Effects of acute exhaustive exercise and chronic exercise training on type 1 and type 2 T lymphocytes

Running Head: Exercise and T lymphocytes

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Abstract

The present study examined the effects of acute exhaustive exercise and chronic exercise training on type 1 and type 2 T lymphocyte distribution and intracellular cytokine production. Seven endurance-trained male cyclists completed exercise trials to exhaustion before, immediately after, and following 2 weeks of resting recovery from a 6-day intensified training period (ITP). During each trial, resting and post-exercise blood samples were incubated with phorbol 12-myristate 13-acetate (PMA) and ionomycin and stained for T lymphocyte surface antigens (CD3). Cells were then permeabilised, stained for intracellular cytokines and analysed using flow cytometry. Acute exhaustive exercise before and following 2 weeks of recovery from the ITP, but not immediately after the ITP, significantly reduced the circulating percentage and number of IFN-γ^+ (type 1) T cells ($P<0.05$). In addition, the amount of IFN-γ produced by stimulated T lymphocytes was decreased ($P<0.05$) post-exercise during each trial. The percentage and number of interferon (IFN)-γ^+ T lymphocytes was decreased ($P<0.05$) at rest immediately after the ITP compared with before and following 2 weeks of resting recovery from the ITP. However, the amount of IFN-γ produced by stimulated T lymphocytes at rest was unaltered following the ITP. Neither acute exercise nor chronic exercise training caused an alteration in the circulating percentage or number of interleukin (IL)-4^+ (type 2) T lymphocytes. These results suggest a possible mechanism for the increased incidence of infection reported during chronic exercise training via modulation of type 1/type 2 T lymphocyte distribution.

Keywords: Exercise, T lymphocytes, cytokines, training, interferon-γ

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Introduction

Prolonged strenuous exercise is followed by transient alterations in several indices of immune function (30), and these changes have been associated with an increased incidence of respiratory tract infections (29). T cell cytokine production plays a critical role in the development of immune responses against invading pathogens. Different types of pathogens induce characteristic patterns of cytokine production that are optimally protective against specific pathogens. The cellular basis for this heterogeneity in cytokine production is due to the presence of two distinct cytokine-producing $CD4^+$ T helper and $CD8^+$ T cytotoxic cell phenotypes that have been designated type 1 or type 2 T lymphocytes based on their distinct profile of cytokine production (26). Type 1 T lymphocytes secrete interferon (IFN)-γ, interleukin (IL)-2 and tumour necrosis factor-β and are responsible for defence against intracellular pathogens (32), whereas type 2 T lymphocytes predominantly secrete IL-4, IL-5 and IL-13 and are responsible for defence against extracellular pathogens (32).

It has been demonstrated that following both prolonged strenuous exercise (19, 37) and major surgery (6) there is decrease in the percentage of circulating type 1 T lymphocytes, but not type 2 T lymphocytes. Furthermore, the shift in type 1 T lymphocytes appears to be related to a specific decline in memory T lymphocytes (19). Indeed, it has been suggested that alterations in the type 1/ type 2 T lymphocyte balance in favour of a type 2 dominance may provide an explanation for the increased susceptibility to infection following prolonged exercise (37) and surgery (5). Although the functional status (i.e. the amount of cytokine produced following stimulation) of the type 1 and type 2 T lymphocytes was not determined in these studies (5, 19, 37), a transient exercise-induced suppression in the amount of IFN-γ produced by stimulated T lymphocytes has been observed at both the single cell level (35) and in the supernatant of *in vitro* mitogen-stimulated blood (4, 34, 41). However, the IL-4 concentration in the supernatant of *in vitro* mitogen-stimulated blood is unaltered following exercise (27).

Glucocorticoids (GC) play an essential physiological role in the regulation of immune responses in health and disease (40). However, over-stimulation of the HPA axis results in chronically elevated systemic glucocorticoid concentrations. This occurs in Cushing's syndrome, chronic psychological stress, the Fischer-rat model, the third trimester of pregnancy and in long-term excessive exercise and results in suppression of immune responses and enhanced susceptibility to infection (9, 10, 21, 40). Furthermore, GCs induce a shift from a type 1 to a type 2 pattern of immunity (40) via downregulation of monocyte/macrophage and professional antigen presenting cell production of IL-12, a potent promoter of type 1 immunity (14), but also directly via their suppressive and stimulatory actions on type 1 and type 2 T lymphocytes, respectively (2).

Intense periods of exercise training are often associated with depressed immunity (15, 24, 25), and an increased incidence of infection (17). Indeed, recurrent infections of the respiratory tract are a common symptom of overtrained athletes (8). While it can be argued that humoral immunity (e.g. levels of secretory immunoglobulin A) is more important in preventing upper respiratory tract infection (17), cell mediated immunity is recognised as being more important in clearing infections (26). A depression of cell mediated immunity may lead to a failure to completely clear viral infections of the respiratory tract causing recurrent infections to occur every few weeks or months. Given that type 1 and type 2 T lymphocytes are critical to the generation of host immunity, the purpose of the present study was to examine the regulation of type 1 and type 2 T lymphocyte distribution and function at rest, and in response to an acute bout of exhaustive exercise performed before, immediately after and following 2 weeks of resting recovery from an intensified training period. Specifically, given the critical role played by the type 1 T cells in antiviral defence and clearance of infections, and since respiratory infections are most commonly caused by viruses, we hypothesised that following the intensified training period there would be a decrease in the resting number of circulating type 1 T lymphocytes compared with before the intensified training period and following 2 weeks of resting recovery. In addition, we hypothesised that following the intensified training period there would be a decrease in the amount of IFN-γ produced by stimulated T lymphocytes at rest compared with before the intensified training period and following 2 weeks of resting recovery. Finally, we hypothesised that following the intensified training period the effects of acute exercise on type 1 and type 2 T lymphocyte trafficking would be attenuated compared with acute exercise both before, and following 2 weeks of resting recovery from the intensified training period.

Materials and Methods

Experimental Subjects

Seven healthy, moderately to well endurance-trained male cyclists [age 30 ± 2 years, body mass 75 ± 3 kg, maximal oxygen uptake ($Vo_{2\ max}$) 4.55 ± 0.11 $l.min^{-1}$; means ± SEM] volunteered to participate in the study. Subjects were non-smokers, not taking any medication and had remained free of respiratory infection for 4 weeks prior to participation in the study. Subjects were fully informed as to the purposes and risks of the experiment before voluntarily giving their written informed consent. The study was approved by the South Birmingham Local Research Ethics Committee of The Queen Elizabeth Hospital (Birmingham, West Midlands, UK).

