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ORIGINAL ARTICLE

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The haematological, biochemical and immunological profile of athletes suffering from the overtraining syndrome

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Abstract To help clarify the overtraining syndrome (OTS), a combination of parameters were measured in ten athletes who were suffering from OTS. Blood samples were obtained at rest and a range of haematological, biochemical and immunological tests were carried out on the samples. For each parameter, the mean value for the group was compared to an established normal range amongst age-matched controls. The subjects were also asked to complete a questionnaire to establish the severity of their condition. The data indicated that the debilitating fatigue experienced by the OTS sufferers was not related to any of the blood parameters traditionally associated with chronic exercise stress, since levels were normal in OTS. The only parameter measured which deviated significantly from the normal range for both the sedentary controls and the athletes was the plasma concentration of glutamine. Although the data in this study would suggest that plasma glutamine concentrations represented an objective, measurable difference between OTS subjects and normal controls, it remains to be shown that there is any correlation between glutamine concentrations and other clinical symptoms of OTS such as physical capability.

Key words Glutamine · Overtraining

Introduction

In recent years much research in the field of exercise physiology has been directed at unravelling the aetiology of the overtraining syndrome (OTS) which can afflict a wide range of athletes. The syndrome manifests itself as an inability to perform or train to an acceptable level, prolonged periods of fatigue, exacerbated by activity or exercise and has often been associated with recurrent infections (Budgett 1990). Research has often been directed towards responses of athletes to either repeated periods of intense exhausting exercise (Fry et al. 1992) or to a deliberate increase in the amount of training or its intensity (Lehmann et al. 1992). Other studies have investigated the overtrained athletes themselves (Barron et al. 1985; Morgan et al. 1987; O'Connor et al. 1989; Koutedakis et al. 1990; Parry-Billings et al. 1992). Close links have been drawn between OTS among athletes and the chronic fatigue syndrome (CFS) among the population as a whole (Fry et al. 1991a, b). Indeed, recent speculation has suggested that affected athletes may in fact be suffering from CFS (Parker and Brukner 1994). However, similarly to OTS, the diagnosis of CFS has remained primarily one of exclusion rather than inclusion (Holmes et al. 1988; Sharpe et al. 1991), and no single biological parameter has been found to be universally useful for diagnosis of either OTS or CFS.

There exists a plethora of data on restricted aspects of CFS and OTS which have concluded that the syndrome is associated with central fatigue (Kent-Braun et al. 1993; Gibson et al. 1993), abnormal muscle metabolism (Arnold et al. 1984; Wong et al. 1992), hypothalamic dysfunction (Barron et al. 1985), abnormalities of the peripheral part of the motor unit (Jamal and Hansen 1985) and a host of different immune dysfunctions including both depressed (Gupta and Vayuvegala 1991; Barker et al. 1994) and raised (Klimas et al. 1990; Ho-Yen et al. 1991) natural killer cell counts, elevated B-cell counts (Klimas et al. 1990), immune activation (Klimas et al. 1990; Landay et al. 1991) and excessive cytokine production (Cheney et al. 1989; Lloyd et al. 1989). Researchers have also linked OTS with low haemoglobin (Hb) concentrations, haematocrit (HCT) and red blood cell counts (RBC) (Rushall 1980; Ryan

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et al. 1983), hormonal changes; decreased catecholamine excretion (Lehmann et al. 1992) and particularly increased concentrations of plasma cortisol (Adlercreutz et al. 1986), increased plasma creatine phosphokinase activity (Ryan et al. 1983), altered plasma amino acid concentrations (Parry-Billings et al. 1992) and psychological disturbances (Morgan et al. 1987; O'Connor et al. 1989). Therefore, a multidisciplinary approach is needed to help clarify OTS and in this study we utilised a combination of physiological, biochemical, immunological, haematological and psychological parameters to try and characterise the nature of OTS in a group of affected subjects.

Methods

Subjects

Ten subjects diagnosed as suffering from OTS gave informed consent following clearance to conduct the study from the Committee of Human Rights of the University of Western Australia. The athletes (four men and six women) were from a wide range of sporting backgrounds (Table 1).

