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STRENUOUS EXERCISE AND IMMUNOLOGICAL CHANGES: A MULTIPLE-TIME-POINT ANALYSIS OF THE LEUKOCYTE SUBSESTS, CD4/CD8 RATIO, IMMUNOGLOBULIN PRODUCTION AND NK CELL RES

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P498766.PDF [Page: 3 of 12]

Strenuous Exercise and Immunological Changes:

A Multiple-Time-Point Analysis of Leukocyte Subsets, CD4/CD8 Ratio, Immunoglobulin Production and NK Cell Response

P. N. Shek[1,2,3], B. H. Sabiston[1], A. Buguet[4], M. W. Radomski[1,2]

[1] Defence and Civil Institute of Environmental Medicine, North York, Ontario, Canada
[2] Programme in Exercise Sciences, Graduate Department of Community Health, and
[3] Department of Clinical Biochemistry, Faculty of Medicine, University of Toronto, Canada
[4] Centre de Recherches du Service de Sainté des Armées Emile Pardé, La Tronche, France

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This study was designed to examine the impact of exhaustive endurance exercise on a number of immune parameters of physically fit male subjects ($\dot{V}O_2$max 66.5 ± 5.3 ml/min/kg) who performed treadmill exercise at 65 % of their $\dot{V}O_2$max for 120 min. Serial blood samples were taken before, during and after exercise and changes in leukocyte and lymphocyte subset concentrations; immunoglobulin production *in vitro;* and natural killer (NK) cell response were measured. The exercise regimen was found to induce the well-known phenomenon of leukocytosis which consisted primarily of a granulocytosis and lymphocytosis. Among the lymphocyte subsets, peripheral pan T cells ($CD3^+$) as well as helper ($CD4^+$) and suppressor ($CD8^+$) T cells were found to be elevated. A relatively smaller increase in $CD4^+$ than $CD8^+$ cells resulted in depressed CD4/CD8 ratios throughout the exercise period. After exercise, T cells declined progressively and, 2 h post-exercise, were less than 60 % of their pre-exercise level. In contrast, the CD4/CD8 ratio demonstrated a progressive increase, thus representing a reversal in the pattern observed during exercise and a trend towards an elevated ratio during recovery. B cells ($CD19^+$) were relatively unaffected by exercise, although IgM production by pokeweed mitogen-stimulated lymphocytes obtained from blood samples after 120 min of exercise was significantly depressed. NK cells were affected dramatically by exercise. Both $CD16^+$ cell numbers and NK cytotoxicity were increased during exercise, followed by a persistent depression in the post-exercise period. The strenuous exercise induced a profound effect on NK cells as evidenced by a 40 % depression of the NK cell count for as long as 7 days after the cessation of exercise. Our results provide direct kinetic evidence demonstrating that exhaustive exertion alters both lymphocyte distribution pattern and effector function, suggestive of possible exercise-induced immune compromise, particularly in the post-exercise recovery period.

■ Key words: Exercise, leukocytes, lymphocytes, immunoglobulin, B cells, T cells, NK cells, CD4/CD8 ratio

Introduction

Physical exertion has been shown to induce immunological changes which have been implicated as a possible explanation for increased susceptibility to illness and infections (18,22,43). Exercise-induced alterations in immune functions are usually preceded by the redistribution of leukocytes in the circulation, notably characterized by a granulocytosis and lymphocytosis (30,46). The two compartments of T and B lymphocytes constitute the key cornerstones of the immune system. T cells play a pivotal role not only in cell-mediated immunity, but also in synergizing with B cells in antibody production (28). Helper/inducer T cells, which recognize antigenic epitopes in the context of major histocompatibility complex (MHC) class II molecules, express the cell-surface associated CD4 phenotype (2). Cytotoxic/suppressor T cells, on the other hand, are characterized by the expression of CD8 antigen; $CD8^+$ T cells recognize foreign antigens in association with MHC class I molecules on transformed or virus-infected targets (4,10). It is believed that a proper balance of $CD4^+$ and $CD8^+$ cells is essential to maintain full immune competence of the host (5) and an improper balance may lead to disease (54). There have been many anecdotal reports linking physical activities to post-exercise infections (43) and an imbalance in the helper/suppressor (CD4/CD8) ratio has been implicated as the possible culprit (13).