Preliminary Procedures

When subjects were initially recruited to the study they continued their 'normal' training regimen. During this period subjects were provided with a heart rate monitor (Polar Vantage NV, Polar Electro, Kempele, Finland) and were asked to record all training undertaken over a continuous 7-day 'normal' training period. At least 2 weeks before the first experiment trial $Vo_{2\ max}$, maximal work rate (W_{max}), maximal heart rate (HR_{max}) and plasma lactate curves were determined for each subject by means of an incremental cycling exercise test to volitional exhaustion as described previously (20). W_{max} values were used to determine the workload employed during the experimental trials. At least 1 week prior to the first experimental trial subjects performed a familiarisation trial at 65% W_{max} to exhaustion. To ensure a relatively prolonged duration of the experimental trials it was necessary to reduce the workload to ~61% W_{max} for four of the subjects.

To determine the intensities at which subjects exercised during the intensified training period (ITP), training 'zones' were calculated based on individual lactate and heart rate responses obtained during the incremental exercise test. Five train-

ing zones were then constructed and expressed as percentages of each subject's individual HR_{max}. The volume of training prescribed during the ITP was based on the duration of training undertaken during the 7-day 'normal' training period with the aim to approximately double the 'normal' training volume.

In the week preceding the first experimental trial subjects were instructed to avoid any strenuous exercise and they performed a $Vo_{2\ max}$ test on the day immediately preceding the first experimental trial.

Experimental Procedures

On day 1, subjects reported to the Human Performance Laboratory at the University of Birmingham after an overnight fast (~10 h) and an indwelling 21G Teflon catheter (Baxter, Norfolk, UK) was inserted into an antecubital vein of one arm, connected to a 3-way stopcock (Sims Portex, Kent, UK) and a resting blood sample was drawn (Pre-exercise). Subjects then commenced cycling exercise to exhaustion (Trial 1) at a pre-determined workload of ~63% W_{max} (~74% $Vo_{2\ max}$). Further blood samples were obtained after 60 min of exercise (60-min), at exhaustion (Post-exercise) and following 1-h of resting recovery (1-h post-exercise). The catheter was kept patent by flushing with ~1.5 ml of sterile saline solution (0.9 % NaCl) after sampling.

On day 2 subjects began the 6-day ITP. During the ITP subjects were instructed to train every day and on day 7 subjects reported to the laboratory for a second $Vo_{2\ max}$ test. On day 8, subjects reported to the laboratory once more and performed the second experimental trial (Trial 2), the procedures for which were identical to the first. On day 9 subjects began a 2-week easy training period consisting of 3 or 4, 1-h exercise sessions per week. On day 22 subjects reported to the laboratory for a third $Vo_{2\ max}$ test. Finally, on day 23 the subjects reported to laboratory to perform the third experimental trial (Trial 3), again the procedures for which were identical to the first. A schematic representation of the experimental procedures is shown in Figure 1. Subjects were instructed to maintain a diet high in carbohydrate throughout the experimental period. During the experimental trials subjects were allowed to consume water *ad libitum*. Temperature and relative humidity during the experimental trials were ~21∞C and ~55%, respectively.

During each experimental trial whole blood (6 ml) was divided into three sterile K_3EDTA vacutainer tubes (Becton Dickinson, Oxford, UK). Two tubes were kept at room temperature until the end of the trial and analysed for total and differential leukocyte counts and lymphocyte surface marker staining. The remaining whole blood was separated immediately by centrifugation (1500 g) for 10 min and aliquots of plasma were stored at -20ºC until analysis for adrenaline (excluding 1-h post-exercise) and cortisol. A further 7 ml of whole blood was placed in sterile lithium-heparin vacutainer tubes (Becton Dickinson, Oxford, UK) and kept at room temperature until the end of the trial for analysis of intracellular cytokine production.

Leukocyte Counts

In addition to the leukocyte count, absolute numbers of neutrophils, lymphocytes and monocytes were determined. This procedure was performed at the Haematology laboratory, University Hospital Birmingham using a Technicon H-2 laser system.

Assessment of Intracellular Cytokine production

Cell activation. Whole blood (500 µl) was added to 500 µl RPMI-1640 medium supplemented with 2 $mmol.l^{-1}$ L-glutamine in each of two 12 x 75-mm polystyrene tubes (Becton Dickinson Labware). To one set of sample tubes (stimulated) 10 $mg.\mu l^{-1}$ Brefeldin A, 25 $ng.ml^{-1}$ phorbol 12-myristate 13-acetate (PMA) and 1 $\mu g.ml^{-1}$ ionomycin was added. To the second set of sample tubes (unstimulated) 10 $\mu g.ml^{-1}$ Brefeldin A only was added. Brefeldin A is a potent inhibitor of intracellular protein transport and was added to samples to ensure cytokines produced during stimulation would be retained within the cell. Unstimulated and stimulated samples were then incubated at 37ºC for 4 h in a 5% CO_2 humidified atmosphere.

Staining for intracellular cytokines. Aliquots (100 µl) of unstimulated and stimulated whole blood were added to separate polystyrene tubes containing 4 µl peridinin chlorophyll protein (PerCP)-CD3 conjugated monoclonal antibody (Becton Dickinson Biosciences, San Jose, CA) and incubated for 15 min. Erythrocytes were lysed by adding 2 ml lysing solution (Becton Dickinson Biosciences) and incubated for 10 min. Samples were centrifuged (1000 rpm) for 5 min and the supernatant decanted. Cells were permeabilized by adding 500 µl permeabilizing solution (Becton Dickinson Biosciences) and incubated for a further 10 min. Samples were then washed (0.5% bovine serum albumin, phosphate buffered saline and 0.1% NaN_3) centrifuged (1000 rpm) for 5 min and the supernatant decanted. Samples were then incubated with R-phycoerythrin (PE)-IFN-γ, PE-IL-4 or control mouse PE-$IgG\gamma_{2a}$ conjugated monoclonal antibodies for 30 min. This resulted in the following combinations of antibodies: $CD3^+$/IFN-γ^+/$IL\text{-}4^+$. Samples were then washed, centrifuged (1000 rpm) for 5 min and the supernatant decanted before being resuspended in 200 µl of phosphate buffered saline (1% paraformaldehyde) and analysed by flow cytometry. All incubations took place at room temperature in the dark.

