (Rowbottom et al., 1996), then diet, timing of the blood sample in relation to the last bout of exercise and food intake must be standardised and other factors (e.g. infection, tissue injury) must be taken into account.

Plasma creatine kinase activity

Exercise-induced muscle damage is a likely candidate for the temporary fall in exercise performance during periods of over-reaching and many athletes that have been diagnosed as suffering from overtraining have reported that their muscles feel sore. The consequences of exercise-induced muscle damage include muscle pain, soreness and stiffness; reduced range of motion; higher than normal blood lactate concentration and perceived exertion during exercise, loss of strength and reduced maximal dynamic power output that can last for 5-10 days (Jones et al., 1986; Gleeson et al.,



1995). A practical index of muscle damage in athletes performing heavy training is elevation of muscle proteins (e.g. myoglobin, creatine kinase or lactate dehydrogenase) in the blood plasma. However, well-trained athletes who perform eccentric muscle actions do not usually show large increases in plasma creatine kinase activity, though they still experience soreness, perhaps as a result of damage and inflammation of connective tissue structures in muscle. Another detrimental effect of exercise-induced muscle damage is that it impairs the restoration of muscle glycogen (O'Reilly et al., 1987). Stores of glycogen become depleted after prolonged strenuous exercise. Damaged muscle has an impaired ability to take up blood-borne glucose that is required to resynthesize glycogen in the muscle. This would be expected to result in decreased endurance performance in subsequent exercise bouts. Assessment of plasma creatine kinase activity is therefore potentially useful, not as a marker of impending overtraining, but as a means of identifying a state of recent muscle damage or temporary over-reaching.

Plasma urea

Some authors have suggested that the concentration of nitrogenous wastes in the blood plasma (e.g. urea, 3-methyl histidine and uric acid) may provide a measure of muscle protein breakdown and hence may be markers of overtraining (Kindermann, 1986) because of the assumed association with a catabolic state (presumably caused by chronically elevated levels of glucocorticoid hormones). The main problem here is that prolonged acute exercise is associated with a temporary elevation of the plasma concentrations of uric acid and urea and that the latter is also markedly influenced by the recent dietary protein intake. Hence, plasma urea does not fulfil the requirements for a reliable indicator for the onset of overtraining.

Plasma hormones

The cortisol/testosterone ratio has also been put forward as a potential marker of overtraining based on the surmise that cortisol is a catabolic hormone and testosterone is an anabolic one. However, the logic of this argument is not convincing and several studies reviewed by Eichner (1995) have failed to observe a significant change in this ratio during progressive increases in the training loads of already well-trained athletes.

Nocturnal urinary catecholamine excretion appears to be lower than normal in overtrained athletes which may reflect a decrease in sympathetic drive (Foster and Lehmann, 1999). This could offer some hope of a hormonal marker of impending overtraining, but an even more promising approach

could be to measure the stress hormone response to one or more bouts of high intensity exercise. If during the development of overtraining syndrome there is a down-regulation of peripheral hormone receptors and/or a central dysfunction in hormonal regulation at the level of the hypothalamus-pituitary, then one would expect to see a blunted cortisol response to exercise in athletes at risk of impending overtraining. There is some evidence that this is the case (Figure 1), though further research is needed to establish which exercise testing protocols are most sensitive in identifying this problem. The fact that cortisol can be easily measured in saliva using commercially available radioimmunoassay or enzyme-linked immunosorbent assay kits could offer a means of identifying athletes at risk of overtraining without the need for blood sampling.

Plasma cortisol (nM)

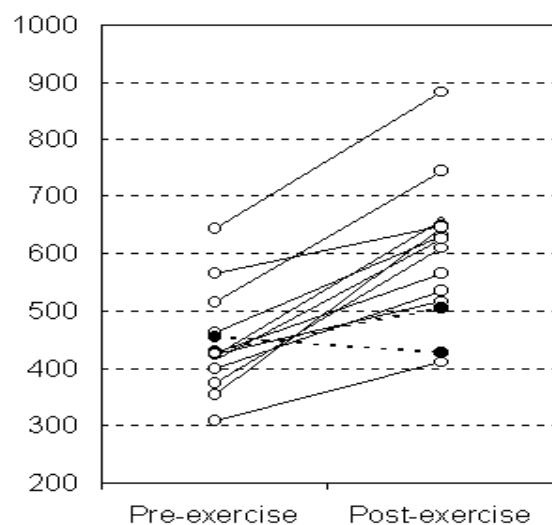


Figure 1. Changes in the plasma cortisol concentration in professional cyclists following two 10-minute bouts of strenuous hill climbing (heart rates 185-195 bpm). Open symbols represent healthy trained cyclists. Closed symbols represent two cyclists suspected of suffering from overtraining. Note the small or absent cortisol response to exercise in these two individuals. Data from Gleeson et al. (2000).