One of the earliest studies on exercise-induced changes in lymphocyte distribution was conducted by Steel et al. (48) who demonstrated that a brief (10 min) exercise was sufficient to cause a significant lymphocytosis, primarily due to an elevation of B cells with no appreciable changes in T cells. A decrease in the CD4/CD8 ratio immediately after exercising for 15, 45, 60 and 120 min has been reported (19,34,37,42). All these reported studies typically compared the change in CD4/CD8 ratio before and immediately after exercise. More recently, using the indwelling-catheter technique for multiple blood sampling, the concomitant elevation of CD4 and CD8 cell concentrations during and immediataely after 60 min of exercise at 60 % $\dot{V}O_2$max has been reported (46). Because of a relatively higher increase in $CD8^+$ cell count, a corresponding decrease in the CD4/CD8 ratio was observed at the mid-point (30 min) of exercise. A multiple-time-point analysis of the T cell sub-populations during and after an exercise bout longer than 60 min in duration has not been well studied.

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P498766.PDF [Page: 4 of 12]

fore, the 30 g of carbohydrate ingested prior to exercise appears to be adequate when a 1 h high-intensity model of endurance performance is used. This could explain the lack of additional benefit when carbohydrate was ingested during exercise.

It is also possible that the addition of CHO during high-intensity exercise did not provide any additional benefit due to a reduction in gastric emptying. Exercise does not delay gastric emptying rates up to 70–75% VO_2max, but does significantly reduce gastric emptying above this intensity (16). In the present study exercise intensity in the G/G treatment consistently exceeded 75% VO_2max. However, Bonen et al. (6) reported that blood lactate levels and carbohydrate oxidation were increased by >75% following CHO in glycogen depleted subjects during intense exercise. This suggests that with glycogen depletion an increased rate of blood glucose uptake by the muscle can occur.

In summary, results of the present study indicate that pre-exercise ingestion of 30 g of glucose polymer in a 10% solution results in less drop off in power output during 1 h of maximal effort cycle ergometer endurance performance. This was demonstrable only during the final 20-min of exercise. No further benefit is observed when the same amount of carbohydrate is also ingested every 15 min during exercise.

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Corresponding Author:

A. Weltman
Exercise Physiology Laborataoary
Memorial Gymnasium
Department of Human Services
Curry School of Education
University of Virginia
Charlottesville, VA 22903
U.S.A.

P498766.PDF [Page: 5 of 12]

Immunoglobulin production is the primary effector function of plasma cells which are descendants of B cells. There have been relatively few studies examining the impact of physical exercise on humoral immunity and B cell functions. Decreased salivary IgA levels were observed in competitive cross-country skiers after a race (51) and in university swimmers after a training session (49). *In vitro* immunoglobulin production by cultured lymphocytes obtained from subjects after a short (15 min) bout of exercise was found to be depressed (19). Peripheral blood B cell concentration has been reported to be either unchanged (42) or moderately elevated (46) after 60 min of exercise at about 60 % VO_2max; immunoglobulin production, however, was not measured in both studies. There have been very few studies relating exercise-induced changes in peripheral B cell concentration to associated immunoglobulin production.

Exercise-induced lymphocytosis invariably results in an altered distribution pattern of both T and B cells (44). Meaningful interpretation of T and B cell changes among most studies has been rendered difficult by mostly pre- and post-exercise blood sampling procedures, thus failing to provide conceivably important data during exercise (1,7,37,42,53). Among studies where a three-point (before, during and after exercise) analysis was performed, there were no prolonged follow-up recovery measurements of the exercise subjects (12,16).

Natural killer (NK) cells are large granular lymphocytes bearing the distinct cell surface-associated CD16 and/or CD56 markers without the co-expression of CD3 antigen (17,23) NK cells have the capacity to destroy a wide range of transformed or virus-infected targets of malignant or benign origin (20) and unlike cytotoxic T cells, NK cells can engage a target without the restriction of MHC recognition (31,32). It is believed that NK cells serve a crucial function in terms of immune surveillance against non-self and transformed targets (50).