Assessment of Lymphocyte Surface Marker Expression

Surface marker staining on unstimulated lymphocytes. Aliquots (50 µl) of whole blood were added to polystyrene tubes and incubated for a minimum of 15 min with the following combinations of conjugated monoclonal antibodies (all antibodies were obtained from Becton Dickinson Immunocytometry, San Jose, CA, unless otherwise stated): PerCP-CD3, FITC-CD4 & PE-CD8; PE-CD4 (DAKO, Glostrup, Denmark), PerCP-CD8 & FITC-CD45RO (DAKO). Erythrocytes were lysed (1 ml lysing solution) and samples washed (3 ml phosphate buffered saline, 0.1% NaN_3) essentially as described above, and then analysed by flow cytometry. All incubations took place at room temperature in the dark.

Flow cytometric analysis. Labelled cells were analysed by flow cytometry using a fluorescence activated cell sorter (Becton Dickinson FACSCalibur). Cells were selected on the basis of their forward vs. side light scatter and subsequently analysed (Cell Quest, Becton Dickinson) with gates set to identify positive and negative staining set on isotype controls (<1% positive). Lymphocyte subset counts were expressed as percentages of cells among total lymphocytes. Absolute numbers of lymphocyte subsets were determined by multiplying the correspon-

ding percentages by the total lymphocyte count. Intracellular cytokine production results were expressed as the percentage of IFN-γ and IL-4 positive $CD3^+$ T cells among total T cells. Absolute numbers of cytokine positive T cells were determined by multiplying the corresponding percentages by the total T cell concentration. To quantify the amount of cytokine within positive cells the geometric mean fluorescence intensity (GMFI) was obtained.

Stress Hormones

Plasma cortisol (DRG instruments, Germany) and adrenaline (IBL, Hamburg, Germany) concentrations were determined by enzyme linked immunosorbent assay (ELISA).

Statistical Analysis

A two-way (trial × time) repeated measure analysis of variance (RM ANOVA) was used to compare means. A significant interaction effect would indicate that a change in any specific parameter in response to acute exercise is dependent on the specific time in the experimental period at which that trial took place. Where the RM ANOVA revealed a significant F ratio differences where inspected with Tukey's honestly significant difference post hoc test. SPSS version 10 for Windows (SPSS inc., Chicago, Illinois) was used to calculate these statistics. The level of significance accepted to reject the null hypothesis was $P<0.05$. Data in the text, tables and figures are mean ± SEM.

Results

The subjects' $Vo_{2\,max}$ values were not different prior to Trials 1, 2 and 3 (data nor shown). The mean exercise time to exhaustion in the experimental trial before the 6-day ITP (Trial 1) was 107 ± 7 min. In the experimental trial immediately after the ITP (Trial 2) , during which subjects average training volume increased by ~73% (Fig. 2), there was a decrease ($P<0.01$) in the mean exercise time to exhaustion to 85 ± 5 min. In addition, following the ITP all subjects met established criteria defining them as overreached (17), i.e. decreased exercise performance capacity and increased mood disturbance. Following two weeks of low volume exercise training (Trial 3) the mean exercise time to exhaustion returned to 103 ± 11 min.

Exercise during Trails 1 and 3 resulted in an increase ($P<0.01$) in the circulating lymphocyte, neutrophil and monocyte concentrations at 60-min and post-exercise compared with pre-exercise, resulting in an increase in the total circulating leukocyte concentration (Table 1). The increased leukocyte concentration was maintained at 1-h post-exercise ($P<0.01$) primarily due to the increased neutrophil and monocyte concentrations (Table 1). At 1-h post-exercise, lymphocyte concentrations returned to pre-exercise levels (Table1). Compared with exercise during both Trials 1 and 3, exercise during Trial 2 resulted in lower ($P<0.01$) post-exercise and 1-h post-exercise neutrophil, lymphocyte and monocyte ($P<0.01$) concentrations and consequently a lower ($P< 0.01$) total leukocyte concentration (Table 1).

In each of the experimental trials, the circulating $CD3^+$ T lymphocyte concentrations increased ($P<0.01$) at 60-min and post-exercise compared with pre-exercise and then returned to pre-exercise levels at 1-h post-exercise (Table 2). The response to exercise of the $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes shared a similar pattern to the total $CD3^+$ lymphocyte population (Table 2). Furthermore, the increase in $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes was primarily due to an increase in those $CD4^+$ and $CD8^+$ lymphocytes expressing $CD45RO^+$, i.e. memory T cells (Table 2).

During each trial there was a tendency for the concentration of stimulated $CD3^+$ T lymphocytes staining positive for IFN-γ ($CD3^+IFN\text{-}\gamma^+$) to increase ($P=0.07$) at 60-min compared with pre-exercise (Table 3). Exercise during Trials 1 and 3 resulted in a decrease ($P<0.05$) in both the percentage and concentration of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes at 1-h post-exercise compared with pre-exercise (Table 3). Exercise during Trial 2 resulted in no statistically significant alterations in either the percentage or concentration of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes. Furthermore, the percentage and concentration of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes at rest in Trial 2 was decreased ($P<0.05$) compared with the resting values in both Trials 1 and 3 (Table 3). There was a significant main trial effect such that the amount of IFN-γ produced by stimulated $CD3^+$ T lymphocytes was decreased ($P<0.05$) post-exercise compared with pre-exercise (Fig. 3A). The percentage of un-stimulated $CD3^+$ T lymphocytes staining positive for either IFN-γ or IL-4 was always less than 1%. Therefore, exercise *per se* had no effect on IFN-γ or IL-4 production from $CD3^+$ T lymphocytes, and stimulation with PMA + ionomycin dramatically up-regulated the

Table 1. Circulating concentrations of total leukocytes and subsets in response to exercise, before (Trial 1), immediately after (Trial 2) and following 2 weeks recovery (Trial 3) from an intensified training period.

