Effect of glutamine supplementation on exercise-induced changes in lymphocyte function

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EXERCISE INDUCES NUMEROUS EFFECTS on the immune system. In essence, a bout of exercise induces mobilization of immunocompetent cells to the circulation. After strenuous exercise the lymphocyte count declines, and lymphocyte proliferative responses and cytotoxic activity of natural killer (NK) and lymphokine-activated killer (LAK) cells decrease (10).

The mechanisms underlying exercise-associated immune changes are multifactorial and include neuroendocrinological factors such as epinephrine, norepinephrine, growth hormone, cortisol, and β-endorphin (24) as well as physiological factors such as increased body temperature during exercise (11).

Metabolic changes during exercise may also play a role. Declined glutamine concentration in plasma as a result of muscular activity has been suggested to influence lymphocyte function (17), and a decreased level of plasma glucose has been suggested to increase stress-hormone levels and thereby influence immune function (20). The purpose of the present study was to explore the possibility that glutamine supplementation might abolish exercise-induced immunosuppression.

It has been established that cells of the immune system obtain their energy by metabolism of glucose. However, it is also known that glutamine constitutes an important fuel for lymphocytes, macrophages, and neutrophils (15, 16, 21). Several lines of evidence suggest that glutamine is used at a very high rate by these cells, even when they are quiescent (16). It has been proposed that the glutamine pathway in lymphocytes may be under external regulation, due partly to the supply of glutamine itself (1).

Skeletal muscle is the major tissue involved in glutamine production and is known to release glutamine into the blood stream at a high rate. It has been suggested that skeletal muscle plays a vital role in maintaining the plasma glutamine concentration, thus affecting the availability of glutamine to the immune cells. Consequently, the activity of the skeletal muscle may directly influence the immune system. It has been hypothesized (the so-called “glutamine hypothesis”) that under intense physical exercise, the demands on muscle and other organs for glutamine is such that the lymphoid system may be forced into a glutamine debt, which temporarily affects its function. Thus factors that directly or indirectly influence glutamine blood levels could theoretically influence the function of lymphocytes, neutrophils, and monocytes (15, 16, 21). After intense long-term exercise and other physical stress conditions, the glutamine concentration in plasma declines (3, 4, 7, 12, 14, 22). In conditions where the

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plasma glutamine concentration is decreased, provision of glutamine could be advantageous for cells of the immune system. Several studies (8, 32, 35) have examined the effect of glutamine, as a part of total parenteral nutrition, on cells of the immune system. In humans, it was shown that glutamine-enriched intravenous feeding to patients with hematological malignancies in remission decreased the amount of positive microbial cultures and diminished the number of clinical infections (32). In septic rats, it was shown that glutamine supplemented total parenteral nutrition, partially prevented the decrease in lymphocyte blastogenesis, and increased the phagocytic index compared with standard parenteral nutrition (35). Fahr et al. (8) showed that oral glutamine supplementation of tumor-bearing rats decreased the tumor growth, which was associated with an increase in LAK cell activity.

In vitro, optimal lymphocyte proliferation is dependent on the presence of glutamine (23, 29, 31). Furthermore, Rohde et al. (31) showed that the presence of glutamine augmented the LAK cell activity in vitro, and in relation to strenuous exercise, the time course of changes in serum glutamine was paralleled by changes in LAK cell activities (29). In two recent placebo-controlled glutamine intervention studies (28, 30), glutamine was given postexercise. In the latter studies, glutamine abolished the exercise-induced decline in glutamine but did not abolish the postexercise decline in immune function. However, the possibility that the level of glutamine had declined in compartments other than blood during exercise cannot be excluded. In the present study, therefore, glutamine supplementation was given during as well as after exercise to investigate the hypothesis that glutamine given throughout the exercise would be able to restore postexercise impairment of the immune system.

MATERIALS AND METHODS

Subjects. The experimental protocol was approved by the ethical committee of Copenhagen Community, and written informed consent was obtained from all subjects. Ten healthy, elite athletes of ages 25–48 yr (mean age 37 yr) with a maximal oxygen consumption (V\textsubscript{\text{O2 max}}) of 47.4–68.4 ml·min\textsuperscript{-1}·kg\textsuperscript{-1} (mean V\textsubscript{\text{O2 max}} 59.6 ml·min\textsuperscript{-1}·kg\textsuperscript{-1}) participated in the study.