Diagnostic criteria

All the subjects met the CFS case study definition criteria (Holmes et al. 1988) at the time of inclusion into the study. The subjects reported debilitating fatigue, lack of energy and extreme tiredness as universal symptoms. All stated that in their sport they were performing below an acceptable level, and were having difficulty maintaining their training programme. Only three subjects were still exercising at the time of their inclusion into the study, albeit at a severely reduced level. In all cases the symptoms were not relieved by a period of either rest or bed-rest depending on the severity of the condition. All the subjects had previously been undertaking an exercise training programme and competing in their respective sport in either amateur or professional competition, some up to international level. They reported the onset of their condition to have occurred over a period ranging from 2 days to 6 weeks. In some cases, OTS occurred following a period of increased volume or intensity of training, but this was not universal. The most commonly reported nonfatigue symptoms included muscle pain, sleep disturbances, depression, irritability, lapses in memory or concentration, lack of co-ordination, and an increased susceptibility to infections, particularly colds and stomach complaints.

Blood sampling

Prior to sampling, each subject was required to rest quietly in a supine position for 10 min. An 18-ml blood sample was then collected from an antecubital vein, using a winged cannula attached to a vacutainer (Becton Dickinson, Rutherford, N.J., USA) bleeding system. A 7-ml sample was collected into a clotting tube to obtain serum, 4 ml into ethylenediaminetetra-acetic acid (EDTA) and 7 ml into lithium heparin. Samples were immediately chilled on ice. Serum was obtained by allowing the blood to stand on ice for 1 h and then centrifuging at 1000*g* for 15 min. Serum was stored at $-20°$ C prior to assaying and all assays were completed within 20 days of sample collection. The EDTA-treated samples were used to determine complete haematology and five cell leucocyte differential counts using a Coulter STKS (Coulter Electronics, Hialeah, Fla., USA) analyser which incorporated aperture impedence with volume, conductivity and light scatter measurements for the determination of cell characteristics, and spectroscopy for the determination of Hb concentration. This analysis was conducted at the Department of Clinical Haematology, Sir Charles Gairdner Hospital (SCGH), Perth, Western Australia. A portion of the EDTA-treated blood was used for lymphocyte subpopulation analysis using flow cytometry [fluorescently activated cell scanner (FACScan), Becton Dickinson, Sunnyville, Calif., USA]. Serum and plasma samples were used for determination of a range of biochemical parameters. Commercially available kits were used for the determination of uric acid (Kinetic ALDH, Boehringer Mannheim, Germany) and creatine phosphokinase (CBR CK-NAC, Boehringer Mannheim, Germany) using a Beckman Synchron CX5 (Becton Instruments Inc., Gladesville, Calif., USA) automatic analyser. Commercially available radio-immunoassay kits were used for the determination of cortisol (Amersham, Buckinghamshire, England), and ferritin (Bioclone, Marrickville, Australia). Automated analysis was used for the determination of urea, creatinine, albumin, calcium and magnesium. The instrument used was a Technicon SMAC II (Technicon Instruments Corporation, Tarrytown, N.Y., USA) and the methods were those recommended in the SMAC II procedures manual. This analysis was conducted at the Department of Clinical Biochemistry, SCGH. A 2-ml sample of lithium heparin treated plasma was extracted with ice-cold 15% perchloric acid ($HClO_4$), left on ice for 30 min, and centrifuged (1000*g*) for 10 min. The supernatant was neutralised with 5M-KOH (plus 7.7-mM triethanolamine), and stored at $-20°$ C prior to assaying for glutamine, which was carried out within 10 days of sample collection.