	Cell Count ($x10^9$ cells.l^{-1})			
	Pre-exercise	60-min	Post-exercise	1-h post-exercise
Leukocytes				
Trial 1	4.9 (0.4)	7.8 (0.5)	10.4 (0.6)*	11.6 (0.6)*†
Trial 2	4.6 (0.2)	7.3 (0.4)	7.5 (0.2)	8.1 (1.3)*
Trial 3	4.5 (0.3)	6.9 (0.3)	11.6 (1.5)*†	12.8 (1.3)*†
Neutrophils				
Trial 1	2.9 (0.3)	4.5 (0.6)	7.2 (0.7)*	9.5 (0.4)*†
Trial 2	2.5 (0.2)	4.0 (0.4)	4.9 (0.2)	6.2 (1.1)*
Trial 3	2.5 (0.2)	3.7 (0.3)	8.2 (1.4)*†	10.7 (1.2)*†
Lymphocytes				
Trial 1	1.4 (0.2)	2.5 (0.3)*	2.3 (0.3)*†	1.3 (0.2)
Trial 2	1.6 (0.2)	2.4 (0.3)*	1.9 (0.2)	1.4 (0.2)
Trial 3	1.5 (0.1)	2.3 (0.2)*	2.4 (0.3)*†	1.3 (0.1)
Monocytes				
Trial 1	0.5 (0.1)	0.7 (0.1)*	0.8 (0.1)*	0.7 (0.1)*
Trial 2	0.5 (0.1)	0.7 (0.1)*	0.6 (0.1)	0.5 (0.1)
Trial 3	0.4 (0.1)	0.6 (0.1)*	0.8 (0.1)*	0.7 (0.1)*

Table 1. Values are circulating total leukocyte, neutrophil, lymphocyte and monocyte concentrations (× 10^9 cells.l^{-1}) in peripheral blood at rest (Pre-exercise), after 60 min of exercise (60-min), at exhaustion (Post-exercise) and following 1-h of recovery from exercise (1-h post-exercise); before (Trial 1), immediately after (Trial 2) and following 2 weeks of recovery (Trial 3) from an intensified training period (ITP). Values are means ± S.E.M.; $n = 7$ subjects. * Interaction effect, significantly different from pre-exercise ($P<0.01$). † Interaction effect, significantly different from Trial 1 ($P<0.01$).

Table 2. Circulating concentrations of T lymphocytes in response to exercise, before (Trial 1), immediately after (Trial 2) and following 2 weeks recovery (Trial 3) from an intensified training period.

	Cell Count (x10⁹ cells.l⁻¹)			
	Pre-exercise	60-min	Post-exercise	1-h post-exercise
$CD3^+$				
Trial 1	0.85 (0.08)	1.14 (0.12)¶#	1.11 (0.13)¶#	0.72 (0.10)
Trial 2	0.91 (0.10)	1.21 (0.18)¶#	0.97 (0.11)¶#	0.84 (0.11)
Trial 3	0.85 (0.07)	1.07 (0.10)¶#	1.15 (0.11)¶#	0.76 (0.08)
$CD3^+CD4^+$				
Trial 1	0.54 (0.07)	0.67 (0.08)¶#	0.66 (0.09)¶#	0.45 (0.05)
Trial 2	0.56 (0.07)	0.70 (0.12)¶#	0.59 (0.08)¶#	0.53 (0.07)
Trial 3	0.52 (0.06)	0.63 (0.08)¶#	0.64 (0.08)¶#	0.50 (0.06)
$CD3^+CD8^+$				
Trial 1	0.30 (0.04)	0.42 (0.08)¶#	0.42 (0.08)¶#	0.28 (0.08)
Trial 2	0.31 (0.06)	0.46 (0.10)¶#	0.31 (0.05)¶#	0.27 (0.06)
Trial 3	0.31 (0.05)	0.40 (0.05)¶#	0.45 (0.07)¶#	0.24 (0.05)
$CD4^+CD45RO^+$				
Trial 1	0.39 (0.07)	0.58 (0.11)¶#	0.54 (0.10)¶#	0.35 (0.08)
Trial 2	0.43 (0.07)	0.57 (0.11)¶#	0.48 (0.08)¶#	0.42 (0.08)
Trial 3	0.39 (0.07)	0.50 (0.10)¶#	0.51 (0.11)¶#	0.37 (0.07)
$CD8^+CD45RO^+$				
Trial 1	0.15 (0.02)	0.24 (0.04)¶#	0.24 (0.03)¶#	0.12 (0.03)¶
Trial 2	0.15 (0.03)	0.25 (0.04)¶#	0.19 (0.02)¶#	0.13 (0.03)¶
Trial 3	0.17 (0.03)	0.28 (0.08)¶#	0.26 (0.05)¶#	0.14 (0.03)¶

Table 2. Values are circulating total T cell ($CD3^+$) and T cell subset ($CD3^+CD4^+$, $CD3^+CD8^+$, $CD4^+CD45RO^+$, $CD8^+CD45RO^+$) concentrations ($\times 10^9$ cells.L^{-1}) in peripheral blood obtained at rest (Pre-exercise), after 60 min of exercise (60-min), at exhaustion (Post-exercise) and following 1-h of recovery from exercise (1-h post-exercise); before (Trial 1), immediately after (Trial 2) and following 2 weeks of recovery (Trial 3) from an intensified training period (ITP). Values are means ± SEM; $n = 7$ subjects. ¶ Main time effect, significantly different from pre-exercise ($P<0.05$). # Main time effect, significantly different from 1-hr post-exercise ($P<0.05$).

production of IFN-γ from $CD3^+$ T cells.

Neither acute exercise, nor chronic exercise training resulted in a statistically significant alteration of either the concentration or percentage of stimulated $CD3^+$ T lymphocytes staining positive for IL-4 (Table 3). In addition, the amount of IL-4 produced by stimulated $CD3^+$ T lymphocytes following stimulation was unchanged following exercise (Fig. 3B).

Plasma adrenaline concentration was elevated ($P<0.05$) after 60-min of exercise and post-exercise compared with pre-exercise (Table 4). Plasma cortisol concentration increased after 60-min of exercise ($P<0.05$), and was elevated post-exercise ($P<0.05$) compared with 1-h post-exercise (Table 4). In addition, there was a trial main effect ($P<0.05$) such that plasma cortisol concentration was higher during both Trials 1 and 3 compared with Trial 2 (Table 4).

Discussion

The major findings of the present study were that firstly, a decrease in both the percentage and number of circulating type 1 T lymphocytes was observed following acute exercise during the normal training and recovery training periods, how-

Table 3. Circulating concentrations and percentages of stimulated T cells positive for IFN-γ and IL-4 in response to exercise, before (Trial 1), immediately after (Trial 2) and following 2 weeks recovery (Trial 3) from an intensified training period.

