Strenuous exercise decreases the percentage of type 1 T cells in the circulation

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Received 22 March 2001; accepted in final form 11 June 2001

Strenuous exercise decreases the percentage of type 1 T cells in the circulation. J Appl Physiol 91: 1708–1712, 2001.—Prolonged strenuous exercise is followed by a temporary functional immune impairment. Low numbers of CD4+ T helper (Th) and CD8+ T cytotoxic (Tc) cells are found in the circulation. These cells can be divided according to their cytokine profile into type 1 (Th1 and Tc1), which produce interferon-γ and interleukin (IL)-2, and type 2 (Th2 and Tc2) cells, which produce IL-4. The question addressed in the present study was whether exercise affected the relative balance between the circulating levels of these cytokine-producing T cells. Nine male runners performed treadmill running for 2.5 h at 75% of maximal oxygen consumption. The intracellular expression of cytokines was detected following stimulation with ionomycin and phorbol 12-myristate 13-acetate in blood obtained before, during, and after exercise. The percentage of type 1 T cells in the circulation was suppressed at the end of exercise and 2 h after exercise, whereas no changes were found in the percentage of type 2 T cells. Plasma epinephrine correlated negatively with the percentage of circulating CD8+ T cells producing IL-2, whereas peak IL-6 correlated with the percentage of CD8+ IL-4-producing T cells in the circulation. Peak plasma IL-6 correlated with plasma cortisol postrunning. In conclusion, the postexercise decrease in T lymphocyte number is accompanied by a more pronounced decrease in type 1 T cells, which may be linked to high plasma epinephrine. Furthermore, IL-6 may stimulate type 2 T cells, thereby maintaining a relatively unaltered percentage of these cells in the circulation compared with total circulating lymphocyte number.

interleukin-4; interleukin-2; interleukin-6; interleukin-12; interferon-γ

PROLONGED STRENUEOUS EXERCISE is followed by a temporary functional immune impairment (10) and an increased sensitivity to upper respiratory tract infections (URTI) (8). In the postexercise period, the number of circulating T lymphocytes is low (10). However, it is not known whether all T lymphocyte subsets are affected to the same extent. The CD4+ T helper (Th) and the CD8+ T cytotoxic (Tc) cells can be divided into type 1 (Th1 and Tc1) and type 2 (Th2 and Tc2) cells according to their cytokine profile. Type 1 T cells produce interferon (IFN)-γ and interleukin (IL)-2, whereas type 2 T cells produce IL-4, IL-5, IL-6, and IL-10 (6). Type 1 T cell responses are stimulated by IL-12 (6) and have been shown to protect against intracellular pathogens such as several viruses (5). IL-6 has been shown to induce Th2 polarization by stimulating the initial production of IL-4. Type 2 T cells are important in the defense against extracellular parasites such as several helminths and schistosomiasis (5). Studies suggest that both cortisol and epinephrine inhibit the production of type 1 T cell cytokines (3, 4). However, little is known about the physiological regulation of type 1 and type 2 T cell balances. Exercise has been accepted as a model to study interactions between endocrine and immune systems during physical stress (10). Intense and prolonged exercise induces significant changes in a number of immune parameters (10). Thus, during exercise, lymphocytes are recruited to the blood. However, in the postexercise period, the number of circulating lymphocytes declines below preexercise values. Concomitantly, the plasma levels of proinflammatory cytokines are elevated. Thus the level of plasma IL-6 may be enhanced more than 100-fold (9), and plasma IL-12 has been demonstrated to increase (1). To understand how the type 1 and type 2 T cell balances are regulated, it is of interest to investigate the effect of exercise. The observation of an increased risk of URTI after an acute bout of heavy exercise may be a consequence of an impaired type 1 T cell response. Viruses often cause URTI, and type 1 T cells are crucial in the defense against intracellular pathogens.