Barron et al. (1985) have also presented evidence of an adrenocortical deficiency in athletes suffering from overtraining syndrome. They found that growth hormone, prolactin and ACTH responses to insulin-induced hypoglycaemia (a potent stimulus to sympathetic nervous activity) were lower in a small group (n=4) of overtrained athletes compared with healthy well-trained controls. However, for obvious reasons, this would not be useful as part of a routine test battery to detect impending overtraining.

Blood lactate profiles

Some studies have reported lower blood lactate responses during submaximal exercise tests in overtrained athletes (Figure 2). This has been explained on the basis of low muscle glycogen levels, a decreased catecholamine response to exercise or a decreased muscle tissue responsiveness to the effects of catecholamines (Jeukendrup et al., 1992). This contrasts with the elevated blood lactate response to exercise following exercise-induced muscle damage described by Gleeson et al. (1998), and may offer a means of distinguishing between overtraining and over-reaching. Thus, as shown in Figure 3, a standardized high intensity exercise test with post-exercise blood lactate measurement could be used as a tool to identify athletes at risk of overtraining, if such a test was performed on a regular basis (say every 2 weeks).

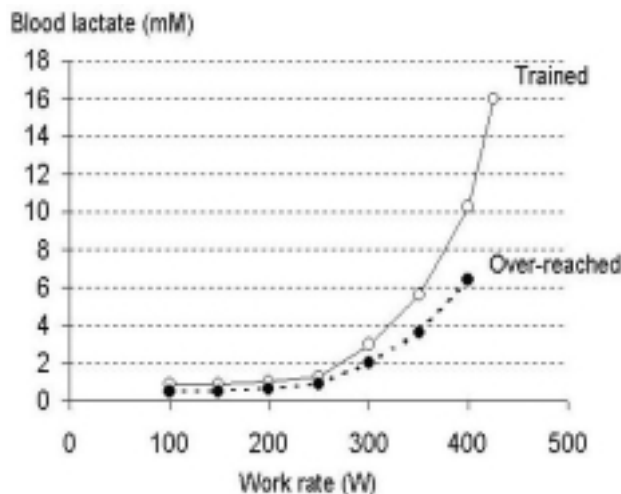


Figure 2. Example of the lower blood lactate response to incremental cycle ergometer exercise following a period of over-reaching in elite cyclists. Data from Jeukendrup et al. (1992).

IMMUNOLOGICAL MARKERS

The immune system is extremely sensitive to stress - both physiological and psychological - and thus, potentially, immune variables could be used as an index of stress in relation to exercise training. Regular blood monitoring could in future provide a diagnostic window for evaluating the impact of acute and chronic exercise on health (Smith and Pyne, 1997). The main drawback here is that measures of immune function are expensive and usually limited to just one aspect of what is a multi-faceted system which contains much redundancy. Several aspects of immune function are affected by both acute and chronic exercise and, of course, by tissue injury and infection.

Blood lactate (mM) at 300 W

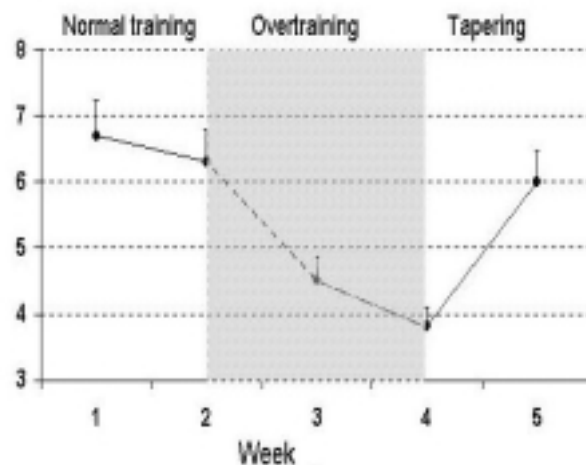


Figure 3. Reduction in the blood lactate concentration when cycling at a work rate of 300 W during a 2-week period of overtraining compared with normal training and a recovery taper period in a group of elite cyclists. Data from Jeukendrup et al. (1992).

