Overtraining may be best described as an imbalance between stress, involving training as well as non-training sources, and recovery. As many athletes incorporate high training volumes and limited recovery periods into their training regimen, they risk the development of overreaching. Overreaching is defined as an accumulation of training and/or nontraining stress resulting in a short-term decrement in performance capacity, in which restoration of performance capacity may take from several days to several weeks (OR) (9). It is generally believed that if the imbalance between training and recovery persists this may result in a long-term decrement in performance capacity, in which restoration of performance capacity may take several weeks or months. This condition is termed overtraining (OT) (9).

Long-duration, high-intensity exercise has been associated with immunosuppression (18,20), including a reduction in the circulating lymphocyte concentration and suppression of natural killer cell activity and secretory IgA in mucosal fluid (12,20). Given the high volume of training and limited recovery periods often associated with overreaching and overtraining, it has been suggested that immunosuppression may occur in overtrained athletes.

Glutamine is a neutral amino acid found in high levels in a number of human tissues (21) and is the most abundant amino acid in human muscle tissue and plasma (1). Under normal conditions, glutamine levels are maintained by a balance between the release and utilization of glutamine by various organs (21). The brain, lungs, liver, skeletal muscle, and possibly adipose tissue release glutamine, whereas cells of the immune system, the liver, kidneys, and gastrointestinal tract are the primary utilizers (21). The determination of whether a cell is a net producer or consumer of glutamine is based on the direction of a single reversible reaction (21,28). Glutamine is synthesized from ammonia and glutamate by glutamine synthetase. Glutaminase catalyzes the reverse reaction to form ammonia and glutamate from glutamine (21).

According to Rowbottom et al. (21), glutamine may be the most versatile of the amino acids. This is evidenced by the differing roles that glutamine has in a number of tissues.
and organs. These include the transfer of nitrogen between organs and detoxification of ammonia, maintenance of acid-base balance during acidosis, as a nitrogen precursor for the synthesis of nucleotides, a fuel for gut mucosal cells, a fuel for cells of the immune system, and as a possible direct regulator of protein synthesis and degradation (21).

A decline in plasma glutamine concentration as a result of intensified training suggests that there is either an increased demand for glutamine by tissues that require glutamine as a fuel and/or a decreased production or altered transport kinetics of this amino acid (28). A recent hypothesis suggests that increased hepatic and gastrointestinal uptake of glutamine for gluconeogenesis is occurring at a time when muscle release of glutamine remains constant or is decreasing (28), thereby explaining the fall in postexercise plasma glutamine.

As the mechanisms behind the performance decrements associated with overtraining are unclear, a combination of a number of markers is needed for early diagnosis. Changes in the plasma glutamine/glutamate ratio (Gln/Glu) have recently been suggested as a predictor of overreaching or overtraining in athletes (23). Elevated plasma glutamate and hence a reduced Gln/Glu ratio was observed in athletes who were classified as overtrained (23), and Parry-Billings et al. (19) reported lower glutamine and increased glutamate levels in overtrained athletes. However, no studies have investigated changes in glutamine, glutamate, and reported concurrent performance measures during a period of intensified training that has resulted in overreaching.

Currently, there is no unifying hypothesis to adequately explain the mechanisms behind the variety of changes that are associated with overtraining. Recently in an attempt to integrate the available information regarding overtraining, Smith (24) proposed the cytokine hypothesis of overtraining. It is suggested that exercise-induced microtrauma to the musculoskeletal system leading to a local inflammatory response is the initiating event in the development of overtraining. Inadequate recovery and a continuation of the athletes’ training regimen compound this initial local inflammation leading to chronic inflammation. This results in the release of inflammatory mediators and the subsequent release of pro-inflammatory cytokines from activated monocytes, which causes systemic inflammation. This induces “sickness” behavior (fatigue, appetite suppression, depression), activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal-axis, suppression of the hypothalamic-pituitary-gonadal-axis, up-regulation of liver function, and possibly immunosuppression (24). Only one investigation has examined interleukin-6 (IL-6) in overtrained athletes (N = 4) and found that levels were within normal ranges and unaffected by overtraining (19). However, until now there has been no assessment of the changes in plasma cytokines and performance levels in response to overreaching, a state considered to be the precursor of the overtraining syndrome.