The hypothesis tested in this study is whether changes in stress hormones and cytokines during prolonged strenuous exercise induce a suppression of type 1 T cells compared with type 2 T cells. Thus the present
study investigates how 2.5 h of treadmill running affects the number of circulating type 1 and type 2 T cells using flow cytometry. This method allows detection of intracellular cytokines within CD4\(^+\) and CD8\(^+\) T cells (14). Furthermore, a possible relation between IL-6, IL-12, and stress hormones on one hand and circulating type 1 and type 2 T cells on the other is investigated by using correlations.

**METHODS**

**Subjects.** Nine endurance-trained male runners aged 25–50 yr (median = 30 yr) with a maximal pulmonary oxygen consumption (VO\(_{2\max}\)) of 3.61–5.20 l/min (median = 4.48 l/min), corresponding to 52.2–68.3 ml·kg\(^{-1}\)·min\(^{-1}\) (median = 60.1 ml·kg\(^{-1}\)·min\(^{-1}\)), were included. The subjects did not take any medication. The study was approved by the local ethical committee for Copenhagen and Frederiksberg Communities (no. 01-111/97). Subjects were informed of the risks of the experiment before their voluntary written consent was obtained.

**Exercise protocol.** For each subject, VO\(_{2\max}\) was determined approximately 1 wk before the experiment by an incremental exercise test on the same treadmill (Technogym, HC1200) and CPX express (MedGraphics) as used in the experiment. In the experiment, subjects ran for 2.5 h at a speed determined in the VO\(_{2\max}\) test to give an oxygen consumption of 75% VO\(_{2\max}\). Actual oxygen consumption was sampled during the experiment as a control.

**Experimental protocol.** At 8:00 AM, subjects reported to the laboratory after an overnight fast, during which they allowed to drink water ad libitum. They were instructed to arrive well rested and to abstain from any extraordinary training in the week before and no training at all for 2 days before the experiment. Blood samples were drawn from the antecubital vein of both arms. Blood was obtained before, after 0.5 and 1.5 h of running, and at the end of running (2.5 h). When sampling was done during exercise, the speed of the treadmill was lowered to walking speed [average duration = 3 min (range = 2–4 min)]. For the next 2 h, the subjects stayed at the laboratory, and blood was sampled at 0.5, 1, 1.5, and 2 h postrunning. The next day, subjects reported back to the laboratory for the last blood sample. In the recovery period, subjects were allowed to eat and drink.

**Lymphocyte number.** This measurement was performed at the Central Laboratory, University Hospital of Copenhagen, Rigshospitalet, using standard laboratory procedures.

**Isolation of blood mononuclear cells.** Blood mononuclear cells (BMNC) were isolated by density-gradient centrifugation (Lymphoprep Nyegaard, Oslo, Norway) on LeucoSep tubes (Greiner, Friekenhausen, Germany) and washed three times in medium RPMI. Analyses were performed on nonfrozen cells.

**Flow cytometry analyses of intracellular cytokines.** We stimulated 10\(^6\) BMNC with 20 μl of ionomycin (0.1 μmol/l) and 50 μl of phorbol 12-myristate 13-acetate (1 μg/ml) for 4 h at 37°C in the presence of 10 μl monensin (0.2 μmol/l). Stimulated cells were harvested, washed in staining buffer, and incubated with surface antibodies for 20 min at 4°C. After wash and fixation in 4% paraformaldehyde for 10 min in a dark place, cells were spun down and resuspended in PBS with 1% FCS. The next day, cells were incubated with 200 μl of saponin buffer (0.1 g of saponin in 100 ml PBS) for 10 min at room temperature to make the cell membranes permeable. The cytokine antibodies were then added and incubated in the presence of saponin buffer for 30 min at 4°C and subsequently washed twice in saponin buffer. Labeled cells were analyzed by flow cytometry using a fluorescence-activated cell sorter analyzer (Epics XL-MCL, Coulter, Miami, FL). The data were analyzed using WinList PC software (Verity Software House).