Physical exercise has been shown to affect the distribution and cytotoxic function of circulating NK cells (26). The hallmark of the exercise-induced change is a highly reproducible elevation of NK cell concentration (3,8,11) and cytotoxic activity (6,55). Because of the experimental design to include only pre- and post-exercise blood sampling in most studies of NK cell changes, data on the status of NK cells during the mid-exercise period are relatively scarce (46). There have also been very few, if any, attempts to examine the full kinetics of exercise-induced effects on NK cells in the post-exercise recovery period. Such analysis is considered important in assessing the potential impact of prolonged and intense exercise on the NK effector function and surveillance capacity following strenuous physical exertion.

In this study, we examined the effect of strenuous exercise (at 65 % VO_2max for 120 min) on selected immune parameters – $CD3^+$ T cells, CD4/CD8 ratio, $CD19^+$ B cells, pokeweed-mitogen-stimulated immunoglobulin production *in vitro*, and NK cell status. The experimental protocol was designed to include a multiple blood-sampling scheme before, during and after exercise. Recovery blood samples were also taken 1 and 7 days post-exercise.

Materials and Methods

Subjects, exercise, and blood sampling

Six male volunteers (Age: 22± years, VO_2max: 66.5±5.3 ml/kg/min) were recruited for the experiment and the experimental protocol was approved by the Institute's Human Ethics Committee according to the principles set forth in the Declaration of Helsinki of the World Medical Association. Each subject ran on a treadmill at 65 % of his VO_2max for up to 120 min or until his core temperature reached 40 °C. The core temperature was monitored continuously with a rectal probe connected to a thermal monitor. Each subject drank 200 ml of mineral water every 20 min during the run. Heparinized blood samples (20 ml per sample) were collected, at preselected time-intervals, from an indwelling catheter in the antecubital vein of the arm of the subject.

Preparation of mononuclear cells from peripheral blood

Five ml of heparinized blood, mixed with an equal volume of phosphate-buffered saline (PBS), were layered over 5 ml of Ficoll-Paque (Pharmacia LKB, Uppsala, Sweden) and centrifuged at 400 g for 30 min at 20 °C. The mononuclear cell layer was removed and washed twice with 10 ml of RPMI 1640 culture medium (GIBCO, NY) supplemented with 10 % fetal calf serum, at 330 g for 10 min at 20 °C. The washed peripheral blood mononuclear cells (PBMC) were resuspended and the cell concentration determined by using an electronic cell counter (Royco Cell-Crit 920-A).

Quantitation of circulating leukocyte and leukocyte subpopulations

The leukocyte concentration in each blood sample was determined by using the automated Coulter JT Hematology System. The cell concentration of each leukocyte subpopulation, i.e., granulocyte, lymphocyte, or monocyte, was calculated using the corresponding percentage of total leukocytes for that subpopulation, determined by flow cytometry analysis as described below. The hemoglobin concentration of each blood sample, determined simultaneously by the same Coulter system, was used to calculate the corresponding change in blood volume according to the method of Dill and Costill (9). All concentrations of the blood cell parameters were adjusted for any changes in blood volume.

Determination of leukocyte and lymphocyte subsets by flow cytometry

Cell samples (100 µl whole blood or 50 µl PBMC at a concentration of 5×10^6 cells/ml) were each mixed with 10 µl of a monoclonal antibody conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE). All monoclonal antibody reagents were purchased from Immunotech S. A. (Marseille, France). After a 15-min incubation on ice in the dark, 2 ml of a FACS lysing solution (Becton Dickinson) were added to the staining tube and vortexed, and the mixture was left at room temperature for no more than 10 min in the dark to lyse the red cells. Non-lysed cells were centrifuged for 5 min at room temperature at 300 g; washed once with 2 ml of Cell Wash (Becton Dickinson); and centrifuged for 5 min at room temperature at 500 g. The resultant pellet was resuspended in

P498766.PDF [Page: 6 of 12]

0.3 ml of cold 1 % paraformaldehyde (Polysciences, Warnington, PA) in Cell Wash and stored in the dark at 4 °C for analysis by flow cytometry the next day.