The aims of the present study were threefold. First, to investigate the possibility of altered plasma cytokine concentrations in response to overreaching and recovery. Second, to determine whether the Gln/Glu ratio is altered as result of intensified training. Finally, measurements of a range of immunological, biochemical, and hematological parameters were performed to examine other possible indicators of overreaching. It is hypothesized that plasma cytokines will remain unchanged after a 2-wk period of intensified training and the ratio of glutamine to glutamate is expected to decline.

**METHODS**

**Approach to the Problem and Experimental Design**

To determine whether changes in various immunological and hematological indices occur with overreaching, a 6-wk training period was employed (Fig. 1). During this period, training was manipulated to induce a state of overreaching identified by a decline in performance and an increase in mood disturbance. Immunological and hematological measures were made before intensified training, after intensified training, and during a period of recovery to determine whether changes in these measures occurred alongside the reduction in performance. Resting plasma cytokines were examined during normal, intensified and recovery training to investigate possible changes in cytokines during overreaching. Additionally, resting Gln and Glu concentrations were measured to ascertain their use as a marker of overreaching. Routine hematological measures were also made as such measures can be relatively easily obtained and monitored in athletes.

Eight endurance-trained cyclists completed a 6-wk training protocol described below. Physical characteristics are...
outlined in Table 1. The study was approved by the South Birmingham Local Research Ethics Committee. Before participation, and after both comprehensive verbal and written explanations of the study, all subjects gave written informed consent. Each subject completed six incremental maximal oxygen uptake tests to volitional exhaustion (MT), six simulated time trials (TT), and eight intermittent tests (IT).

### Training

Subjects completed 6 wk of training, which consisted of two weeks of normal training (N), 2 wk of an intensified training period (ITP), and 2 wk of recovery (R). Subjects wore a heart rate monitor during all training sessions. This was so the researchers could document training intensity and to ensure that all subjects completed the prescribed training. Training during the ITP was based on each individual’s normal training, which was quantified by heart rate monitoring (Vantage NV, Polar, Kempele, Finland) during training during N as well as questionnaire assessment of normal training volumes. Blood lactate concentrations and heart rate responses from maximal cycle ergometer tests were used to calculate training zones. For additional information see Halson et al. (5).

### Maximal Cycle Ergometer Test (MT)

Subjects attended the laboratory after an overnight fast, and a Teflon catheter (Becton Dickinson, Quickcath) was inserted into an antecubital vein. After this, the subjects performed an incremental test to exhaustion on an electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine maximal aerobic power output (W_max), submaximal and maximal oxygen consumption, and heart rate. Work rate began at 95 W and increased by 35 W every 3 min until volitional exhaustion. Blood samples were collected at the end of each stage and blood lactate concentration was immediately determined (YSI 2300 STAT Plus, Yellow Springs, OH).

### Time Trial (TT)

After a 5-min warm-up at 50% W_max, subjects performed a simulated time trial in which they were asked to complete a target amount of work as fast as possible. The amount of work to be performed was calculated by assuming that subjects could cycle at 75% of their W_max for ~60 min at a cadence of 80 rpm and thus these time trials lasted approximately 60 min for all subjects.

### Intermittent Test (IT)

Unlike the TT, the IT was of a set duration and the change in work performed and mean power output was assessed. Subjects completed a 5-min warm-up at 50% W_max, followed by two 10-min bouts of maximal exercise. Each subject was given 5-min rest between bouts. Subjects were asked to produce a maximal effort for each of the 10-min bouts, i.e., to produce the maximal amount of work possible, which could be viewed on a computer screen in front of the subject.