Determination of glutamine

An *Escherichia coli* (YMC11, Coli Genetic Stock Center, Department of Biology, Yale University, New Haven, CT, USA) which has been shown to be totally dependent on glutamine for replication (Mayers et al. 1975) was used in a unique bio-assay system previously described (Keast et al. 1995). Briefly, a standard volume of *E. Coli*, grown overnight in brain and heart infusion broth, was washed in phosphate buffered saline (PBS) and transferred into fresh assay medium consisting of Vogel and Bonner salts solution supplemented with 100 $\mu g \cdot ml^{-1}$ of thiamine, 10 $\mu g \cdot ml^{-1}$ of tryptophan and 10 $\mu g \cdot ml^{-1}$ glucose with a range of concentrations of glutamine to provide a standard graph of growth versus glutamine concentration. Test samples were added to the assay medium in place of glutamine and the cultures were incubated at 37° C for 18 h. The micro-organisms were then centrifuged, resuspended in 3 ml of PBS and the growth of the *E. Coli* measured as optical density changes at 600 nm using a DMS 70 UV-visible spectrophotometer (Varian Techtron, Mulgrave, Vic., Australia). Growth of the micro-organism was directly proportional to the amount of glutamine available. All assays were carried out in duplicate and the glutamine present in test samples, which always included a 1 $mmol \cdot l^{-1}$ standard plasma sample, was determined from a standard graph prepared with each run.

Determination of lymphocyte subsets

A 3-ml sample of blood collected in an EDTA tube was diluted with 6-ml PBS and centrifuged (300*g*) over ficoll-paque (Pharmacia Fine Chemicals, Uppsala, Sweden). The mononuclear cells were then removed and washed twice in medium consisting of Roswell Park Memorial Institute (medium) (RPMI-1640; Grand Island Company, N.Y., USA) supplemented with 10% foetal calf serum (FCS; Gibco Laboratories, Penrose, Auckland, New Zealand) and 100-$\mu g \cdot ml^{-1}$ gentamicin (Delta West, Bentley, Western Australia). The cells were

then resuspended at a concentration of 2×10^6 cells·ml^{-1} in RPMI:10% FCS. A 50-µl sample of the cell suspension was then placed in 12 × 75 mm tubes (Becton Dickinson). To each tube, 5 µl of the required conjugated monoclonal antibody (kindly provided by Dr. J. Wotherspoon, Becton Dickinson, Sydney, Australia) was added. Each antibody was either conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE). Following incubation in the dark, on ice, for 30 min, 2-ml PBS was added and the suspension was centrifuged for 5 min at 300*g*. The cells were subsequently resuspended in 300-µl PBS and surface marker analysis was conducted on a FACScan flow cytometer (Becton Dickinson). The same forward and side scatter parameters were used for each trial as established for human peripheral leucocytes. Standard gating procedures were used to select mainly lymphocytes and minor contributions of monocytes, and to differentiate between labelled and unlabelled cells. The natural fluorescence of unstained preparations and its contribution to the fluorescence channels was determined using an unstained proportion of the cell suspension. Data were then analysed using Lysis software. Single marker studies were completed for T-cells ($CD3^+$), B-cells ($CD19^+$), natural killer cells ($CD56^+$) and activated lymphocytes ($CD25^+$ and HLA-DR^+). Dual marker studies were completed for T-helper/inducer cells ($CD4^+$) labelled with FITC and cytotoxic/suppressor cells ($CD8^+$) labelled with PE. Absolute numbers of cells expressing each marker were determined by multiplying the percentage of the cell of interest by the absolute number of lymphocytes derived from the Coulter counter data.

Normal values

A normal range of values for an age-matched population was obtained for each parameter from the Department of Clinical Haematology, SCGH (haematology), the Department of Clinical Biochemistry, SCGH (biochemistry) and the published literature (Hannet et al. 1992; lymphocyte subsets). The ranges quoted represented the 5th and 95th percentiles of the normal distribution for the control group. Hence, individual values outside of this range were significantly different from the control mean ($P < 0.05$). The quoted lymphocyte subset normals were recalculated from published data for the 25th and 75th percentiles of a representative sample of 101 subjects (Hannet et al. 1992). A normal range for plasma glutamine concentration was established for the unique glutamine bio-assay by obtaining blood samples from 30 age-matched subjects (14 women and 16 men) within the Department of Microbiology, SCGH. These data were analysed separately for the male and female groups to establish whether a sex difference existed.

Statistical analysis

For each parameter the null hypothesis was tested that there was no significant difference between the established normal mean and the mean of the values obtained from the group of subjects, tested by Student's *t*-test analysis. For each subject's individual values the null hypothesis was tested that the value fell within 5th and 95th percentiles of the normal distribution of control values.