dead cells, platelets, and monocytes were excluded by forward and side-scattered light angle. Monocytes were also excluded by being CD14\(^+\). CD3\(^-\) and CD8\(^-\) cells were accepted as CD4\(^+\) T cells because stimulation with phorbol 12-myristate 13-acetate induces downregulation of the CD4 receptor (11). The following antibodies were used: FITC-conjugated CD45 (clone T29/33, DAKO), mIgG1 FITC (clone MOPC-21, PharMingen), tumor necrosis factor-α FITC (clone Mab11, PharMingen), rIgG1 FITC (clone R3-34, PharMingen), IL-4 FITC (clone MP4-25D2, PharMingen), R-phycocerythrin (PE)-conjugated CD14 (clone TUK4), rIgG2a PE (clone R35–95, PharMingen), IL-2 PE (clone MQ1-17H12, PharMingen), mIgG1 (clone TC647, DAKO), and IFN-γ PE (clone 4S.B3, PharMingen). PE covalently linked to Texas red ethyl cysteinate dimer (ECD)-conjugated IgG1 (clone 679 1MC7 Coulter) and CD3 ECD (clone UCHT1, Coulter). PE covalently linked to Cy5 (Cy5)-conjugated IgG1 (clone DAK-GO1, DAKO) and CD8 Cy5 (clone DK25, DAKO).

**Extracellular cytokine measurements.** Blood samples for cytokine measurements (IL-6, IL-12) were drawn into precooled glass tubes containing EDTA. The tubes were spun immediately at 2,200 g for 15 min at 4°C. The plasma was stored at −80°C until analyses were performed. Enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems (Minneapolis, MN) were used. According to R&D Systems, the IL-6 ELISA kit is insensitive to the addition of the recombinant forms of the soluble receptor (sIL-6R), and the measurements, therefore, correspond to both soluble and receptor-bound cytokine. The intra-assay coefficient of variation (CV) for the IL-6 kit was 5.9%.

**Measurements of epinephrine.** Blood samples for measurements of epinephrine were drawn into ice-cold glass tubes containing glutathione (1.3 mg/ml blood) and EGTA (1.5 mg/ml blood) with a pH of 6–7 and spun immediately. Plasma was stored at −80°C until analyzed by high-performance liquid chromatography (Hewlett-Packard HPLC, Waldbronn, Germany) with electrochemical detection.

**Measurements of cortisol.** Blood samples for cortisol measurement were drawn into precooled glass tubes containing EDTA. The tubes were spun immediately at 2,200 g for 15 min at 4°C. The plasma was stored at −80°C until analyses were performed. For cortisol measurement, ELISA kits from Diagnostic Laboratories (Webster, TX) were used (DSL-10-2000). The intra-assay CV was 5%.

**Statistics.** None of the intracellular cytokine data were distributed normally; therefore, these data are presented as medians and quartiles. Changes over time were tested using a nonparametric Friedman test; if this was significant, a pairwise comparison was done using a nonparametric Wilcoxon test.

Data showing a normal distribution, i.e., lymphocyte concentrations and log plasma IL-6, log plasma epinephrine, and log plasma cortisol, are shown as means and SE. The normally distributed data were tested for changes over time using repeated-measurements ANOVA. Pairwise comparisons were done using a paired t-test with Bonferroni correction. A Spearman rank data correlation analysis was performed to test for correlations. \( P < 0.05 \) was accepted as significant.