### Questionnaires

Every day for the duration of the study, subjects completed both the Daily Analysis of Life Demands of Athletes (DALDA) (22) and Profile of Mood States Short Form Questionnaire (POMS-22) (16). The DALDA is divided into parts A and B, which represent the sources of stress and the manifestation of this stress in the form of symptoms, respectively. Subjects were asked to complete these questionnaires at the same time of each day before training. Subjects also completed the 65-question version of the POMS (16) once a week on the morning of the MT. Global mood state was determined using the method described by Morgan et al. (17).

### Blood Handling, Storage, and Analysis

Measurements were performed on resting, overnight-fasted samples, which were collected in the morning, once per week over the 6 wk of the study. Samples were collected immediately before the maximal cycle ergometer tests after insertion of a Teflon catheter (Becton Dickinson, Quickcath) into an antecubital vein. Venous blood was collected into K2EDTA tubes and centrifuged at 1500 g for 10 min at 4°C; plasma was stored at −20°C. All samples were measured in duplicate with the exception of hematological variables. To avoid interassay variation, all samples were analyzed in one batch at the end of the study, with the exception of hematological measures, which were performed on the day of collection. The intra-assay coefficient of variation for the metabolites, creatine kinase, and cytokines measured were all less than 5%, with the exception of glutamine and glutamate, which was 7% and salivary IgA which was 10%.

Saliva samples were collected before and after the 8th IT. Subjects were fasted for at least 3 h before testing, and saliva samples were collected immediately before the first bout and immediately after the second bout. Subjects were instructed to swallow, and then unstimulated whole saliva was collected over a 3-min period into tubes before exercise and immediately postexercise. Subjects were instructed to allow saliva to dribble into the collecting tubes unaided by spitting. All saliva collections were made with subjects seated, leaning forward with their heads down.

#### Plasma urea

Plasma urea was measured using an enzymatic colorimetric endpoint method (Kit No. 640-A, Sigma, Poole, UK).

#### Plasma creatine kinase activity

Plasma creatine kinase (CK) activity was determined at 30°C using an enzymatic kit (No. 47–10, Sigma, Poole, UK).

#### Plasma ammonia and glutamine

Plasma glutamine was analyzed enzymatically by first determining the plasma...
ammonia concentration based on the reductive amination of 2-oxoglutarate, using glutamate dehydrogenase (EC 1.4.1.3) and reduced nicotinamide adenine dinucleotide (Sigma, Poole, UK). Plasma was then incubated for 60 min at 37°C with glutaminase (EC 3.5.1.2), converting free glutamine to ammonia and glutamate (10), and the ammonia concentration measured. Plasma glutamine levels were calculated by subtracting the untreated plasma ammonia concentration from the ammonia concentration in the sample treated with glutaminase.

**Plasma glutamate.** Plasma glutamate was analyzed enzymatically using glutamate dehydrogenase (EC 1.4.1.3) and nicotinamide adenine dinucleotide.

**Hematology.** Venous blood was used for hematological analysis of differential leukocyte counts using a Technicon H-2 laser system (Bayer Diagnostics, Basingstoke, UK). This included determination of the total leukocyte count and neutrophil, lymphocyte, and monocyte counts.

**Plasma cytokines.** Plasma concentrations of IL-6 and tumor necrosis factor-α (TNF-α) were determined in aliquots of plasma with the use of quantitative sandwich-type enzyme-linked immunosorbent (ELISA) kits (R&D Systems, Abingdon, UK). A high-sensitivity kit was used for analysis of IL-6.