Blood leukocyte subset counts

The majority of overtrained athletes have abnormally low blood leukocyte counts which means that regular blood monitoring could provide a guide to when exercise is becoming too stressful. Prolonged exercise in particular causes a large release of neutrophils from the bone marrow and it seems entirely plausible that repeated bouts of prolonged exercise over weeks or months could actually deplete the bone marrow of its reserves of mature neutrophils. This could account for the unusually low blood neutrophil numbers observed in many overtrained athletes. This combined with the depressant effect of acute bouts of exercise on neutrophil function (Smith and Pyne, 1997; Robson et al., 1999a) is likely to make such individuals much more susceptible to infection. Several recent studies indicate that feeding carbohydrate during prolonged exercise markedly reduces the rise in circulating neutrophil numbers (Nehlsen-Cannarella et al., 1997; Nieman, 1998) and also prevents the functional failure of neutrophils (Bishop et al., 2000) observed in exercise tests without carbohydrate supplementation.

Since in the hours following recovery from exercise the blood neutrophil count continues to increase and the blood lymphocyte count decreases, it has been suggested that the neutrophil/lymphocyte (N/L) ratio can provide a good measure of exercise stress and subsequent recovery (Nieman, 1998). The N/L ratio usually returns to normal within 6-9 hours after exercise, but where the exercise has been particularly prolonged and stressful, the N/L ratio

may still be elevated at 24 hours post-exercise (Figure 4). One advantage of this marker is that the N/L ratio can be easily estimated under a light microscope using a blood smear stained with Wright's stain.

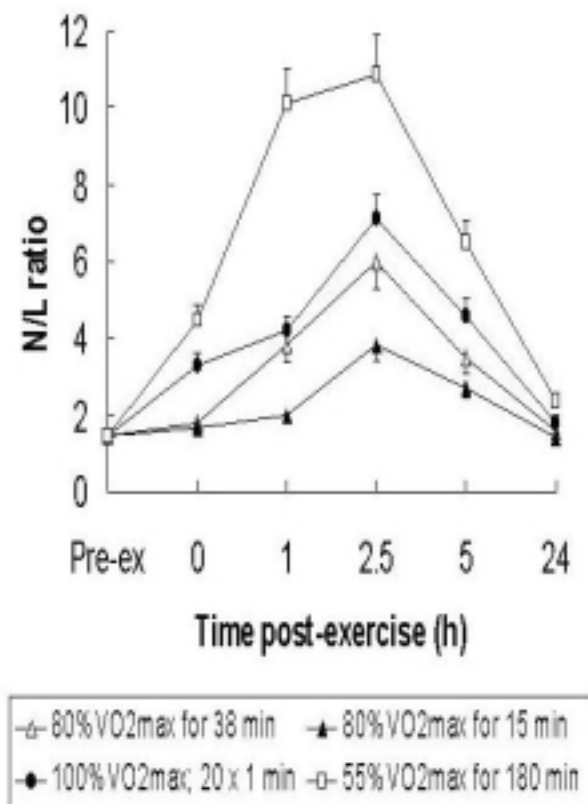


Figure 4. Changes in the neutrophil/lymphocyte (N/L) ratio following different intensities and durations of cycle ergometer exercise. Data from Robson et al. (1999a) and Walsh et al. (1998b). VO₂max, maximal oxygen uptake.

Circulating numbers of lymphocyte subsets change with exercise and training. With heavy training, the T-lymphocyte CD4+/CD8+ (helper/suppressor) ratio falls. However, this has not been shown to be different in athletes diagnosed as suffering from overtraining syndrome compared with healthy well-trained athletes. A recent study (Gabriel et al., 1998) has shown that the expression of other proteins on the cell surface of T-lymphocytes does seem to be sensitive enough to distinguish between the majority of overtrained athletes and healthy athletes (Figure 5). The expression of CD45RO+ on CD4+ cells (but not the circulating numbers of CD45RO+ T-cells) was significantly higher in athletes suffering from overtraining syndrome compared with healthy well-trained controls. Using this indicator, overtraining could be classified with high specificity and sensitivity.