Experimental design. Each subject performed two exercise trials separated by 2 wk. At each experimental day the subjects reported to the laboratory at 8:00 AM, after an overnight fast. The subjects were told to avoid strenuous exercise the day before the trial and were not allowed to perform any exercise 8 h before the trial. At the first appointment, a training and diet history was obtained, and subjects were asked to keep the same scheme of training and dieting before the second trial. Furthermore, any disease presenting within 1 wk before or during the experiment excluded the subject.

Three days before their first trial, the subjects performed a graded exercise test determining V\textsubscript{\text{O2 max}} and maximal heart rate. The test was carried out on the same Krogh bicycle ergometer used in the experiments. Oxygen uptake and ventilation were measured with the MedGraphics CPF-S and CPX metabolic system (St. Paul, MN). Heart rate was measured with the Polar advantage NV system (Kepele, Finland). The subjects cycled for 2 h at 75% V\textsubscript{\text{O2 max}} on a Krogh ergometer cycle. Oxygen uptake was measured three times during the trial, and if necessary, the load was adjusted to reach the desired workload. Heart rate was continuously monitored throughout the exercise. During the 4-h postexercise phase, the subjects were asked to keep sedentary but awake.

Supplementation. The study used a randomized, double-blind, placebo-controlled crossover design. The subjects consumed either isocaloric L-glutamine or maltodextrin (placebo) beverages. The placebo drink was based on maltodextrin, and the dosage was chosen to be isoenergetic with the glutamine supplementation. The total carbohydrate was only ~10% of the dosage used in classic carbohydrate supplementation studies (19). After 60 min of exercise, the subjects consumed 0.5 liter of an aqueous solution of either 3.5 g of glutamine or 3.5 g of maltodextrin; the subsequence four doses of the beverage were ingested at intervals of 45 min. The intervals were based on pilot studies showing that this supplementation protocol would give stable plasma glutamine concentrations. Subjects were asked to finish each drink within 4 min. The beverages were prepared in the morning by adding 50–60°C hot water to bags containing either glutamine or placebo provided by the Nestlé Research Center (Lausanne, Switzerland). The beverages were identical in appearance and taste. After having consumed the fifth beverage, the subjects were offered a standardized meal consisting of ~200 g of white bread, 65 g of cheese, 150 g of tomato, 150 g of cucumber, 50 g of lettuce, and 1 banana. Subjects were allowed to drink water ad libitum.

Blood samples. Blood samples were obtained from an antecubital vein at rest before the exercise (start), immediately after the exercise (finish), and 2 h postexercise.

Isolation of blood mononuclear cells. Blood mononuclear cells (BMNC) were extracted from heparinized blood by using density gradient centrifugation (Lymphoprep; Nyegaard, Oslo, Norway) on Leucosep tubes (Greiner, Frickenhausen, Germany). After isolation, cells were washed three times in RPMI 1640 (GIBCO, Grand Island, NY).

Freezing and thawing of BMNC. BMNC were frozen in a medium consisting of 50% RPMI, 30% fetal calf serum (FCS; GIBCO), and 20% DMSO (Bie and Berntsen, Redovre, Denmark) and were kept in liquid nitrogen until thawed for analysis. BMNC were thawed in a water bath at 37°C and washed twice immediately afterward in RPMI containing 10% FCS. Viable cells were counted, and the cell concentration was adjusted to 5 × 10\textsuperscript{6} cells/ml with RPMI medium containing 10% FCS. BMNC from both trials for each subject were thawed and analyzed at the same time to eliminate interassay variability between samples. Assays of proliferation, NK and LAK cell activity, and labeling of cell surface markers were performed simultaneously.