**Salivary IgA.** After thawing, stored saliva samples were analyzed for IgA using a sandwich-ELISA method (27). Briefly, flat-bottomed microtitration plates (Linbro EIA plates, Flow Laboratories Inc., McLean, VA) were coated with the primary antibody, rabbit antihuman IgA (I-8760, Sigma), at a dilution of 1 in 800 in carbonate buffer, pH 9.6. After washing with phosphate-buffered saline (PBS, pH 7.2), the plates were coated with blocking protein solution (2% w/v casein in PBS). Sample analysis was performed in duplicate using saliva samples diluted 1 in 1000 with deionized water and a range of standards (Human colostrum IgA, 1-2636, Sigma) up to 400 μg·L−1. A reference sample was incorporated into each microwell plate, and all samples from a single subject were analyzed on a single plate. The plates were incubated for 60 min at 20°C. After a washing step, peroxidase-conjugated goat antihuman IgA (A-4165, Sigma) was added and the plate incubated for a further 60 min at 20°C. After another washing step, the substrate, ABTS (Boehringer Mannheim, Lewes, UK), was added and after 30 min the absorbance was measured at 405 nm.

**Statistical Analysis**

Changes in all variables over time was analyzed using a repeated measures analysis of variance, with least significance difference comparison performed to identify significant differences between the individual means. The level of statistical significance was set at P < 0.05.

**RESULTS**

**Responses to training.** Subjects completed 2 wk of normal training (N, 7 ± 2 h·wk−1), 2 wk of intensified training (ITP, 14 ± 5 h·wk−1), and a final 2 wk of recovery training (R, 3.5 ± 2 h·wk−1). Performance on MT, TT, and IT all significantly declined after the intensified training period and subjects demonstrated altered psychological state, with significant changes in global mood state and both the source and manifestation of stress. Both performance and mood state returned to baseline or near baseline values after the recovery period. From this information, it was concluded that all subjects were overreached upon completion of the intensified training.

At the end of ITP, maximal power output during MT significantly declined by 5.4% and maximal work produced during the IT was also significantly reduced from 181 ± 10 to 166 ± 12 kJ. At the same time point, TT time significantly increased from 59.4 ± 1.9 min during N to 64.0 ± 2.3 min. For additional information on performance changes see Halson et al. (5).

**Questionnaires.** All subjects demonstrated altered mood state with significantly increased scores on the POMS-65 from 90.4 during N to 116.4 during ITP. Upon completion of R, scores returned to 91.5. From this questionnaire, the subscales of tension, fatigue and confusion were also significantly elevated during ITP, whereas vigor significantly declined. No changes were evident in the depression or anger subscales. Increased total mood disturbance was also identified by the short version of the POMS questionnaire, with significantly elevated total scores during ITP. Parts A and B of the DALDA also increased during ITP. The most common changes in sources of stress, as identified by part A of the DALDA, were related to sport training, sleep, and health. The most common alterations in responses to part B were increased problems associated with the following areas: need for a rest, recovery, irritability, between session recovery, general weakness, and training effort.

**Immunology.** Saliva IgA concentration was 121 ± 14 mg·L−1 during N and fell during ITP to 91 ± 14 mg·L−1, with some recovery by the end of R (110 ± 14 mg·L−1) (Table 2); however, these changes were not statistically significant, even when statistical analysis was performed on normalized data. No significant changes were observed in resting plasma IL-6 or TNF-α concentrations throughout the duration of the study (Table 2).

**Biochemistry.** Plasma creatine kinase activity was significantly elevated during the ITP and returned to baseline levels during R (Table 2). Plasma urea concentration tended to be slightly elevated during the ITP (P = 0.057) and also declined to preintensive training levels after recovery (Table 2). However, this increase was not statistically significant. Plasma ammonia also showed a trend for increased levels during ITP (P = 0.067) (Table 2).

There were no significant changes in plasma glutamine concentration over the 6-wk period; however, values declined to 475 ± 40 μM after ITP (Fig. 2a). Plasma glutamate was significantly elevated during ITP and returned to baseline levels during R (Fig. 2b). Hence, the Gln/Glu ratio was significantly lower in the ITP compared with N. Although the ratio had not returned to pretraining values after...
R, there was no statistically significant difference between R and N (Fig. 2c).

**Hematology.** During the ITP red blood cell count (RBC), Hb, and packed cell volume (PCV) significantly declined and after R had returned to initial levels (Table 3). No changes were observed in mean red blood cell volume (MCV), platelets, white blood cell count, neutrophils, lymphocytes, monocytes, or neutrophil/lymphocyte ratio (Table 3).