	Cell Count (%)				*Cell Count (x10⁹ cells.l⁻¹)*			
	Pre-exercise	60-min	Post-exercise	1-h post-exercise	Pre-exercise	60-min	Post-exercise	1-h post-exercise
$CD3^+$ IFN-γ^+								
Trail 1	23 (2)†	22 (2)†	15 (2)*	15 (2)*	0.19 (0.02)†	0.24 (0.01)	0.16 (0.03)†	0.11 (0.03)*
Trial 2	14 (4)	15 (4)	10 (4)	16 (4)	0.12 (0.03)	0.18 (0.05)	0.09 (0.04)	0.14 (0.05)
Trial 3	21 (2)†	21 (2)	22 (2)	13 (2)*	0.17 (0.01)	0.22 (0.02)	0.26 (0.03)†	0.10 (0.2)*
$CD3^+CD4^+$								
Trail 1	1.6 (0.3)	1.8 (0.4)	1.2 (0.4)	1.5 (0.3)	0.014 (0.003)	0.019 (0.004)	0.014 (0.005)	0.010 (0.002)
Trial 2	1.0 (0.2)	1.7 (0.8)	0.8 (0.2)	1.2 (0.3)	0.008 (0.002)	0.016 (0.006)	0.007 (0.002)	0.009 (0.002)
Trial 3	1.2 (0.3)	1.2 (0.3)	1.1 (0.3)	1.3 (0.2)	0.010 (0.003)	0.013 (0.004)	0.013 (0.003)	0.010 (0.002)

Values are circulating $CD3^+$IFN-γ^+ and $CD3^+$IL-4^+ T lymphocyte concentrations ($\times 10^9$ cells.L^{-1}) and percentages in peripheral blood obtained at rest (Pre-exercise), after 60 min of exercise (60-min), at exhaustion (Post-exercise) and following 1-h of recovery from exercise (1-h post-exercise); before (Trial 1), immediately after (Trial 2) and following 2 weeks of recovery (Trial 3) from an intensified training period (ITP). Values are means ± SEM; $n = 7$ subjects. * Interaction effect, significantly different from pre-exercise ($P<0.05$). † Interaction effect, significantly different from Trial 1 ($P<0.05$).

Table 4. Circulating adrenaline and cortisol concentrations in response to exercise, before (Trial 1), immediately after (Trial 2) and following 2 weeks of recovery (Trial 3) from an intensified training period.

	Plasma concentration (nmol.l-1)			
	Pre-exercise	60-min	Post-exercise	1-h post-exercise
Adrenaline				
Trial 1	0.45 (0.03)	0.67 (0.05)¶	0.87 (0.13)¶	NM
Trial 2	0.42 (0.02)	0.59 (0.05)¶	0.65 (0.07)¶	NM
Trial 3	0.46 (0.03)	0.69 (0.05)¶	1.11 (0.28)¶	NM
Cortisol				
Trial 1	353 (21)‡	504 (43)¶‡	566 (85)‡	443 (70)‡
Trial 2	343 (31)	403 (57)¶	442 (81)	350 (51)
Trial 3	353 (26)‡	478 (59)¶‡	614 (104)	

Values are plasma adrenaline and cortisol concentrations in peripheral blood obtained at rest (Pre-exercise), after 60 min of exercise (60-min), at exhaustion (Post-exercise) and following 1-h of recovery from exercise (1-h post-exercise); before (Trial 1), immediately after (Trial 2) and following 2 weeks of recovery (Trial 3) from an intensified training period (ITP). Values are means ± SEM; n = 7 subjects. ¶ Main time effect, significantly different from pre-exercise ($P<0.05$). ‡ Main trial effect, significantly different from Trial 1 ($P<0.05$). NM = not measured at this time point.

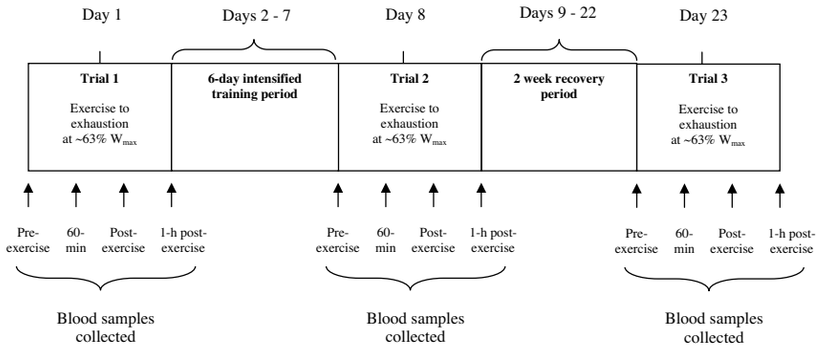

Figure 1. Schematic representation of the experimental procedures. W_{max} = maximal work rate in the $Vo_{2\ max}$ test.

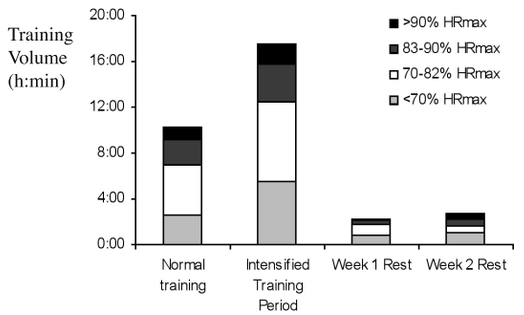

Figure 2. Volume and intensity of training completed during a 7-day 'normal' training period, during the 6-day intensified training period (ITP) and during each week of the 2-week recovery period. HR_{max} = maximal heart rate.

ever no exercise-induced effects were observed on the distribution of type 1 T lymphocytes in the trial performed immediately after the ITP (Table 3). Secondly, although acute exercise caused a decrease in the amount of IFN-γ produced by stimulated $CD3^+$ T lymphocytes, no effect of chronic exercise training was observed on resting T lymphocyte IFN-γ production (Fig. 3A). Thirdly, both the resting percentage and number of circulating type 1 T lymphocytes decreased in the trial immediately after the ITP compared with the normal and recovery training trials (Table 3). Finally, we observed no effects of either acute exhaustive exercise, or chronic exercise training on type 2 T lymphocyte distribution or function (Table 3, Fig. 3B).