Results

Haematology

There were no significant differences between the normal values for an age-matched subject group and the mean values for the ten subjects in this study, for any of the haematological parameters measured (Table 1). It was, however, noticeable that Hb concentrations, RBC and HCT were higher for the four men compared to the six women [15.7 (SD 0.3) vs 13.4 (SD 0.3), 5.13 (SD 0.04) vs 4.53 (SD 0.13), 0.46 (SD 0.01) vs 0.39 (SD 0.01)], but when averaged for the men and women separately, these values still fell within the established normal ranges for men and women, respectively. The mean total white blood cell count, the component five cell differential counts and platelets counts did not differ from the normal range (Table 1). In addition to the mean values for the group of ten subjects, each individual's values for all parameters fell within the established normal ranges as well, with the exception of high monocyte and eosinophil counts for subjects 8 and 9, respectively, which were outside of the 99th percentile ($P < 0.01$) of the normal distribution for control values.

Biochemistry

All mean and individual values for plasma biochemical parameters were within the established normal ranges for both men and women respectively, except for plasma glutamine concentrations (Table 2). The mean plasma glutamine concentration for the group of ten subjects in the study was 704 (SD 236) µmol·l^{-1}. This compared to a mean value of 1000 (SD 92) µmol·l^{-1} for the group of age-matched controls. Using Student's *t*-test analysis, the study group was significantly lower ($P < 0.01$) than this normal range. When the plasma glutamine concentrations among the group of normals were averaged separately for men and women (Table 3), a significantly lower ($P < 0.05$) mean value was obtained for the women than for the men [956 (SD 93) vs 1030 (SD 77)]. When compared to this sex-specific control group, seven of the ten individual values for the OTS subjects were found to be significantly lower than normal ($P < 0.05$; Table 4). Resting plasma glutamine concentrations for groups of both male and female elite triathletes and elite swimmers, described fully elsewhere (Rowbottom et al. to be published/in preparation), were also included for reference (Table 3). When a comparison was carried out between individual values for the ten overtrained athletes and the normal distributions of the sex-specific athlete data, the differences were found to reach greater significance than with the sedentary group, with only one athlete (3) remaining within the 5th and 95th percentile range for the athlete group (Table 4).

Immunology

There were no significant differences between the normal value for an age-matched subject group and the mean values for the ten subjects in this study, for any of

Table 1 Resting haematological data for the ten athletes suffering from the overtraining syndrome. *WBC* total leucocyte number, *NE* neutrophil number, *EO* eosinophil number, *BA* basophil number, *LY* lymphocyte number, *MO* monocyte number, *Hb* haemoglobin concentration, *RBC* erythrocyte number, *HCT* haematocrit, *MCV* mean erythrocyte volume, *MCH* mean erythrocyte haemoglobin concentration, *PLT* platelet number, *MPV* mean platelet volume

Subject Sex Sport	1 Male Triathlon	2 Male Running	3 Male Football	4 Male Surfing	5 Female Ballet	6 Female Swimming	7 Female Swimming	8 Female Swimming	9 Female Tennis	10 Female Rower	Mean	Standard deviation	Normal range
WBC ($\times 10^9 \cdot l^{-1}$)	4.7	6.6	5.2	5.0	8.2	6.0	5.7	6.5	8.0	8.1	6.4	1.3	4.0–11.0
NE ($\times 10^9 \cdot l^{-1}$)	2.6	4.6	2.5	2.2	4.7	3.0	3.0	2.3	4.2	5.5	3.5	1.2	2.0–7.5
EO ($\times 10^9 \cdot l^{-1}$)	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.1	0.7**	0.2	0.2	0.2	0.1–0.5
BA ($\times 10^9 \cdot l^{-1}$)	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.0–0.2
LY ($\times 10^9 \cdot l^{-1}$)	1.6	1.4	2.0	2.2	2.2	2.4	1.7	2.7	2.6	1.6	2.0	0.5	1.2–4.0
MO ($\times 10^9 \cdot l^{-1}$)	0.4	0.6	0.5	0.3	1.0	0.5	0.7	1.4**	0.3	0.7	0.6	0.3	0.2–1.0
Hb ($g \cdot dl^{-1}$)	15.7	15.7	15.3	15.9	13.8	13.2	13.3	13.0	13.6	13.6	14.3	1.2	11.5–16.0
RBC ($\times 10^{12} \cdot l^{-1}$)	5.18	5.08	5.12	5.15	4.65	4.72	4.51	4.39	4.41	4.50	4.77	0.33	3.8–5.8
HCT	0.45	0.46	0.44	0.47	0.39	0.38	0.38	0.38	0.41	0.40	0.42	0.04	0.37–0.47
MCV (fl)	86.0	90.0	86.0	90.0	87.0	81.0	85.0	86.3	91.8	88.0	87.1	3.1	80–100
MCH (pg)	30.3	30.9	29.9	31.0	29.7	28.0	29.5	29.6	30.8	30.1	30.0	0.9	27–32
PLT ($\times 10^9 \cdot l^{-1}$)	165	182	211	311	319	252	250	313	366	236	261	65	150–450
MPV (fl)	7.8	8.9	7.8	7.9	7.9	8.9	8.1	7.7	7.6	9.1	8.2	0.6	6.0–10.0