**DISCUSSION**

The present investigation does not provide evidence for alterations in cytokines and other immune system parameters during overreaching. However, a decline in the Gln/Glu ratio was evident, and this ratio may be useful as a diagnostic tool for overreaching.

Although in the present study there were no statistically significant changes in resting glutamine concentrations, during the second week of ITP, glutamine concentrations were lower compared with N. This is similar to two previous investigations (7,21) that both reported a decline in glutamine concentration in overreached athletes. Glutamine concentration remained unchanged in swimmers who were classified as overreached after 4 wk of intensified training. However, the well-trained athletes had 20% higher concentrations of plasma glutamine compared with those who were overreached (13). A decline in glutamine may be the result of greater uptake of this amino acid for gluconeogenesis (28). An increase in gluconeogenesis may be the result of glycogen depletion due to continued intensified training; however, alterations in glucose kinetics as a result of overreaching has not been examined. Although plasma glutamine concentration may or may not decrease after periods of intensified training, there is still little evidence to link low glutamine levels with impaired immune function and increased susceptibility to illness or infection (1). However, the use of glutamine concentrations as a marker to indicate impending or current overtraining warrants further attention.

The mechanism/s for the elevation in plasma glutamate with intensified training are unknown. High plasma glutamate concentrations have been reported in catabolic conditions such as cancer, human immunodeficiency vi-
rus infection, and sepsis (4). Elevated plasma glutamate concentration in cancer patients was reported to indicate decreased uptake of glutamate into the peripheral muscle tissue, possibly as a consequence of reduced transport activity (4). Kinscherf et al. (8) suggested that high plasma glutamate in combination with insufficient baseline glutamine levels may result in catabolism or a loss of body cell mass (cachexia) in healthy subjects after very high intensity exercise. These authors suggested that glutamate transport activity may be inhibited when there is a high rate of glycolytic activity in skeletal muscle (8). The significance of the elevated glutamate levels is unknown; however, glutamate has been shown to have no effect on the rate of T-lymphocyte proliferation in vitro (19). Elevated plasma glutamate concentrations may be associated with overreaching and overtraining; however, the role of glutamate in the mechanisms of overreaching and overtraining is questionable.

Smith and Norris (23) reported unchanged resting plasma glutamine concentrations in athletes who were classified as overtrained, yet plasma glutamate concentration was significantly elevated in this group. Thus, our observation of an elevated Gln/Glu ratio after intensified training was also shown in this previous investigation. The glutamine and glutamate values reported by Smith and Norris (23) are similar to those of the present investigation. Our data supports their values suggested to indicate overreaching, i.e., a ratio < 3.58. As performance in the present study returned to baseline after 2 wk of recovery, it was concluded that the subjects were overreached as opposed to overtrained. After recovery the Gln/Glu ratio returned to above 3.58 and therefore supports the classification of overreaching based on the Gln/Glu ratio suggested by Smith and Norris (23).

The decrease in RBC, Hb, and PCV during intensified training most likely reflects plasma volume expansion. The unchanged resting blood leukocyte counts are consistent with previous research in this area (14). However, declining leukocyte counts after 4 wk of training have been found alongside lower lymphocyte counts in overreached athletes when compared with well-trained athletes after 2 wk of intensified training (14). This may suggest that leukocyte counts reflect the athlete’s training status and/or may be associated with longer term intensified training, i.e., greater than 2 wk.

Our findings of unchanged resting plasma IL-6 and TNF-α concentrations during a 2-wk period of intensified training despite changes in performance, fatigue, and mood state do not support the role of cytokines in overreaching. According to the cytokine hypothesis of overtraining, the proinflammatory cytokine IL-1β, in addition to plasma IL-6 or TNF-α, is central to the development of overtraining. In the present study, we did not measure IL-1β, and the possibility cannot be excluded that elevated systemic levels of IL-1β may have accounted for some of the changes we observed in our group of overreached subjects. However, as IL-6 and TNF-α were unchanged, it is doubtful that IL-1β would have demonstrated considerable changes.