Stained cell samples were analyzed by the use of a FACScan flow-cytometer (Becton Dickinson). The FACScan system was calibrated and optimized each day using in turn, CaliBrite beads (Becton Dickinson), an isotype negative control, and a CD4/CD8 double-stained sample. The data were acquired and analyzed with Consort 30 and LYSIS softwares (Becton Dickinson). Usually 10,000 cells were scanned per sample and the results were expressed as the percentage of cells yielding a specific fluorescent emission in a gated lymphocyte region. For the enumeration of $CD8^+$ cells, the gated lymphocytes were scanned for $CD8^{bright+}$ cells as previously described (21,35,41). The absolute lymphocyte subset count was derived by multiplying the percentage of cells by the total number of lymphocytes.

An unstained cell sample was also used to determine the proportions of three leukocyte subsets (granulocyte, lymphocyte, and monocyte), based on cell size and granularity, on a dot plot of forward versus side light-scattering. The wavelength of the FACScan laser was 488 nm.

Pokeweed mitogen-stimulated cell culture

Peripheral blood mononuclear cells, at a density of 1×10^6/ml, were cultured in 17×100 mm round-bottomed plastic tubes containing RPMI 1640 medium with 10 % heat-inactivated fetal calf serum (GIBCO) and 100 U Pen/Strep (GIBCO). The cultured cells were incubated at 37 °C in a humidified atmosphere of 5 % CO_2 and 95 % air. Each experimental culture was stimulated with an optimal dose (20 µg/ml) of pokeweed mitogen (PWM, GIBCO) and the control culture was incubated under identical conditions in the absence of PWM. After a culture period of 6 days, the culture tubes were centrifuged at 20 °C at 300 g and the supernatant was harvested and kept frozen at −70 °C.

Determination of immunoglobulin concentrations by enzyme linked immunosorbent assay (ELISA)

The concentrations of IgM, IgG and IgA in tissue culture supernatants were quantitated using the double-antibody ELISA method. All wells of a 96-well Immunlon 2U plate (Dynatech) were each coated with 100 µl of goat anti-human IgM, IgG or IgA antibodies (Cappell, Organon Teknika, Durham, NC) at a concentration of 400 ng/ml. The IgG antibody dilution was prepared with 0.02 M PBS, pH 7.3, while IgM and IgA antibodies were diluted with a 0.05 M bicarbonate buffer, pH 9.6. The antibody-coated plates were covered, incubated overnight at 4 °C in a humid chamber, and then washed 3 times with 0.15 M saline containing 0.05 % Tween 20 (Sigma Chemicals). Tissue culture supernatants and specific immunoglobulin standards were appropriately diluted in PBS containing 0.5 % bovine serum albumin (Sigma Chemicals), pH 7.4, before their separate additions, at a volume of 100 µl, to appropriate wells of the antibody-coated wells in quadruplicate. The reaction plates were covered and incubated for 1 h (IgG, IgM) or 2 h (IgA) in a humidified chamber at 37 °C. At the end of the incubation period, the plates were washed as described above. A volume of 100 µl of a corresponding peroxidase-conjugated antibody (Cappell), at an appropriate dilution (IgG 1 : 6,000, IgA 1 : 8,000, IgM 1 : 10,000), was added to each well. The treated plates were covered, incubated for 1 h at 37 °C in a humid chamber, and washed by the same procedure as above. The substrate, o-phenylenediamine dihydrochloride (OPD), was prepared just prior to use by dissolving a 30-mg OPD tablet (Sigma Chemicals) in 6 ml of distilled water containing 120 µl of 30 % H_2O_2. After the addition of 100 µl of the substrate solution to each well, the plates were covered and incubated in the dark for 30 min at room temperature. Rections were terminated by the addition of 50 µl of 1 N H_2SO_4 to each well and the optical density (at 492 nm) of the contents of each well was determined. The Behring ELISA II Processor (Behring Diagnostics) was used in this assay procedure for the addition of reagents, washing of all plates, and reading of optical densities.