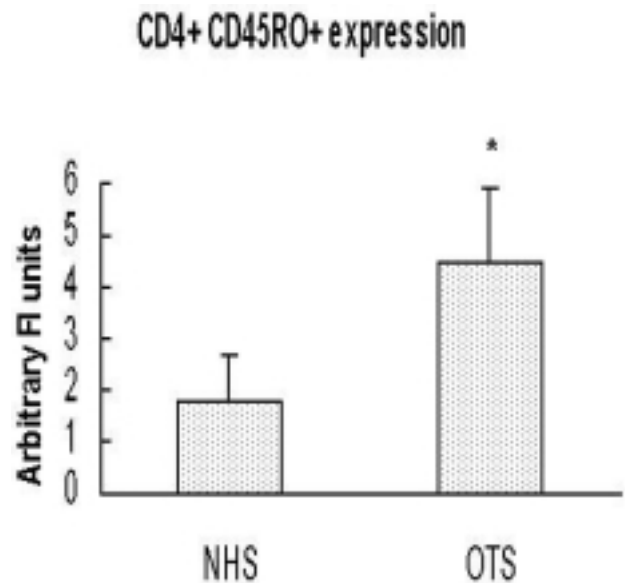


Figure 5. Mean fluorescence intensity (FI) of anti-CD45RO-FITC on CD4+ cells in athletes during periods of normal healthy status (NHS) and overtraining syndrome (OTS). Data from Gabriel et al. (1998); * P<0.001 OTS versus NHS.

Several indices of neutrophil function appear to be sensitive to the training load. A 5-week period of endurance training in previously sedentary subjects caused a 30% reduction in the LPS-stimulated neutrophil degranulation response (Blannin et al., 1997) and a 2-week period of intensified training in already well-trained triathletes was associated with a 20% fall in the LPS-stimulated neutrophil degranulation response (Robson, 1999b; Table 3). Other leukocyte functions including mitogen-stimulated lymphocyte proliferation and antibody synthesis and natural killer cell cytotoxic activity have been shown to sensitive to increases in the training load in already well-trained athletes (Table 3). However, these measures of immune cell function have not been identified as being able to distinguish between overtrained and healthy athletes (Verde et al., 1992). The main antibody or immunoglobulin (Ig) found in external secretions (e.g. mucous, tears, saliva) is IgA and this is considered to be an important mechanism of host defence, particularly against pathogens that cause URTI. Low levels of salivary IgA have been reported in overtrained athletes and progressive falls in saliva IgA concentration can be observed during periods of intensified training in elite swimmers (Mackinnon, 1996). Thus, regular monitoring of saliva IgA levels may be useful as a means of detecting overtraining. However, there are wide variations in resting saliva IgA concentration among different individuals and there is some disagreement in the literature about the acute effects of exercise on saliva IgA secretion (Blannin et al., 1998).



Certainly, for this variable, and indeed for most if not all other variables that have been suggested as potential markers of chronic exercise stress, it is essential to obtain individual profiles and identify what is the normal healthy baseline value for an individual. Simply comparing a “one-off” value for a particular athlete against a group mean or normal range is all too often not sensitive enough, misleading or uninformative.

PSYCHOLOGICAL AND PHYSIOLOGICAL MARKERS

Psychological profiling may also be undertaken to some effect using self-scored profiles of mood states; some scientists believe that the best gauge of overtraining is how the athlete feels: as training advances, athletes tend to develop dose-related mood disturbances with low scores for vigour and rising scores for negative moods such as depression, tension, anger, fatigue and confusion (Morgan et al., 1987). Gauging sensations of muscle soreness and fatigue during and after each training session has also been recommended (Noakes, 1992) and may be an effective way of monitoring the recovery from

deliberate over-reaching and identifying early development of overtraining syndrome.