** $P < 0.01$

Table 2 Resting biochemical data for the ten athletes suffering from the overtraining syndrome. *Creat* creatinine, *CPK* creatine phosphokinase, *UA* uric acid, *Cort* cortisol, *Gln* glutamine

Subject Sex Sport	1 Male Triathlon	2 Male Running	3 Male Football	4 Male Surfing	5 Female Ballet	6 Female Swimming	7 Female Swimming	8 Female Swimming	9 Female Tennis	10 Female Rower	Mean	Standard deviation	Normal range
Urea ($mmol \cdot l^{-1}$)	4.3	4.1	5.5	4.9	4.2	4.9	4.0	5.4	4.3	3.2	4.5	0.7	3.0–8.0
Creat ($\mu mol \cdot l^{-1}$)	93	106	110	91	90	71	67	59	68	68	82	18	0–120
Albumin ($g \cdot l^{-1}$)	45	49	46	46	49	49	47	44	42	44	46	2	35–50
Ca^{2+} ($mmol \cdot l^{-1}$)	2.30	2.33	2.34	2.40	2.42	2.32	2.24	2.29	2.33	2.28	2.33	0.05	2.25–2.60
Mg^{2+} ($mmol \cdot l^{-1}$)	0.72	0.82	0.76	0.78	0.75	0.80	0.88	0.90	0.70	0.80	0.79	0.06	0.7–1.1
CPK ($U \cdot l^{-1}$)	123	126	84	94	116	101	153	34	68	85	98	34	30–180
UA ($mmol \cdot l^{-1}$)	0.21	0.34	0.28	0.31	0.27	0.23	0.21	0.26	0.28	0.19	0.26	0.05	0.14–0.34
Ferritin ($\mu g \cdot l^{-1}$)	125	33	60	93	58	31	32	142	67	58	70	39	20–200
Cort ($nmol \cdot l^{-1}$)	410	270	420	380	490	520	370	420	340	340	396	74	220–750
Gln ($\mu mol \cdot l^{-1}$)	870	870	1068	255	788	649	870	506	536	632	704**	236	850–1150

** $P < 0.01$ by Student's *t*-test analysis

the lymphocyte subset parameters measured (Table 5). All the means for the subject group were very similar to the means for the control group, with the exception of the B-cell ($CD19^+$) count, which was low but not significantly different to the control mean. Individual values were occasionally elevated or depressed: one subject (2)

Table 3 A comparison of the mean plasma glutamine concentrations betweeen sample groups of male and female athletes (data from Rowbottom et al. in preparation), sedentary controls, and athletes suffering from the overtraining syndrome

	$[Glutamine]_{plasma}$	
	$\mu mol \cdot l^{-1}$	
	Mean	SD
Sedentary males ($n = 16$)	1030	77
Male athletes ($n = 17$)	1179	106
Sedentary females ($n = 14$)	956	93
Female athletes ($n = 12$)	1106	103
Overtrained athletes ($n = 10$)	704	236

Table 4 A comparison between the individual plasma glutamine concentrations for the ten overtrained athletes, and normal distributions of sex-specific sedentary and athlete (data from Rowbottom et al. in preparation) groups. Values represent the P value for that data point on the respective normal distribution curve. ns, not significant