The results of the present study, which demonstrate a decline in the percentage and number of type 1 ($CD3^+IFN\text{-}\gamma^+$) T lymphocytes following acute exercise in both the normal training and recovery training trials, support the findings of previous work (19, 37). In addition, similarly to previous studies we observed no exercise-induced effects on the distribution of type 2 ($CD3^+IL\text{-}4^+$) T lymphocytes. Lymphoid cells are particularly sensitive to glucocorticoid (GC)-induced apoptosis (3), and it is possible that the exercise-induced increase in circulating cortisol levels may have promoted apoptosis in the $CD3^+IFN\text{-}\gamma^+$ T lymphocytes thus mediating the decline in these cells from the circulation. However, given that peripheral T lymphocytes are relatively resistant to GC-induced apoptosis (3), that lymphocyte apoptosis has been shown not to contribute to post-exercise lymphocytopenia (36) and the relatively rapid decline in circulating $CD3^+IFN\text{-}\gamma^+$ T lymphocytes following exercise, apoptosis is an unlikely cause of the post-exercise decline in the number and percentage of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes observed in the present study. The differential effects of exercise on type 1 and type 2 T lymphocytes are most likely accounted for by the higher surface expression of β_2-adrenergic receptors on type 1 T lymphocytes compared with type 2 T lymphocytes (31), and that type 1 T lymphocytes are markedly more sensitive to the effects of GCs (6, 16) compared with type 2 T lymphocytes.

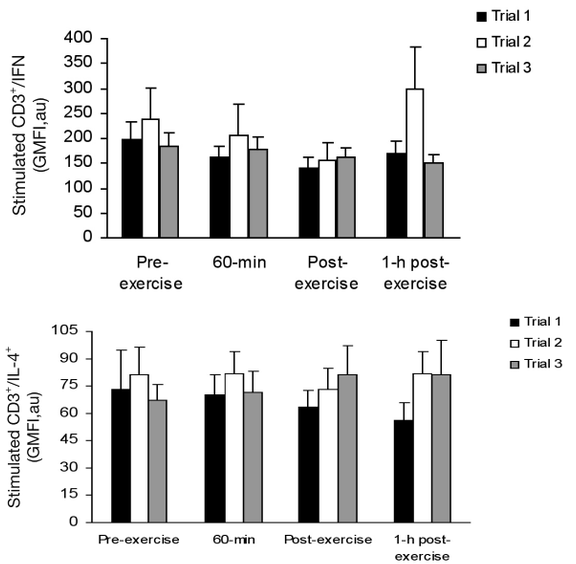

Figure 3 A & B. Values are the geometric mean fluorescence intensity (GMFI) of stimulated $CD3^+$ T lymphocytes positive for interferon (IFN)-γ (Fig. 3A) and interleukin (IL)-4 (Fig. 3B) in peripheral blood obtained at rest (Pre-exercise), after 60 min of exercise (60-min), at exhaustion (Post-exercise) and following 1-h of recovery from exercise (1-h post-exercise); before (Trial 1), immediately after (Trial 2) and following 2 weeks of recovery (Trial 3) from an intensified training period (ITP). Values are means ± SEM; n = 7 subjects. ¶ Main time effect, significantly different from pre-exercise ($P<0.05$).

It has been demonstrated that adrenal stress hormones exert considerable influence over the trafficking of leukocytes between the circulation and peripheral immune compartments (1, 13, 35), and recent data from our laboratory provide support for the role of stress hormones in mediating the exercise-induced decrease in the circulating levels of type 1 T lymphocytes (23). Although a decrease in the percentage of circulating type 1 T lymphocytes has been suggested as a mechanism to explain the increased susceptibility to infection following prolonged strenuous exercise (37), it has been elegantly demonstrated that acute stress may actually enhance type 1 (cell-mediated) immunity (10-12) as a result of an adrenal stress-hormone mediated trafficking of circulating leukocytes from the systemic circulation to the vasculature of peripheral immune compartments (e.g. skin, urogenital and gastrointestinal tracts). It seems reasonable to suggest that following an acute bout of prolonged strenuous exercise the increased circulating adrenal stress hormone concentrations promote the trafficking of type 1 T lymphocytes from the circulation to the vasculature of other immune compartments. Consequently, the percentage and number of type 1 T lymphocytes present in the circulation is decreased, but instead of being immunosuppressive *per se,* this decrease may mediate an enhanced immune response against certain types of pathogen in specific immune compart-

ments by virtue of the greater number of type 1 T lymphocytes in these compartments. Interestingly, while the work of Dhabhar and colleagues has demonstrated that acute stress enhances cell-mediated immunity (as determined by the delayed type hypersensitivity (DTH) response), the DTH response following prolonged strenuous exercise is depressed (7). While the reasons for these inconsistencies are not clear, it is likely that the severity of the stress plays a role. In the study by Bruunsgaard and colleagues, subjects performed a half-ironman triathlon competitive race (approximately 5 h in duration). In the studies by Dhabhar and colleagues 2 h of restraint stress was used, a stress model that the authors state is relatively mild. These differences in both the duration and intensity of stressor will result in dramatic differences in the stress hormone and cytokine responses, and this may account for the different results between these studies.

This study is the first to examine the effect of a period of chronic exercise training on type 1 and type 2 T lymphocyte distribution and function. The results of the present study demonstrate an attenuation of the decrease of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes in response to acute exercise immediately after the chronic exercise training period compared with acute exercise before and following 2-weeks of resting recovery from the chronic exercise training period, and a reduction in both the resting number and percentage of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes following a period of chronic exercise training. In addition, while acute exercise decreased IFN-γ production by stimulated T lymphocytes, no effect of the intensified training period on T lymphocyte IFN-γ production at rest was observed. The current observation of an attenuated decline in the percentage and number of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes in response to acute exercise immediately after the chronic exercise is consistent with the suggestion that while acute exercise may enhance type 1 immunity in certain situations as a result of a stress-hormone mediated trafficking of type 1 T lymphocytes from the circulation to sites of potential infection, chronic exercise training may suppress immune function as a result of a decrease in the stress-induced trafficking of type 1 T lymphocytes. Given that we observed a markedly lower circulating cortisol concentration in response to acute exercise immediately after the ITP compared with before and following 2-weeks of resting recovery from the ITP, and that GCs are the main mediators of the acute stress-induced efflux of lymphocytes from the circulation, it is likely, at least in part, that the attenuated decrease of the circulating number and percentage of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes observed in response to acute exercise immediately after the ITP was a result of an attenuated exercise-induced stress-hormone response. However, it is important to note that the duration of the exercise trial after the ITP (85 min) was significantly less than in the trials before the ITP (107 min) and following 2 weeks of recovery from the ITP (103 min) and exercise duration is an important mediator of lymphocyte trafficking.