Proliferation assay. The BMNC proliferation assay was performed in triplicate in microtiter plates with U-shaped wells (NUNC, Roskilde, Denmark). BMNC were resuspended in RPMI containing 10% normal human serum. Cells (3 × 10\textsuperscript{4} cells/ml) were cultured for 72 h at 37°C, 5% CO\textsubscript{2} with either isotonic NaCl, physohemagglutinin (PHA; 20 μg/ml; Difco Laboratories, Detroit, MI), or interleukin (IL)-2 (Boehringer Mannheim, Mannheim, Germany). During the last 24 h of the culture period, the cells were exposed to [\textsuperscript{3}H]thyidine (NEN, Boston, MA). The cell cultures were collected on glass fiber filters with a harvesting machine (Micromat 196; Packard), and incorporation of [\textsuperscript{3}H]thyidine into the DNA of the cells was measured in a beta counter (Harvester, Matrix 96; Packard). For each triplicate, the mean count per minute was recorded.
NK cell activity. The activity of NK cells was measured using K562 tumor target cells in a 51Cr release assay. Triplicates of 100 µl of BMNC (effector cells) and 100 µl of target cells (1 × 10⁶ cells/ml) were incubated in microtiter plates (NUNC) with U-shaped wells for 4 h at 37°C and 5% CO₂. Effector cells were added in different concentrations giving effector-to-target cell (E/T) ratios of 50:1, 25:1, 12.5:1, and 6.25:1. After incubation, the plates were centrifuged at 700 g for 5 min, 100 µl of supernatant were transferred from each well to new tubes, and radioactivity was determined in a gamma counter. Spontaneous release of 51Cr from the target cells was determined by incubating 100 µl of target cells with 100 µl of medium. Maximum release was determined by incubation of 100 µl of target cells with 100 µl of medium containing 10% Triton X-100 (Sigma Chemical, St. Louis, MO). The activity of NK cells was determined as the percentage of lysis, calculated by the formula

\[
\text{Percent lysis} = 100 \times \left( \frac{\text{Test } 51\text{Cr} - \text{spontaneous } 51\text{Cr}}{\text{Maximum } 51\text{Cr} - \text{spontaneous } 51\text{Cr}} \right)
\]

\[
\times \frac{T}{X_p}
\]

where T is the number of target cells, p is the reference lysis level, and X_p is the E/T ratio required to lyse p% of the targets. In this case, T = 10⁷ cells and p = 25%.

LAK cell activity. BMNC were incubated with interleukin-2 (IL-2, Proleukin; Chiron, Emeryville, CA) in quadruplicate in flat-bottomed microtiter plates (NUNC) for 48 h at 37°C and 5% CO₂. The final concentration of IL-2 in the wells was 6 × 10⁻⁵ U/ml and 1 × 10⁵ BMNC/ml. The activity of LAK cells was measured in a 51Cr release assay using DAUDI target cells. Target cells (100 µl) at a concentration of 2 × 10⁴ cells/ml and 100 µl of effector cells in various concentrations were added to the wells in microtiter plates giving E/T ratios of 50:1, 25:1, 12.5:1, and 6.25:1. The assay was carried out to completion as described for the NK cell activity assay.

Flow cytometry. The following anti-human mouse monoclonal antibodies were used: fluorescein isothiocyanate (FITC)-conjugated antibodies anti-CD16 (clone NKP15) and anti-TCRβ (clone WT31), purchased from Becton Dickinson (San José, CA); and anti-CD4 (clone MT310), anti-CD14 (clone CD28.1), and anti-CD95 (clone DX2), purchased from Becton Dickinson, and anti-CD56 (clone MY31), anti-TCRgδ (clone HD37), purchased from DAKO. Peridinin-chlorophyll protein-conjugated antibodies included an IgG1 negative control (clone DAK-G01) dual-color reagent conjugated with FITC and R-phycocerythrin (R-PE). R-PE-conjugated single-color antibodies included anti-CD56 (clone MY31), anti-TCRβ (clone 11F2), anti-CD28 (clone CD28.1), and anti-CD95 (clone DX2), purchased from Becton Dickinson, and anti-CD45RA (clone 4KB5) and anti-CD19 (clone HD37), purchased from DAKO. Peridinin-chlorophyll protein-conjugated antibodies included an IgG1 negative control (clone X40), anti-CD3 (clone SK7), and antiCD8 (clone SK1), all from Becton Dickinson.