Subject	Sedentary	Athletes
Males		
1	$P < 0.05$	$P < 0.01$
2	$P < 0.05$	$P < 0.01$
3	ns	ns
4	$P < 0.001$	$P < 0.001$
Females		
5	ns	$P < 0.01$
6	$P < 0.01$	$P < 0.001$
7	ns	$P < 0.05$
8	$P < 0.001$	$P < 0.001$
9	$P < 0.001$	$P < 0.001$
10	$P < 0.01$	$P < 0.001$

had a non-significantly low $CD4^+$:$CD8^+$ ratio (0.92) corresponding to a relatively low $CD4^+$ cell count ($0.65 \times 10^9/l$), four subjects (2, 6, 7 and 10) had significantly low B-cell counts, and only one subject showed a significant increased expression of immune activation markers (subject 9, 35.5% $HLA\text{-}DR^+$ expression). Despite these occasional individual abnormalities, differences were by no means universal among all subjects.

Discussion

Apart from the decrement in physical performance, common to all athletes suffering from OTS, many other parameters have been previously considered as possible indicators of an overtrained state among athletes. Whilst many acute changes have been observed following a period of over-reaching (Lehmann et al. 1992; Fry et al. 1992; Keast et al. 1995), the data presented demonstrate that those athletes who were suffering from the prolonged effects of OTS had a normal profile for the majority of parameters considered. This indicated that the debilitating fatigue experienced by the OTS sufferers was not related to many of the factors traditionally associated with over-exercise such as low Hb and ferritin concentrations, and elevated uric acid, creatine phosphokinase and cortisol concentrations, since levels were normal in OTS. Even the level of immune activation (%CD25 and %HLA-DR), which has previously been associated with CFS (Klimas et al. 1990; Landay et al. 1991), was not elevated in the group as a whole. Only one subject showed a significant amount of activation, and as such this cannot be regarded as a universal symptom of OTS. Furthermore, interleukin-1 and interleukin-2 were not detectable in plasma samples from any of the subjects (data not shown).

Table 5 Resting lymphocyte subset data for the ten athletes suffering from the overtraining syndrome. $CD3^+$ T-cells, $CD4^+$ T-helper/inducer cells, $CD8^+$ cytotoxic/suppressor cells, $CD19^+$ B-cells, $CD56^+$ natural killer cells, $\%CD25^+$ percentage of T-cells expressing *CD25* activation marker, $\%HLA\text{-}DR^+$ percentage of T-cells expressing *HLA-DR* activation marker

Subject	1	2	3	4	5	6	7	8	9	10			
Sex	Male	Male	Male	Male	Female	Female	Female	Female	Female	Female			
Sport	Triathlon	Running	Football	Surfer	Ballet	Swimming	Swimming	Swimming	Tennis	Rower	Mean	Standard deviation	Normal range
$CD3^+$ ($\times 10^9 \cdot l^{-1}$)	data	1.19	1.25	1.54	1.54	1.94	1.50	2.10	2.15	1.12	1.59	0.39	0.6–2.3
$CD4^+$ ($\times 10^9 \cdot l^{-1}$)	not	0.65	0.84	0.95	1.29	1.37	1.17	1.20	1.37	0.68	1.06	0.28	0.4–1.4
$CD8^+$ ($\times 10^9 \cdot l^{-1}$)	available	0.71	0.60	0.64	0.76	0.86	0.75	1.13	1.07	0.62	0.79	0.19	0.2–1.2
$CD19^+$ ($\times 10^9 \cdot l^{-1}$)		0.07*	0.11	0.21	0.22	0.08*	0.03*	0.24	0.16	0.08*	0.13	0.07	0.1–0.5
$CD56^+$ ($\times 10^9 \cdot l^{-1}$)		0.16	0.36	0.50	0.28	0.41	0.25	0.20	0.31	0.35	0.31	0.10	0.1–0.5
$\%CD25^+$		25.7	18.4	9.7	22.1	18.6	18.7	8.1	13.0	22.3	17.4	5.6	5.0–32.0
$\%HLA\text{-}DR^+$		13.4	13.6	35.5**	13.6	6.7	14.0	23.3	14.9	16.1	16.8	7.7	1.5–23.5
$CD4^+$:$CD8^+$ (ratio)		0.92	1.40	1.48	1.70	1.59	1.56	1.06	1.28	1.10	1.34	0.27	0.6–1.9