It is possible that apoptosis of the type 1 T lymphocytes as a result of the prolonged and repeated daily exposure to exercise-induced elevations in cortisol may account for the decline in the resting level of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes following chronic exercise training. A second possibility may be that as a result of exercise on the preceding day ($Vo_{2\,max}$ test and training) there was a sustained and selective retention of the type 1 T lymphocytes in certain immune compartments, such that on the following day not all type 1 T cells had returned to the circulation. Previous work by Dhabhar and colleagues has demonstrated that a period of chronic stress prior to an acute stressor resulted in an attenuation of the stress-induced increase in plasma corticosterone levels, an attenuation of the stress-induced decrease in blood lymphocyte concentrations and a reduction in basal blood lymphocyte concentrations compared with acute stress alone (10). The effects of the chronic exercise period on the level of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes in the present study are consistent with these findings.

In the present study we assessed type 1 and type 2 T lymphocyte function by quantifying the amount of IFN-γ and IL-4 produced by stimulated T cells. We observed a decrease in the amount of IFN-γ produced by stimulated T cells following acute exercise, supporting previous work demonstrating a decrease in the concentration of IFN-γ in the supernatant of *in vitro* mitogen-stimulated blood after exercise (4, 34, 41), and the decline observed in IFN-γ production by T lymphocytes quantified at the single cell level (37). In contrast to IFN-γ production, the amount of IL-4 produced by stimulated T cells was unchanged following acute exercise. The mechanism underlying this decrease in T cell IFN-γ production appears to be unrelated to the exercise-induced rise in the circulating catecholamine concentration (35), since adrenergic blockade had no effect on the magnitude of the post-exercise suppression of IFN-γ production. Furthermore, adrenergic blockade suppressed the exercise-induced rise in the plasma cortisol concentration, suggesting a lack of association between cortisol and the post-exercise depression of stimulated T lymphocyte IFN-γ production. Given that adrenal stress hormones are known to inhibit IL-2, IL-4, and IFN-γ production from *in vitro* mitogen-stimulated blood (22, 28, 38), the observation of a lack of association between the exercise-induced stress hormone response and the depression of intracellular cytokine production is surprising. It is possible that a critical threshold for stress hormone induced effects may exist, above which further increases in cortisol or catecholamines have no further suppressive effects on T lymphocyte IFN-γ production. In support of this idea, although the exercise-induced increase in the cortisol concentration was higher in the trials before and following 2 weeks of resting recovery from the ITP, a post-exercise increase remained in the trial immediately after the ITP and this may have been sufficient to account for the observed suppression in T cell IFN-γ production. In contrast to our hypothesis, T lymphocyte IFN-γ production at rest after completion of the ITP was unaltered compared with values before and following 2 weeks of resting recovery from the ITP. However, the resting adrenaline and cortisol concentrations were unaffected by the ITP, and it is possible that this may account for the lack of an observed effect of the ITP on resting T lymphocyte IFN-γ production.

In conclusion, we have observed that the effect of acute exercise following a period of chronic exercise training on the re-distribution of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes was attenuated compared with acute exercise before and following 2 weeks of resting recovery from the chronic intensified exercise training period. Furthermore, we observed a decrease in both the number and percentage of $CD3^+IFN\text{-}\gamma^+$ T lymphocytes at rest following a period of intensified exercise training. Given the critical role played by the type 1 T cells in the generation of antiviral defence, and that respiratory tract infections are typically the result of viruses, our results which illustrate a modulation of type 1 T lymphocyte distribution suggest a possible mechanism for the increased incidence of infection typically reported by athletes during periods of hard training. Finally, differences exist in

the patterns of distribution of type 1 and type 2 T lymphocytes between the circulation and the nasal mucosa (39), and since the predominant site of infection reported by athletes is typically the mucosa of the upper respiratory tract, the effect of exercise on type 1 and type 2 T lymphocyte balance and function within the lymphoid tissue of the upper respiratory and nasal mucosae seems warranted.

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References

1. Ahlborg, B. and G. Ahlborg. Exercise leukocytosis with and without beta-adrenergic blockade. Acta Med Scand. 187:241-246, 1970.
2. Almawi, W. Y., M. M. Abou Jaoude, and X. C. Li. Transcriptional and post-transcriptional mechanisms of glucocorticoid antiproliferative effects. Hematol Oncol. 20:17-32, 2002.
3. Ashwell, J. D., F. W. Lu, and M. S. Vacchio. Glucocorticoids in T cell development and function. Annu Rev Immunol. 18:309-345, 2000.
4. Baum, M., M. Muller-Steinhardt, H. Liesen, and H. Kirchner. Moderate and exhaustive endurance exercise influences the interferon-gamma levels in whole-blood culture supernatants. Eur J Appl Physiol. 76:165-169, 1997.
5. Berguer, R., N. Bravo, M. Bowyer, C. Egan, T. Knolmayer, and D. Ferrick. Major surgery suppresses maximal production of helper T-cell type 1 cytokines without potentiating the release of helper T-cell type 2 cytokines. Arch Surg. 134:540-544, 1999.
6. Blotta, M. H., R. H. DeKruyff, and D. T. Umetsu. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. J Immunol. 158:5589-5595, 1997.
7. Bruunsgaard, H., A. Hartkopp, T. Mohr, H. Konradsen, I. Heron, C. H. Mordhorst, and B. K. Pedersen. In vivo cell-mediated immunity and vaccination response following prolonged, intense exercise. Med Sci Sports Exerc. 29:1176-1181, 1997.
8. Budgett R. Fatigue and underperformance in athletes: the overtraining syndrome. Br J Sports Med 32: 107-110, 1998.
9. Chrousos, G. P. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N Engl J Med. 332:1351-1362, 1995.
10. Dhabhar, F. S. and B. S. McEwen. Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. Brain Behav Immun. 11:286-306, 1997.
11. Dhabhar, F. S. and B. S. McEwen. Enhancing versus suppressive effects of stress hormones on skin immune function. Proc Natl Acad Sci USA. 96:1059-1064, 1999.
12. Dhabhar, F. S. and B. S. McEwen. Stress-induced enhancement of antigen-specific cell-mediated immunity. J Immunol. 156:2608-2615, 1996.