BMNC were resuspended in PBS (JT Baker, Deventer, Holland) containing 3% FCS, and labeled antibodies in excess were added to 100 µl of cell suspension (1 × 10⁶ cells). After incubation in the dark for 30 min at 4°C, the cells were washed twice in PBS containing 3% FCS. After resuspension in 300 µl of washing buffer, the labeled cells were analyzed by flow cytometry using a fluorescence-activated cell sorter analyzer (Epics XL-MCL; Coulter, Hialeah, FL). The subsequent computer analysis was carried out by personal computer lysis software from Becton Dickinson. A lymphocyte gate was used for all analyses. The percentages of CD4 and CD8 were determined as a mean of three and two samples, respectively. The absolute number of cells of a given subpopulation was calculated by multiplying the percentage of the subpopulation made from the total lymphocyte number by the lymphocyte cell count.

Catecholamines. Concentrations of epinephrine and norepinephrine were measured in EDTA- (1.5 mg/ml blood) and glutathione-treated plasma (reduced, 1.3 mg/ml blood) (Boehringer Mannheim) by high-performance liquid chromatography (HPLC; Hewlett-Packard) with electrochemical detection.

Amino acids. Blood was drawn into glass tubes containing EDTA and centrifuged at 2,500 g for 15 min at 4°C. Plasma was stored at −80°C (for a maximum of 6 mo) and analyzed by HPLC.

Clinical chemistry analyses. These tests were carried out using standard laboratory procedures at the Department of Clinical Chemistry, University Hospital of Copenhagen, Denmark. Blood was drawn into tubes containing EDTA for estimation of the concentration of erythrocytes, hemoglobin, glucose, lymphocytes, monocytes, neutrophil granulocytes, and total blood leukocytes. The analyses were carried out with a cell counter (Technicon H.I.; Miles, Tarrytown, NY).

Muscle enzymes were measured in lithium-heparinized plasma using automated enzyme reactions (Hitachi System 717; Boehringer Mannheim Diagnostica; Mannheim, Germany). Corrections were made for changes in the plasma volume according to the method described by Dill and Costill (5a).

Statistical analyses. To test whether the measured parameters were influenced by time and interaction between time and treatment, we carried out a special case of the three-way analysis of variance (ANOVA); because the measurements at different times were on the same subjects and each subject served as its own control (the same subjects repeated the exercise protocol 2 times), a paired, repeated-measures design was employed. The employed model is

\[
Y_{ijk} = \mu + tr_i + s_j + t_k + (tr \times t)_{jk} + (s \times t)_{jk} + E_{ijk}
\]

where Y denotes the dependent variable, µ is the overall mean, and the main effects are treatments (tr), subjects (s), and time (t). Indices i, j, and k indicate the level of the respective factors. Interactions between main effects are indicated by ‘*’. E signifies the remaining (residual) variation that is not explained by the effects of the model. When no significant difference between the two treatments was found, the interaction between time and treatment was considered as if arising from only one treatment. In this case, differences in time are indicated for both treatments in Tables 1–4. If an interaction between time and treatment was significant, a paired t-test was performed at each time point. If a significant effect was found for time, the time trend in relation to the preexercise value was further analyzed with a Bonferroni-adjusted Student’s paired t-test.

In all cases the untransformed data proved to meet the requirements of normal distribution. Statistical calculations were performed using SYSTAT statistical software (v7.0). In all tests P ≤ 0.05 was considered significant.

RESULTS

Exercise characteristics. Oxygen consumption, heart rate, and workload of subjects did not differ between the two exercise trials. Average oxygen consumption, heart rate, and workload were respectively 74, 76, and 69% of maximal values obtained during the preliminary tests (data not shown).
Effect of exercise. The concentration of plasma glutamine significantly decreased 15% 2 h after exercise in the placebo group, whereas the glutamine level did not change in the glutamine trial group (Fig. 1). The individual levels of several amino acids besides glutamine significantly decreased 15% 2 h after exercise from trials where subjects received either placebo (P) or glutamine (S). Data are reported as means ± SD for each group. *Significantly different from start (P ≤ 0.01). #Significant interaction between time and treatment as determined by ANOVA (P ≤ 0.05).