J Appl Physiol • VOL 91 • OCTOBER 2001 • www.jap.org
The median running speed was 12.6 km/h (range = 10–15.1 km/h) at 75 ± 1% (SE) VO\textsubscript{2}max. The total number of circulating lymphocytes, CD4\textsuperscript{+}, and CD8\textsuperscript{+} T cells increased in response to exercise and declined below preexercise values in the recovery period (Fig. 1). The percentage of circulating CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells producing IFN-\gamma decreased by almost 50% after exercise and remained low 2 h postexercise compared with preexercise values. Twenty-four hours later, the percentage of the IFN-\gamma-producing CD8\textsuperscript{+} T cells was back to preexercise values, whereas the percentage of the IFN-\gamma-producing CD4\textsuperscript{+} T cells remained low (Fig. 2A). The percentage of IL-2-producing CD4\textsuperscript{+} T cells in the circulation was slightly increased after 30 min of exercise. The percentage of both CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells producing IL-2 decreased by almost 50% at the end of exercise and remained low, compared with preexercise values, 2 h after exercise but had returned to preexercise values 24 h later (Fig. 2B). The percentage of CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells in the circulation-producing IL-4 did not change in response to exercise (Fig. 3). Plasma IL-6 was increased by ~30-fold at the end of exercise and declined toward preexercise values in the recovery period (Fig. 4). Plasma IL-12 was below detection limits (n = 4). Plasma cortisol increased ~1.7-fold in response to running and declined toward preexercise values in the recovery period (Fig. 5). Plasma epinephrine increased approximately threefold during exercise, and mean plasma epinephrine measured after 0.5, 1.5, and 2.5 h of running was 0.76 ± 0.1 nmol/l (Fig. 6).

Correlations. Mean plasma epinephrine during running correlated negatively with the percentage of circulating IL-2-producing CD8\textsuperscript{+} T cells 2 h postexercise (r = −0.717; P < 0.05). Peak plasma IL-6 correlated with the percentage of IL-4 producing CD8\textsuperscript{+} T cells in the circulation immediately after exercise (r = 0.686; P < 0.05) and 2 h postrunning (r = 0.783; P < 0.05). Plasma cortisol did not correlate with the percentage of type 1 T cells in the circulation. Peak plasma IL-6 positively correlated with plasma cortisol 1 h postrunning (r = 0.703; P < 0.05).
Epinephrine and percentage of IL-2-producing CD8 T cells were correlated with exercise in this study, a negative correlation between mean plasma IL-6 found in this study did not increase the inflammatory response in muscles (7). Therefore, a larger increase in type 1 T cell immigration into the muscles postexercise compared with other CD3^+ T cells might explain the decrease in the percentage of type 1 T cells.

Tsios et al. (16) demonstrated that infusion of recombinant human IL-6 into humans increases the levels of glucocorticoids. IL-6 probably functions through the hypothalamic-pituitary-adrenal axis, thereby increasing secretion of cortisol. In accordance, this study demonstrated a correlation between peak plasma IL-6 and plasma cortisol 1 h postrunning.

The relatively more pronounced decrease in type 1 compared with type 2 T cells in the recovery period may explain the increased sensitivity to URTI following strenuous exercise, as these infections are often caused by viruses.

In conclusion, the present study demonstrates that the postexercise decrease in T lymphocyte number is accompanied by a more pronounced decrease in type 1 T cells. Although the correlational relationship should be taken with some caution (the r values are high, but the n values are only 9), our data suggest that the increase in plasma epinephrine during exercise contributes to the suppression of IL-2-producing T cells and that high plasma IL-6 helps to maintain the IL-4-producing T cells in the circulation. Thus epinephrine and IL-6 may participate in the exercise-induced shift toward a relative type 2 T cell dominance. However, other physiological changes, such as local inflammation, may also play a role. In addition,

![Fig. 5. Plasma cortisol measured before, during, and after 2.5 h of treadmill running. Values are means ± SE (n = 9 subjects). *Significantly different from preexercise values (P < 0.05).](image)

![Fig. 6. Plasma epinephrine measured before, during, and 2.5 h at the end of treadmill running. Values are means ± SE (n = 9 subjects). *Significantly different from preexercise values (P < 0.05).](image)
the present study supports the idea that an exercise-induced increase in plasma IL-6 induces elevated levels of plasma cortisol.

The excellent technical assistance by R. Rousing, H. Willumsen, and B. Mollerup is acknowledged. The study was supported by The Danish National Research Foundation (Grant 504-14) and a scholarship from The Danish Medical Research Council.

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