Heart rate monitoring

Heart rate monitoring could be used to help detect the early stages of overtraining. An increased resting heart rate (usually measured by palpation after waking up in the morning) may indicate fatigue or overtraining but a more sensitive and reliable measure is the heart rate measured by radiotelemetry during sleep. Jeukendrup et al. (1992) had eight well-trained cyclists undergo a training programme in which the weekly training duration was increased by 45% with the duration of high intensity training bouts increased by 350%. After 2 weeks, performance had decreased in all subjects. Maximal heart rate fell significantly with overtraining. Time trial performance decreased and the heart rate during the time trial also decreased but no differences in perceived exertion were observed. The sleeping heart rate was increased in these overtrained cyclists. Furthermore, their heart rate pattern during the night was less regular and peaks were higher after overtraining (Jeukendrup and Van Dieman, 1998).

Table 3. Blood immune and biochemical variables at rest in endurance athletes during normal training and after 2-3 weeks of heavy intensified training. All data are mean (\pm S.E.M.).

	Normal Training	Heavy Training	
LPS-stimulated neutrophil degranulation response \blacklozenge (fg/cell)	166 (13)	111 (7)*	Robson et al. (1999b), (n=8)
Neutrophil/Lymphocyte Ratio	1.4 (0.2)	1.5 (0.2)	<i>Additional interval training sessions</i>
Saliva IgA (mg/l)	115 (21)	104 (25)	
Plasma cortisol (nM)	431 (37)	471 (42)	
Plasma glutamine (μ M)	686 (46)	646 (50)	
Plasma CK (U/l)	137 (33)	564 (189)*	
T-cell CD4+/CD8+ Ratio	2.91 (0.71)	2.05 (0.32)	Verde et al. (1992), (n=10)
IgG synthesis (ng/ml)	644 (207)	537 (130)*	<i>Training distance increased by 35%</i>
IgM synthesis (ng/ml)	730 (190)	585 (445)*	

* $P < 0.05$: significant effect of additional training

LPS, lipopolysaccharide; CK, creatine kinase; CD, clusters of differentiation; Ig, immunoglobulin.

\blacklozenge elastase release in response to stimulation with bacterial LPS.

CONCLUSIONS AND A SUGGESTED BATTERY OF TESTS FOR MONITORING ATHLETES AT RISK OF OVERTRAINING

Practical markers of overtraining would be ones that could be measured routinely in the laboratory and offered to athletes as part of their sports science and medical support. The identification of common factors among overtrained athletes in comparison with well-trained athletes not suffering from under-

performance could permit appropriate intervention to prevent athletes from progressing to a more serious stage of the overtraining syndrome. A possible battery of measures that includes those markers for which there is good scientific evidence of being reliable in detecting overtraining is shown in Table 4. If an athlete is suspected of suffering from over-training syndrome, other measures will also be required, if only to exclude other possible causes of underperformance including post-viral fatigue, glandular fever, clinical depression, poor



diet, anaemia, thyroid disorders, myocarditis and other medical problems interfering with recovery.

Table 4. Individual monitoring: a suggested battery of tests to detect impending overtraining.

-
- Performance
 - Mood state questionnaires
 - Diary of responses to training (fatigue, muscle soreness) and symptoms of illness
 - Sleeping heart rate
 - Blood lactate and plasma cortisol response to high intensity or incremental exercise
 - Plasma creatine kinase activity
 - Cortisol:Testosterone ratio
 - Nocturnal urinary noradrenaline and adrenaline secretion
 - Routine haematology (blood haemoglobin, serum ferritin, leukocyte counts)
 - T-lymphocyte CD4+/CD45RO+ expression
 - Experience of coach and athlete
-

Clearly, athletes fail to perform to the best of their ability if they become infected, stale, sore or malnourished. Excessive training with insufficient recovery can lead to a debilitating syndrome in which performance and well being can be affected for months. Eliminating or minimising these problems by providing advice and guidelines on training loads, recovery times, nutrition or pharmacological intervention and regular monitoring of athletes using an appropriate battery of markers can help prevent the development of an overtraining syndrome in athletes. Although further research is required, it seems that regular monitoring of the heart rate, plasma cortisol and blood lactate response to a standardized bout of high intensity exercise could provide an objective and reliable means of identifying athletes at risk of developing overtraining syndrome.