The cytokine hypothesis of overtraining proposes that trauma to the musculoskeletal system leading to a local inflammatory response is the initiating event in the development of overtraining. Mechanical injury to the musculoskeletal system due to high impact forces, and in particular injury to those muscles that contract eccentrically to absorb foot strike impact forces (2), are the likely source of microtrauma and local inflammation during running. However, during cycling, muscle contraction is almost entirely concentric, and mechanical trauma to the musculoskeletal system from ground impact forces does not occur. Although similar CK levels have been observed during concentric, eccentric, and isometric arm flexion exercise (3), it is recognized that eccentric muscular contractions result in greater muscle fiber injury than concentric contractions. It is therefore proposed that ischemia/reperfusion injury may be a possible source of the microtrauma leading to local inflammation during activities such as cycling which are predominately composed of concentric muscle contractions.

Exercise-induced muscle ischemia/reperfusion injury may occur as a result of fibers within the contracting muscle experiencing hypoxia followed by reoxygenation upon the cessation of exercise and the subsequent generation of reactive oxygen species (29). However, the available scientific evidence does not support such a view (6,15), and it has been suggested that during prolonged exercise, athletes are highly unlikely to experience ischemic muscle injury (2). Without an initial source of microtrauma the development of local and chronic inflammation will not occur. Furthermore, circulating monocytes will not become activated, and

**IMMUNOLOGICAL RESPONSES TO OVERREACHING**

**TABLE 3.** Selected hematological variables during normal training (N), intensified training (ITP), and recovery (R).

<table>
<thead>
<tr>
<th>Week</th>
<th>N</th>
<th>2</th>
<th>3</th>
<th>ITP</th>
<th>4</th>
<th>5</th>
<th>R</th>
<th>6</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC ($10^{12}$ L$^{-1}$)</td>
<td>4.95 ± 0.06*</td>
<td>4.58 ± 0.08</td>
<td>4.40 ± 0.08**</td>
<td>4.27 ± 0.07**</td>
<td>4.57 ± 0.06*</td>
<td>4.55 ± 0.08*</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (g·dL$^{-1}$)</td>
<td>14.7 ± 0.2*</td>
<td>13.7 ± 0.2</td>
<td>13.1 ± 0.3**</td>
<td>12.8 ± 0.2**</td>
<td>13.6 ± 0.2*</td>
<td>13.5 ± 0.3*</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCV (L·L$^{-1}$)</td>
<td>0.44 ± 0.01*</td>
<td>0.41 ± 0.01*</td>
<td>0.39 ± 0.01**</td>
<td>0.39 ± 0.01**</td>
<td>0.41 ± 0.01**</td>
<td>0.41 ± 0.01*</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>89.7 ± 0.7</td>
<td>90.2 ± 0.9</td>
<td>90.6 ± 0.5</td>
<td>90.8 ± 0.5</td>
<td>91.1 ± 0.4</td>
<td>89.8 ± 0.7</td>
<td>0.120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets ($10^{12}$ L$^{-1}$)</td>
<td>238 ± 13</td>
<td>232 ± 10</td>
<td>240 ± 11</td>
<td>256 ± 19*</td>
<td>275 ± 16*</td>
<td>239 ± 21</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC ($10^{9}$ L$^{-1}$)</td>
<td>5.6 ± 0.4</td>
<td>5.1 ± 0.2</td>
<td>5.3 ± 0.4</td>
<td>5.7 ± 0.7</td>
<td>5.2 ± 0.4</td>
<td>5.2 ± 0.5</td>
<td>0.813</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils ($10^{9}$ L$^{-1}$)</td>
<td>2.9 ± 0.3</td>
<td>2.5 ± 0.2</td>
<td>2.6 ± 0.3</td>
<td>3.1 ± 0.7</td>
<td>2.8 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>0.790</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes ($10^{9}$ L$^{-1}$)</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>0.057</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes ($10^{9}$ L$^{-1}$)</td>
<td>0.4 ± 0.04</td>
<td>0.4 ± 0.03</td>
<td>0.5 ± 0.02</td>
<td>0.5 ± 0.06</td>
<td>0.5 ± 0.04</td>
<td>0.5 ± 0.03</td>
<td>0.054</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neut:lymph</td>
<td>2.0 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>2.0 ± 0.7</td>
<td>1.8 ± 0.3</td>
<td>1.2 ± 0.1</td>
<td>0.192</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from normal training (N).
* Significantly different from recovery (R).
therefore elevated resting systemic pro-inflammatory cytokine concentrations would not be expected.