* $P < 0.05$; ** $P < 0.01$

The only parameter measured which deviated significantly from the established normal range was the plasma concentration of glutamine. These results are in agreement with a previous study which reported lower plasma glutamine concentrations in athletes who were suffering from OTS than age-matched controls (Parry-Billings et al. 1992). The fact that our results also reported the normality of a variety of other factors in OTS subjects directs the attention of future OTS research towards the significance of the low level of plasma glutamine. Furthermore, there has also been a recent report of similarly low plasma glutamine concentrations in CFS patients (Pervan et al. in preparation). Particularly pertinent would be to establish a mechanism of dysfunction which would relate abnormally low concentrations of plasma glutamine to the other consistent physiological symptoms observed among patients with these conditions. Most notable of these symptoms was the debilitating fatigue which was present at rest and worsened by exercise. Periods of activity invariably resulted in a subsequent period of prolonged exhaustion which lasted days or even weeks.

Previous studies have reported observations of skeletal muscle abnormalities in CFS patients, particularly noting abnormal mitochondria (Behan et al. 1991) and an early acidosis during aerobic exercise in experiments conducted using phosphate nuclear magnetic resonance (PNMR) techniques (Arnold et al. 1984). Such abnormalities could indicate a disturbance of the mitochondrial based oxidative metabolism. Such a dysfunction may be expected to manifest itself in an early onset of fatigue during prolonged aerobic exercise associated with excessive lactic acid accumulation and a rapid muscle glycogen depletion. However, recent studies using PNMR techniques have refuted the claim that there was any dysfunction of mitochondrial metabolism by comparing results from CFS patients with sedentary normals and patients who suffered from mitochondrial myopathies (Barnes et al. 1993). Their data indicated that only 12 of 46 patients studied had an abnormal post-exercise adenosine diphosphate concentration or a slower than normal rate of recovery of phosphocreatine. They did, however, conclude that there was a possibility that CFS "represents the clinical manifestation of a number of as yet unidentified pathologies" and that there may be some pertinence in further considering the metabolic responses of the small sub-group who fell outside the normal range (Barnes et al. 1993).

Based on the possibility of a mitochondrial lesion within the skeletal muscle in OTS, a cause of the observation of a decreased plasma glutamine concentration can be speculated upon. Glutamine synthetase (GS) is the major enzyme of glutamine biosynthesis and has been found predominantly in skeletal muscle tissue, but also in the lungs, the brain and in smaller quantities in other tissues (Souba 1992). Although glutamine synthesis occurs in the cytoplasm, and not in the mitochondria, GS requires glutamate as a substrate. Glutamate production is undoubtedly via transanimation of other, primarily branched-chain, amino acids. It has been found that this step requires 2-oxoglutarate, a tricarboxylic acid (TCA) cycle intermediate produced from acetyl co-enzme A, as a co-substrate (Newsholme and Leech 1983). It can be speculated that a mitochondrial lesion could limit the availability of 2-oxoglutarate for glutamate, and hence glutamine, production in the muscle. Since skeletal muscle has been shown to be a major site of glutamine production in the body (Newsholme and Parry-Billings 1990) it is proposed that decreased muscle production could lead to a decreased circulating concentration of glutamine. However, the rate of glutamine utilisation has been shown to be very high in a number of different tissues, particularly the gastro-intestinal tract and cells of the immune system (Souba 1992). The possibility exists that the observation of a decreased plasma glutamine concentration could be due to an increased rate of glutamine utilisation among other tissues, rather than a decreased production in the skeletal muscle.

A similar question of decreased production or increased utilisation exists concerning the pattern of plasma glutamine changes during and following exercise. Blood samples taken during the postexercise period after a high intensity interval training session have shown that exercise at intensities of 90% and 120% maximal oxygen uptake was sufficient to bring about a transient, but significant, decrease in plasma glutamine concentration (Keast et al. 1995). The same study has also reported that 10-day period of overload training resulted in a significant reduction in resting plasma glutamine concentrations after only 5 days of training. These data may provide a link between the chronic exercise stress which athletes place themselves under during prolonged periods of training, and our observation of permanently low plasma glutamine concentrations in overtrained athletes. Studies reporting depressed glutamine concentrations after exercise, particularly of a high intensity (Keast et al. 1995), take on greater significance for athletes suffering from OTS. Similar postexercise losses of glutamine from circulation for these athletes, starting from a low resting level, could have a significant role to play, particularly regarding the possibility of immunosuppression after exercise.