Concentrations of leukocyte subpopulations. The concentrations of total leukocytes, neutrophils, and monocytes were elevated during and immediately after exercise compared with preexercise values, whereas the lymphocyte count increased during exercise and declined below preexercise values 2 h postexercise (Table 1). The concentrations of lymphocyte subpopulations in relation to exercise did not differ between the supplementation and placebo trials. Thus only the data from the placebo trial is shown (Table 2). The total CD3+ cell concentration increased at the end of exercise due to an increase in both CD3+TCRβ+ and CD3+TCRδ+; the concentrations of CD3+, CD3+TCRβ+, and CD3+TCRδ+ decreased 2 h postexercise compared with preexercise values. The concentrations of total CD8+, CD8+CD45RA−naive, CD8+CD45RA−memory, CD8+CD28−, CD8+CD95−, and CD8+CD95− cell subpopulations increased at the end of exercise and decreased 2 h postexercise. The concentrations of all NK cell subpopulations (CD3−CD16−CD56+, CD3−CD16+CD56+, and CD3−CD16+CD56−) increased during exercise and declined below preexercise values 2 h postexercise. Regarding the concentrations of CD4+ cell subpopulations, only CD4+CD95− and CD4+ cells lacking CD28 or CD45RA on the surface increased in response to exercise. However, the latter two subsets did not decrease postexercise, in contrast to the CD4+CD45RA+, CD4+CD28+, CD4+CD95−, and CD4+CD95− cell subpopulations and the total CD4+ cells. The CD19+ B cells increased during exercise but returned to preexercise values within 2 h of rest.

Lymphocyte function. The NK and LAK cell activity and the proliferative responses did not differ between the two trials. Thus only data from the placebo trial are shown in Table 3. The NK cell activity increased in response to exercise when expressed as lytic units (LU)

![Fig. 1. Plasma glutamine concentration (in μM) in relation to 2 h of bicycle exercise at 75% maximum O2 consumption. Subjects (n = 9) were supplemented with placebo (P) or glutamine (S). Data are reported as means ± SD for each group. *Significantly different from start (P ≤ 0.01). #Significant interaction between time and treatment as determined by ANOVA (P ≤ 0.05).](http://ajpcell.physiology.org/download/doi/10.1152/ajpcell.00010.2001/DC1/C1262_1-C1262_1.jpg)

**Table 1. Concentration of leukocytes, lymphocytes, neutrophils, and monocytes measured in blood from trials where subjects received either glutamine or placebo**

<table>
<thead>
<tr>
<th></th>
<th>Start</th>
<th>Finish</th>
<th>2 h Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>5.2±1.0</td>
<td>14.0±4.5*</td>
<td>14.4±4.1*</td>
</tr>
<tr>
<td>P</td>
<td>5.6±1.4</td>
<td>16.3±4.9*</td>
<td>14.3±3.6*</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>2.0±0.5</td>
<td>3.1±1.3*</td>
<td>1.4±0.5*</td>
</tr>
<tr>
<td>P</td>
<td>1.9±0.5</td>
<td>3.2±1.2*</td>
<td>1.3±0.8*</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>9.8±3.7†</td>
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</tr>
<tr>
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<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.8±0.3*</td>
</tr>
<tr>
<td>P</td>
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<td>0.9±0.3*</td>
<td>0.7±0.3*</td>
</tr>
</tbody>
</table>

Values are concentrations (billion cells/l) reported as means ± SD; n = 10 subjects. *Significantly different from start (P ≤ 0.001). †Significant interaction between time and treatment (P ≤ 0.05) as determined by ANOVA in a paired, repeated-measures design. A pairwise comparison at each time point reveals a significant difference (P = 0.022) at finish. However, this difference is only borderline significant (P = 0.005) when adjusted data are adjusted for multiple comparisons. S, glutamine supplement; P, placebo.

**Table 2. Concentration of lymphocytes and monocytes in whole blood from subjects receiving placebo**

<table>
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<th>Start</th>
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<td>CD3+</td>
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<td>1.6±0.4†</td>
<td>0.9±0.6†‡</td>
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<td>0.3±0.4*</td>
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</tr>
<tr>
<td>CD4+</td>
<td>0.8±0.3</td>
<td>0.9±0.3</td>
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</tr>
<tr>
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<td>0.5±0.2</td>
<td>0.4±0.3*</td>
</tr>
<tr>
<td>CD4+CD45RA−</td>
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<td>0.9±0.3</td>
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<td>0.2±0.1†</td>
<td>0.1±0.0†</td>
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<tr>
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<td>0.3±0.2†</td>
<td>0.1±0.1‡</td>
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<td>0.6±0.4†</td>
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<tr>
<td>CD3−</td>
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<td>CD3−CD16+CD56−</td>
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<td>CD3−CD16−CD56+</td>
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<td>CD19+</td>
<td>0.1±0.1</td>
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</tr>
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</table>