REFERENCES

- Barron, J.L., Noakes, T.D., Levy, W., Smith, C. and Millar, R.P. (1985) Hypothalamic dysfunction in overtrained athletes. *Journal of Clinical Endocrinology and Metabolism* **60**, 803-806.
- Bishop, N.C., Blannin, A.K., Rand, R., Johnson, R. and Gleeson, M. (2000) Effects of carbohydrate supplementation on the blood neutrophil degranulation responses to prolonged cycling. *International Journal of Sports Medicine* **21 (Suppl 1)**, S73.
- Blannin, A.K., Chatwin, L.J., Cave, R., and Gleeson, M. (1996) Effects of submaximal cycling and long term endurance training on neutrophil phagocytic activity in middle aged men. *British Journal of Sports Medicine* **30**, 125-129.
- Blannin, A.K., Gleeson, M., Brooks, S. and Cave, R. (1997) The effect of endurance training on the bacterially stimulated degranulation of human neutrophils *in vitro*. *Journal of Sports Sciences* **15**, 38.
- Blannin, A.K., Robson, P.J., Walsh, N.P., Clark, A.M., Glennon, L. and Gleeson, M. (1998) The effect of exercising to exhaustion at different intensities on saliva immunoglobulin A, protein and electrolyte secretion. *International Journal of Sports Medicine* **19**, 547-552.
- Bosenberg, A.T., Brock-Utne, J.G., Gaffin, S.L., Wells, M.T.B. and Blake, G.T.W. (1988) Strenuous exercise causes systemic endotoxemia. *Journal of Applied Physiology* **65**, 106-108.
- Budgett, R. (1990) Overtraining syndrome. *British Journal of Sports Medicine* **24**, 231-236.
- Eichner, E.R. (1994) Overtraining: Consequences and prevention. *Journal of Sports Sciences* **13**, S41-S48.
- Foster, C. and Lehmann, M. (1999) Overtraining syndrome. *Insider (Isostar Sport Nutrition Foundation)* **7**, 1-5.
- Fry, R.W., Morton, A.R. and Keast, D. (1991) Overtraining in athletes: An update. *Sports Medicine* **12**, 21-65.
- Gabriel, H.H.W., Urhausen, A., Valet, G., Heidelbach, U. and Kindermann, W. (1998) Overtraining and immune system: A prospective longitudinal study in endurance athletes. *Medicine and Science in Sports and Exercise* **30**, 1151-1157.
- Gleeson, M., Blannin, A.K., Zhu, B., Brooks, S. and Cave, R. (1995) Cardiorespiratory, hormonal and haematological responses to submaximal cycling performed 2 days after eccentric or concentric exercise bouts. *Journal of Sports Sciences* **13**, 471-479.
- Gleeson, M., Blannin, A.K., Walsh, N.P., Field, C.N.E. and Pritchard, J.C. (1998) Effect of exercise-induced muscle damage on the blood lactate response to incremental exercise in humans. *European Journal of Applied Physiology* **77**, 292-295.
- Gleeson, M., Jeukendrup, A.E., Blannin, A.K. and Bishop, N.C. (2000) Plasma and saliva cortisol responses to hill climbing in elite road race cyclists. *Medicine and Science in Sports and Exercise* **32 Suppl**, S271.
- Greenhaff, P.L., Gleeson, M. and Maughan, R.J. (1988) The influence of an alteration in diet composition on plasma and muscle glutamine levels in man. *Clinical Science* **74**, 20P.
- Jeukendrup, A.E., Hesselink, M.K.C., Snyder, A.C., Kuipers, H. and Keizer, H.A. (1992) Physiological changes in male competitive cyclists after two weeks of intensified training. *International Journal of Sports Medicine* **13**, 534-541.
- Jeukendrup, A.E. and Van Dieman, A. (1998) Heart rate monitoring during training and competition in cycling. *Journal of Sports Sciences* **17**, S591-S599.
- Jones, D.A., Newham, D.J., Round, J.M. and Tolfree, S.E.J. (1986) Experimental human muscle damage:



- morphological changes in relation to other indices of damage. *Journal of Physiology* **375**, 435-448.
- Keen, P., McCarthy, D.A., Passfield, L., Shaker, H.A.A. and Wade, A.J. (1995) Leucocyte and erythrocyte counts during a multi-stage cycling race ('The Milk Race'). *British Journal of Sports Medicine* **29**, 61-65.
- Khansari, D.N., Murgu, A.J. and Faith, R.E. (1990) Effects of stress on the immune system. *Immunology Today* **11**, 170-175.
- Kindermann, W. (1986) Overtraining – an expression of faulty regulated development. translated from *Deutsche Zeitschrift Fur Sportmedizin* **37**, 238-245.
- Lehmann, M., Foster, C., Dickuth, H.-H. and Gastmann, U. (1998) Autonomic imbalance hypothesis and overtraining syndrome. *Medicine and Science in Sports and Exercise* **30**, 1140-1145.
- Mackinnon, L.T. (1996) Exercise, immunoglobulin and antibody. *Exercise Immunology Review* **2**, 1-35.
- Mackinnon, L.T. (1998a) Effects of overreaching and overtraining on immune function. In: *Overtraining In Sport*. Ed: Kreider, R.B., Fry, A.C., O'toole, M.L. Champaign Il: Human Kinetics. 219-241.
- Mackinnon, L.T. (1998b) *Exercise And Immunology*. 2nd Edition. Champaign Il: Human Kinetics.
- Morgan, W.P., Brown, D.R. and Raglin, J.S. (1987) Mood disturbance following increased training in swimmers. *British Journal of Sports Medicine* **21**, 107-114.
- Nehlsen-Cannarella, S.L., Fagoaga, O.R., Nieman, D.C., Henson, D.A., Butterworth, D.E., Schmitt, R.L., Bailey, E.M., Warren, B.J., Utter, A. and Davis, J.M. (1997) Carbohydrate and the cytokine response to 2.5 h of running. *Journal of Applied Physiology* **82**, 1662-1667.
- Nieman, D.C. (1994) Exercise, infection and immunity. *International Journal of Sports Medicine* **15**, S131-S141.
- Nieman, D.C. (1998) Influence of carbohydrate on the immune response to intensive, prolonged exercise. *Exercise Immunology Review* **4**, 64-76.
- Noakes, T.D. (1992) *Lore Of Running* 2nd Edition. Cape Town: Oxford University Press.
- O'reilly, K.P., Warhol, M.J. and Fielding, R.A. (1987) Eccentric exercise-induced muscle damage impairs muscle glycogen repletion. *Journal of Applied Physiology* **63**, 252-256.
- Parry-Billings, M., Budgett, R., Koutedakis, Y., Blomstrand, E., Brooks, S., Williams, C., Calder, P.C., Pilling, S., Baigre, R. and Newsholme, E.A. (1992) Plasma amino acid concentrations in the overtraining syndrome: Possible effects on the immune system. *Medicine and Science in Sports and Exercise* **24**, 1353-1358.
- Pedersen, B.K. and Bruunsgaard, H. (1995) How physical exercise influences the establishment of infections. *Sports Medicine* **19**, 393-400.
- Robson, P.J., Blannin, A.K., Walsh, N.P., Castell, L.M. and Gleeson, M. (1999a) Effects of exercise intensity, duration and recovery on *in vitro* neutrophil function in male athletes. *International Journal of Sports Medicine* **20**, 128-135.
- Robson, P.J., Blannin, A.K., Walsh, N.P., Bishop, N.C. and Gleeson, M. (1999b) The effect of an acute period of intense interval training on human neutrophil function and plasma glutamine in endurance-trained male runners. *Journal of Physiology* **515P**: 84-85P.
- Rowbottom, D.G., Keast, D. and Morton, A.R. (1996) The emerging role of glutamine as an indicator of exercise stress and overtraining. *Sports Medicine* **21**, 80-97.
- Smith, J.A. and Pyne, D.B. (1997) Exercise, training, and neutrophil function. *Exercise Immunology Review* **3**, 96-116.
- Verde, T., Thomas, S. and Shephard, R.J. (1992) Potential markers of heavy training in highly trained endurance runners. *British Journal of Sports Medicine* **26**, 167-175.
- Walsh, N.P., Blannin, A.K., Robson, P.J. and Gleeson, M. (1998a) Glutamine, exercise and immune function: links and possible mechanisms. *Sports Medicine* **26**: 177-191.

AUTHOR'S BIOGRAPHY:



Michael GLEESON

Employment:

Professor of Exercise Biochemistry,
University of Birmingham.

Degrees:

BSc, PhD

Research interests:

Exercise immunology and
metabolism; sports nutrition

E-mail: m.gleeson@bham.ac.uk

✉ **Professor Michael Gleeson**

School of Sport and Exercise Sciences, University of
Birmingham, Edgbaston, Birmingham B15 2TT, England