13. Dhabhar, F. S., A. R. Satoskar, H. Bluethmann, J. R. David, and B. S. McEwen. Stress-induced enhancement of skin immune function: A role for gamma interferon. Proc Natl Acad Sci U S A. 97:2846-2851, 2000.
14. Elenkov, I. J. and G. P. Chrousos. Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. Trends Endocrinol Metab. 10:359-368, 1999.
15. Fitzgerald, L. Exercise and the immune system. Immunol Today. 9:337-339, 1988.
16. Franchimont, D., E. Louis, W. Dewe, H. Martens, Y. Vrindts-Gevaert, D. De Groote, J. Belaiche, and V. Geenen. Effects of dexamethasone on the profile of cytokine secretion in human whole blood cell cultures. Regul Pept. 73:59-65, 1998.
17. Gleeson, M. Mucosal immune responses and risk of respiratory illness in elite athletes. Exerc Immunol Rev. 6:5-42, 2000.
18. Halson, S. L., G. I. Lancaster, J. Achten, M. Gleeson, and A. E. Jeukendrup. Effect of carbohydrate supplementation on performance and carbohydrate oxidation following intensified cycling training. J Appl Physiol. 97: 1245-1253, 2004.
19. Ibfelt, T., E. W. Petersen, H. Bruunsgaard, M. Sandmand, and B. K. Pedersen. Exercise-induced change in type 1 cytokine-producing CD8+ T cells is related to a decrease in memory T cells. J Appl Physiol. 93:645-648, 2002.
20. Jentjens, R. L., A. J. Wagenmakers, and A. E. Jeukendrup. Heat stress increases muscle glycogen use but reduces the oxidation of ingested carbohydrates during exercise. J Appl Physiol. 92:1562-1572, 2002.
21. Kronfol, Z., M. Starkman, D. E. Schteingart, V. Singh, Q. Zhang, and E. Hill. Immune regulation in Cushing's syndrome: relationship to hypothalamic-pituitary-adrenal axis hormones. Psychoneuroendocrinology. 21:599-608, 1996.
22. Kunicka, J. E., M. A. Talle, G. H. Denhardt, M. Brown, L. A. Prince, and G. Goldstein. Immunosuppression by glucocorticoids: inhibition of production of multiple lymphokines by in vivo administration of dexamethasone. Cell Immunol. 149:39-49, 1993.
23. Lancaster GI, K. Q., Drysdate P, Jeukendrup AE, Drayson MT & Glesson M. Effect of feeding different amounts of carbohydrate during prolonged exercise on human T-lymphocyte intracellular cytokine production. J Physiol. 548P (Abstract): O98, 2003.
24. Mackinnon, L. T. Chronic exercise training effects on immune function. Med Sci Sports Exerc. 32:S369-376, 2000.
25. MacKinnon, L. T. Special feature for the Olympics: effects of exercise on the immune system: overtraining effects on immunity and performance in athletes. Immunol Cell Biol. 78:502-509, 2000.
26. Mosmann, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 136:2348-2357, 1986.
27. Moyna, N. M., G. R. Acker, J. R. Fulton, K. Weber, F. L. Goss, R. J. Robertson, D. J. Tollerud, and B. S. Rabin. Lymphocyte function and cytokine production during incremental exercise in active and sedentary males and females. Int J Sports Med. 17:585-591, 1996.
28. Moynihan, J. A., T. A. Callahan, S. P. Kelley, and L. M. Campbell. Adrenal hormone modulation of type 1 and type 2 cytokine production by spleen cells: dexamethasone and dehydroepiandrosterone suppress interleukin-2, interleukin-4, and interferon-gamma production in vitro. Cell Immunol. 184:58-64, 1998.
29. Nieman, D. C. Immune response to heavy exertion. J Appl Physiol. 82:1385-1394, 1997.

30. Pedersen, B. K. and L. Hoffman-Goetz. Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev. 80:1055-1081, 2000.
31. Sanders, V. M., R. A. Baker, D. S. Ramer-Quinn, D. J. Kasprowicz, B. A. Fuchs, and N. E. Street. Differential expression of the beta2-adrenergic receptor by Th1 and Th2 clones: implications for cytokine production and B cell help. J Immunol. 158:4200-4210, 1997.
32. Seder, R. A. Acquisition of lymphokine-producing phenotype by CD4+ T cells. J Allergy Clin Immunol. 94:1195-1202, 1994.
33. Smith LL. Overtraining, excessive exercise, and altered immunity. Is this a T Helper-1 versus T Helper-2 lymphocyte response? Sports Med. 33: 347-364, 2003.
34. Smits, H. H., K. Grunberg, R. H. Derijk, P. J. Sterk, and P. S. Hiemstra. Cytokine release and its modulation by dexamethasone in whole blood following exercise. Clin Exp Immunol. 111:463-468, 1998.
35. Starkie, R. L., J. Rolland, and M. A. Febbraio. Effect of adrenergic blockade on lymphocyte cytokine production at rest and during exercise. Am J Physiol Cell Physiol. 281:C1233-1240, 2001.
36. Steensberg, A., J. Morrow, A. D. Toft, H. Bruunsgaard, and B. K. Pedersen. Prolonged exercise, lymphocyte apoptosis and F2-isoprostanes. Eur J Appl Physiol. 87:38-42, 2002.
37. Steensberg, A., A. D. Toft, H. Bruunsgaard, M. Sandmand, J. Halkjaer-Kristensen, and B. K. Pedersen. Strenuous exercise decreases the percentage of type 1 T cells in the circulation. J Appl Physiol. 91:1708-1712, 2001.
38. Steer, J. H., D. T. Ma, L. Dusci, G. Garas, K. E. Pedersen, and D. A. Joyce. Altered leucocyte trafficking and suppressed tumour necrosis factor alpha release from peripheral blood monocytes after intra-articular glucocorticoid treatment. Ann Rheum Dis. 57:732-737, 1998.
39. Till, S., L. Jopling, P. Wachholz, R. Robson, S. Qin, D. Andrew, L. Wu, J. van Neerven, T. Williams, S. Durham, and I. Sabroe. T cell phenotypes of the normal nasal mucosa: induction of Th2 cytokines and CCR3 expression by IL-4. J Immunol. 166:2303-2310, 2001.
40. Webster, J. I., L. Tonelli, and E. M. Sternberg. Neuroendocrine regulation of immunity. Annu Rev Immunol. 20:125-163, 2002.
41. Weinstock, C., D. Konig, R. Harnischmacher, J. Keul, A. Berg, and H. Northoff. Effect of exhaustive exercise stress on the cytokine response. Med Sci Sports Exerc. 29:345-354, 1997.