Values are concentrations (billion cells/l) reported as means ± SD; n = 9 subjects unless indicated otherwise. *Significantly different from start (P ≤ 0.05). †Significantly different from start (P ≤ 0.01). ‡Significantly different from start (P ≤ 0.001). §Data are reported from n = 8 subjects.
and lymphocyte trafficking. During glutamine supplementation, however, the exercise-induced increase in neutrophil count was slightly lower. The exercise-induced increase in growth hormone and catecholamines is thought to mediate the exercise-induced neutrocytosis (25). In line with this suggestion, there was a slight trend toward a lower level in growth hormone and epinephrine at the end of exercise in the glutamine group compared with the placebo group. However, these differences between treatments were not significant. Nieman et al. (19) found that carbohydrate supplementation affects neutrophil trafficking. In the present study, the glutamine supplementation was compared with carbohydrate-containing placebo. Thus the effect of glutamine supplementation on neutrophils might have been confounded by the carbohydrate in the placebo. The effect on neutrophils could not be ascribed to an effect of increased carbohydrate or energy supply because the placebo and glutamine supplementations were isocarbohydrated and isoenergetic.

The present study describes several new findings regarding exercise effects on lymphocyte subpopulations. The NK cell subsets were more sensitive to exercise stress than any other cell subtypes, and among the heterogeneous population of NK cells, the CD3+CD16+CD56+ cells were recruited in the highest number. Among total CD3+ T cells, cells expressing TCRαβ and TCRγδ were both recruited to the circulation in response to exercise. The CD8+ cells increased more than the CD4+ cells in response to exercise, and it appeared that within the CD4+ subset, only CD4+ cells lacking CD45RA, CD28, and CD95 (Fas) were recruited.

Furthermore, although all CD8+ cell subpopulations were mobilized to the blood, especially cells lacking CD45RA, CD28, and CD95 (Fas) were mobilized in response to exercise. Thus memory cells (defined as

and at all E/T ratios and declined at E/T ratios of 25:1 and 50:1 after 2 h of rest. The LAK cell activity increased during exercise only when expressed as LU and declined below preexercise values 2 h postexercise at E/T ratios of 1:6.25, 1:12.5, and 1:25 and when expressed as LU. The PHA-stimulated proliferative response was decreased immediately after exercise, whereas the IL-2-stimulated response declined 2 h postexercise compared with preexercise values. The unstimulated control did not change.

Glucose, insulin, epinephrine, norepinephrine, and growth hormone. The glucose and insulin levels decreased in response to exercise and were below preexercise values immediately after and 2 h postexercise (Table 4). The levels of epinephrine, norepinephrine, and growth hormone were increased after exercise. The epinephrine level was still slightly elevated after 2 h of rest, whereas the level of norepinephrine had returned to preexercise values. The growth hormone level was slightly below preexercise levels 2 h postexercise. The results did not differ between the two groups.

Effect of supplementation. The neutrophil count was significantly less enhanced at the end of exercise in the glutamine group compared with the placebo group (Table 1).

**DISCUSSION**

The main finding of the present study was that glutamine supplementation during and after 2 h of strenuous bicycle exercise abolished the exercise-induced decline in plasma concentration of glutamine without abolishing the exercise-induced changes in cytotoxic activity, lymphocyte proliferative responses,

and lymphocyte trafficking. During glutamine supplementation, however, the exercise-induced increase in neutrophil count was slightly lower. The exercise-induced increase in growth hormone and catecholamines is thought to mediate the exercise-induced neutrocytosis (25). In line with this suggestion, there was a slight trend toward a lower level in growth hormone and epinephrine at the end of exercise in the glutamine group compared with the placebo group. However, these differences between treatments were not significant. Nieman et al. (19) found that carbohydrate supplementation affects neutrophil trafficking. In the present study, the glutamine supplementation was compared with carbohydrate-containing placebo. Thus the effect of glutamine supplementation on neutrophils might have been confounded by the carbohydrate in the placebo. The effect on neutrophils could not be ascribed to an effect of increased carbohydrate or energy supply because the placebo and glutamine supplementations were isocarbohydrated and isoenergetic.