Natural killer (NK) cell cytotoxic assay

NK cell activity of PBMC was measured by a ^{51}Cr-release assay using labelled K562 target cells (36). The viability of K562 cells was typically about 98 %, as determined by the trypan blue exclusion test. Triplicates of 100 µl PBMC, at concentrations of 2×10^6, 1×10^6 and 0.5×10^6 cells/ml, respectively, were incubated with 100 µl of target cells (1×10^5/ml) in a 96-well microculture plate at 37 °C for 4 h in a CO_2 incubator, each cell mixture corresponding to an effector/target ratio of 20 : 1, 10 : 1 and 5 : 1, respectively. After incubation, the plate was centrifuged for 5 min at 300 g at 4 °C. A volume of 100 µl of supernatant was withdrawn from each well and the radioactivity was determined by a gamma counter (Cobra Autogamma, Packard Instrument Co., Downers Grove, IL). The spontaneous release of ^{51}Cr was determined by the incubation of target cells in 100 µl of medium, and the maximum release was assessed by incubating 100 µl of target cells in 100 µl of medium containing 10 % Triton. Cytotoxic NK activity was expressed as the percentage lysis: (Experimental − Spontaneous Release) cpm/(Maximum − Spontaneous Release) cpm × 100.

Results

Changes in leukocyte counts

Within 30 min after the onset of exercise, there was a significant (greater than 50 %) increase in the total leukocyte count and the leukocytosis continued to surge, reaching peak levels of more than 2.5 times of resting level, 30 − 120 min after the cessation of exercise (Fig. **1**). This exercise-induced leukocytosis was primarily caused by the development of a parallel granulocytosis. Like granulocytes, monocytes also displayed significant elevated levels during the exercise and two-hour recovery periods. In contrast, circulating lymphocytes displayed a different kinetics patterns: elevated lymphocyte levels, as high as 1.8 times of resting level, were attained during the exercise period, returning precipitously to normal at 30 min after exercise and dropping to at least 30 % below normal at 60 and 120 min into recovery (Fig. **1**). All the leukocyte subpopulations examined returned to normal after a resting period of 1 to 7 days.

P498766.PDF [Page: 7 of 12]

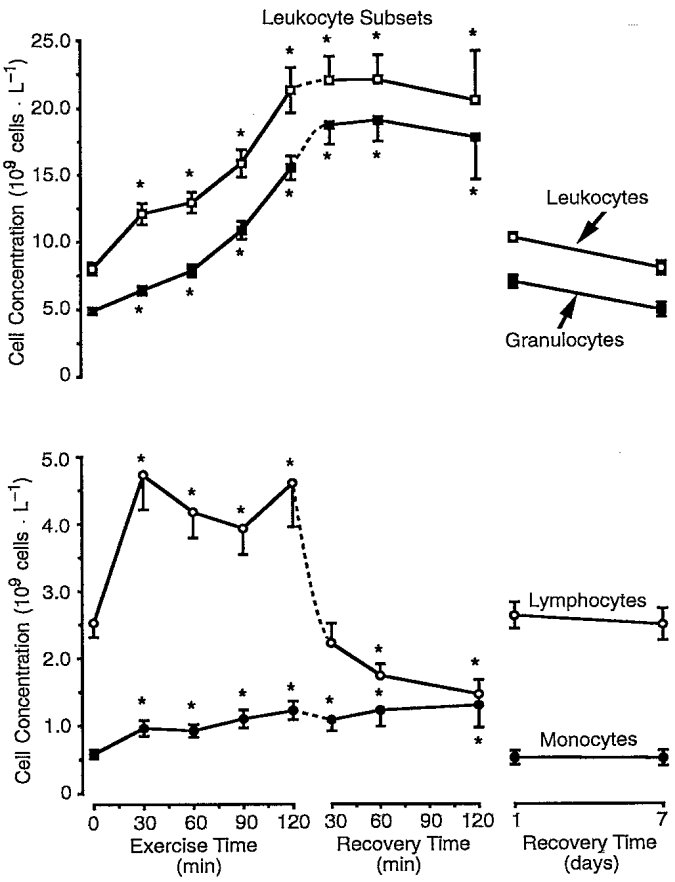

Fig. 1 Exercise-induced changes in circulating white blood cells and leukocyte subsets. All the cell concentrations were corrected for blood volume changes according to the method of Dill and Costill (9) based on the corresponding changes in hemoglobin levels. Each point represents the mean ± SEM determined at a given time from the blood samples of 6 exercising subjects. Each asterisk denotes a significant difference ($p < 0.05$, Student's t-test) between the mean value and the value at time zero.