A tendency toward elevated resting plasma CK was observed after the first week of intensified training, and CK was significantly elevated at the end of the second intensified training week. The current study employed cycling exercise, which excludes eccentric muscle contractions; therefore, the rise in resting plasma CK we observed is unlikely to have been the result of muscle trauma. One possibility might be that the cumulative effect of repeated bouts of prolonged exercise may induce sufficient oxidative stress to impair the body’s antioxidant defense systems and perhaps induce membrane peroxidation resulting in the leakage of CK from the muscle into the circulation (26). Results of a previous study (25) do not support this notion; however, the study employed only three consecutive days of prolonged cycling exercise compared with 14 d in the present study. Although statistically significant, the rise in resting plasma CK was quantitatively small and the ability of this marker to discriminate between normal, intensified training and intensified training that results in overreaching is doubtful.

Mucosal IgA is an important factor in host defense and has been examined in relation to increased upper respiratory tract infection incidence and immune depression in endurance-trained athletes (11). To date, there is limited data on changes in mucosal IgA as a result of overreaching with only Mackinnon et al. (12) reporting 18–32% lower salivary IgA concentrations in athletes showing symptoms of overreaching compared with those who were well trained. We found lower IgA concentrations during ITP compared with N; however, this was not statistically significant.

The results of the present investigation do not provide clear evidence to either definitively confirm or refute the recently proposed cytokine hypothesis of overtraining. Although the underlying causative mechanisms of overreaching and overtraining still remain unclear, it does not appear that elevations in circulating cytokines are primarily responsible for the fatigue and decreased performance associated with overreaching. It was possible to induce a state of overreaching, evident by underperformance and changes in mood state, yet resting plasma cytokine concentrations remained unchanged. However, it cannot be stated that changes in cytokines will not occur in overtrained athletes. Although it is generally assumed that continued training while in a state of overreaching will lead to overtraining, it cannot be said that the symptoms and characteristics of both states are identical. If the athlete continues to train while overreached and does not incorporate adequate recovery between exercise sessions, this acute inflammatory response may develop into a chronic response, ultimately resulting in the activation of circulating monocytes. Pro-inflammatory cytokines released by these activated monocytes may result in systemic inflammation, perhaps accounting for some of the multitude of symptoms observed in overtrained athletes. Furthermore, it is possible that during running, where the stimulus for the initial microtrauma to the musculoskeletal system is greater, a local acute inflammatory response may occur. Finally, subjects in the present study were trained endurance athletes with a moderately high fitness level and it is not known if the results are applicable to athletes of differing fitness levels.

Taken together, the current information regarding the immune system and overreaching seems only to confirm the role of intensified training in immune depression. Many cell numbers do not appear to change during overreaching and those cells that do alter appear to simply reflect the nature of the training performed. Thus, immune parameters may change in response to intensified training independent of whether the training results in overreaching. Hence, the role of changes in the immune system in the etiology of overreaching is in doubt. This study supports the classification of overreaching based on changes in the Gln/Glu ratio. A lowering of the Gln/Glu ratio in conjunction with a decline in performance and altered mood state may be a useful tool for the diagnosis of overreaching.

REFERENCES