The present study describes several new findings regarding exercise effects on lymphocyte subpopulations. The NK cell subsets were more sensitive to exercise stress than any other cell subtypes, and among the heterogeneous population of NK cells, the CD3+CD16+CD56+ cells were recruited in the highest number. Among total CD3+ T cells, cells expressing TCRαβ and TCRγδ were both recruited to the circulation in response to exercise. The CD8+ cells increased more than the CD4+ cells in response to exercise, and it appeared that within the CD4+ subset, only CD4+ cells lacking CD45RA, CD28, and CD95 (Fas) were recruited.

Furthermore, although all CD8+ cell subpopulations were mobilized to the blood, especially cells lacking CD45RA, CD28, CD45RA and CD95 (Fas) were mobilized in response to exercise. Thus memory cells (defined as

and at all E/T ratios and declined at E/T ratios of 25:1 and 50:1 after 2 h of rest. The LAK cell activity increased during exercise only when expressed as LU and declined below preexercise values 2 h postexercise at E/T ratios of 1:6.25, 1:12.5, and 1:25 and when expressed as LU. The PHA-stimulated proliferative response was decreased immediately after exercise, whereas the IL-2-stimulated response declined 2 h postexercise compared with preexercise values. The unstimulated control did not change.

Glucose, insulin, epinephrine, norepinephrine, and growth hormone. The glucose and insulin levels decreased in response to exercise and were below preexercise values immediately after and 2 h postexercise (Table 4). The levels of epinephrine, norepinephrine, and growth hormone were increased after exercise. The epinephrine level was still slightly elevated after 2 h of rest, whereas the level of norepinephrine had returned to preexercise values. The growth hormone level was slightly below preexercise levels 2 h postexercise. The results did not differ between the two groups.

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CD45RO+ or CD45RA−) and not naive (CD45RA+) cells increased in response to exercise. Cell cultures of CD8+ T cells that have reached replicative senescence after multiple rounds of cell division lack expression of the CD28 costimulatory molecule (6). Thus the initial increase in CD4+ and CD8+ cells after exercise is not likely to represent repopulation by newly generated cells but may be a redistribution of activated cells, in agreement with kinetics of CD4+ repopulation after anti-HIV (human immunodeficiency virus) treatment (13) and chemotherapy (9) and CD4 and CD8 repopulation after bone marrow transplantation (2). When a fixed number of BMNC was stimulated with mitogen (PHA), the proliferative response declined in response to exercise in agreement with previous findings (18). The explanation of the decreased PHA response could be that PHA preferentially stimulated CD4+ cells and that the percentage of these cells declined during exercise, because the number of CD8+ and CD16− cells increased more. Furthermore, the novel finding that cells that have reached their end-proliferative stage constitute a larger proportion among BMNC after exercise than before also explains the declined proliferative response. The NK and LAK cell activities are mediated by a heterogeneous population of cells, but exercise than before also explains the declined proliferative capacity.

During the past few years, attempts have been made to identify nutritional supplements that could abolish exercise-induced immune changes (26). Until now, only carbohydrate loading has demonstrated significant findings (26). Thus it has been demonstrated that carbohydrate loading diminishes exercise-mediated effects on neutrophil and lymphocyte trafficking as well as levels of stress hormones (19). Supplementation with antioxidant vitamins (27) or fish oil (33) have not demonstrated any effect on circulating proinflammatory cytokine, lymphocyte, or neutrophil numbers.

The previous finding of an effect of glutamine supplementation on upper respiratory tract infections (5) is not mechanistically explained by an effect of glutamine on lymphocyte function. Thus glutamine supplementation abolished exercise-induced decrease in plasma glutamine but not exercise-induced impaired lymphocyte function.

In conclusion, the present study demonstrated that exercise primarily induces mobilization of cells that have reached their replicative senescence. Furthermore, the study showed that glutamine supplementation during and after exercise abolishes the exercise-induced decline in plasma glutamine concentration without influencing the exercise-induced change in lymphocyte subpopulations, cytotoxic activity, or proliferative capacity. The finding that the exercise-induced neutrocytosis was less pronounced during glutamine supplementation is not likely to be of any clinical significance. Thus the present study adds to previous findings by Rohde et al. (28, 30) showing that glutamine supplementation during as well as after exercise has no effect on exercise-induced lymphocyte function and trafficking.

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REFERENCES