Glutamine has been shown to be a key substrate for cells of the immune system, and considered to be particularly essential for the immune response (Newsholme and Parry-Billings 1990), both as a precursor for nucleotide biosynthesis and as a major energy source via partial oxidation of glutamine. Inadequate amounts of circulating glutamine may lead to an impaired immune function, and an increased susceptibility to infection among athletes suffering from OTS (Keast and Morton 1995). In addition, glutamine consumption by the small intestine has also been found to occur at a very high

rate (Souba 1992) and would appear to be essential for the maintenance of gut mucosal integrity. Observations of gastro-intestinal disorders, particularly diarrhoea and food allergies, may be due, at least in part, to low concentrations of circulating glutamine.

Elite athletes have been reported to have higher glutamine concentrations than sedentary controls (Rowbottom et al. in preparation). Since the ten subjects in this study were athletes previously undertaking intensive training programmes, this athletic background must be taken into consideration. Therefore, it was of greater interest to compare their glutamine concentrations to those of well-trained athletes, instead of a sedentary group (Table 4). We suggest that the elevated plasma glutamine concentrations amongst athletes represent a positive adaptation to a well-balanced training programme, while the lower concentrations observed in OTS athletes could be a negative effect of excessive exercise or overtraining. Considering the essential role of glutamine in immune function, previous reports of a "J-shaped" model of the relationship between the amount of exercise and the risk of upper respiratory tract infection (Nieman 1994) would fit with this glutamine adaptation to exercise stress (Fig. 1).

Although the data in this study would suggest that plasma glutamine concentrations represented an objective, measurable difference between the OTS subjects and the normal controls, it remains to be shown that there is any correlation between glutamine concentrations and other clinical symptoms such as physical capability. However, subject 3 was the most physically active of the subjects who presented with OTS, and his plasma glutamine concentration (1065 $\mu mol \cdot l^{-1}$) was the only one not significantly different to either the sedentary or the athlete group. Subsequent to his inclusion in the study, he recovered quickly to the stage of trying to resume moderate training. Conversely, those subjects with the lowest glutamine concentrations, particularly subjects 4 (255 $\mu mol \cdot l^{-1}$), 6 (649 $\mu mol \cdot l^{-1}$) and 8 (505 $\mu mol \cdot l^{-1}$) were those with extremely low physical capability, being unable to walk for more than a few paces before feeling fatigued to the extent of returning to bed for a number of days. The data suggested that such a link may exist, and warrants further investigation. We concluded that the debilitating fatigue experienced by the OTS sufferers was not related to any of the blood parameters traditionally associated with overexercise, with the exception of plasma glutamine concentration, and as such glutamine concentration may represent an effective diagnostic criterion for OTS in the future.

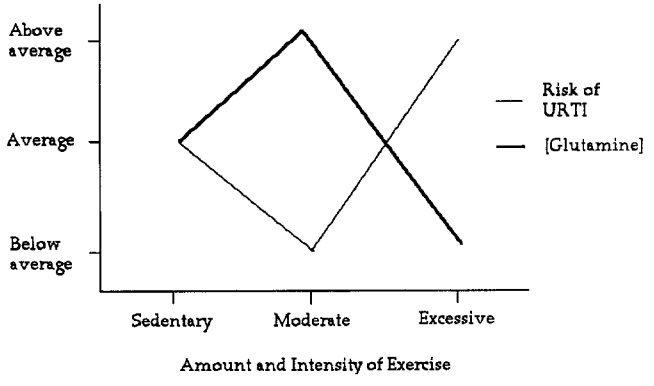

Fig. 1 Modified "J-shaped" model of the relationship between varying amounts of exercise and both the risk of upper respiratory tract infection (*URTI*) and the plasma glutamine concentration. [Risk of URTI modified from Nieman (1994) plus our current data]

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