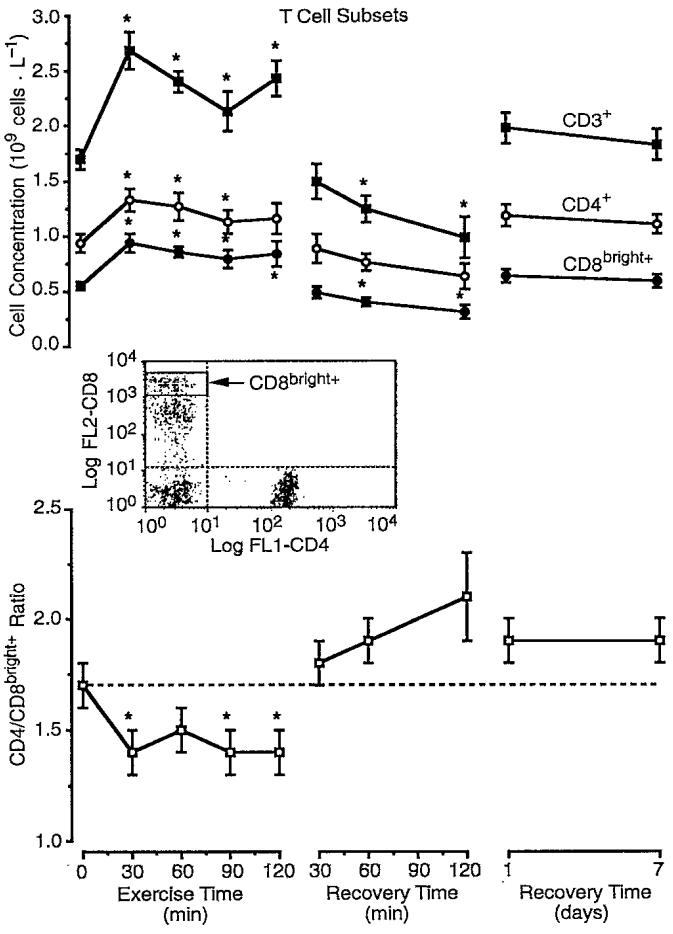

Fig. 2 Changes in peripheral blood T cell subsets and CD4/$CD8^{bright+}$ ratio with exercise. Only $CD8^{bright+}$ cells (see inset) which represent predominantly a $CD8^+$ T cell subset were enumerated for this analysis. Each point represents the mean ± SEM determined at a given time from the blood samples of 6 exercising subjects. Each asterisk denotes a significant difference ($p < 0.05$, Student's t-test) between the mean value and the value at time zero.

T and B lymphocyte subpopulations

$CD3^+$ T cells

The exercise regimen triggered an initial increase in $CD3^+$ T cells and the highest T cell concentration, attained after 30 min of exercise, was 58 % higher than the baseline level at time zero (Fig. **2**). The $CD3^+$ T-cell count remained elevated during the whole exercise period, but declined to normal level 30 min after the cessation of exercise. Thereafter, the T cell decline continued and by 2 h in the post-exercise period, T cell concentration dropped significantly to 42 % below the normal resting level. The $CD3^+$ cell concentration returned to normal 24 h after exercise.

CD4/CD8 ratio

Helper ($CD4^+$) and suppressor ($CD8^{bright+}$) T cell concentrations were also monitored and the results are shown in Fig. **2**. In this study, only $CD8^{bright+}$ cells (Fig. **2** inset) which essentially represent the suppressor/cytotoxic T cell subset (29,35) were enumerated from gated lymphocytes. The kinetics curves for $CD4^+$ and $CD8^{bright+}$ cells were very simlar to that of $CD3^+$ cells during and after exercise. The two T-cell subpopulations followed a remarkably parallel pattern of exercise-induced changes. Like the kinetics curve of $CD3^+$ cells, there was a peak elevation of $CD4^+$ and $CD8^{bright+}$ cells, by 40 and 70 %, respectively, after exercising for 30 min. This was followed by a smaller increase for each cell type during the remaining exercise period. A gradual decline in helper and suppressor T cell concentrations was also observed during the 2-h post-exercise period.

Despite a parallel kinetics pattern for $CD4^+$ and $CD8^{bright+}$ cells, a difference in the magnitude of change between the two cell types gave rise to different trends of CD4/$CD8^{bright}$ ratio in the exercise and recovery phases (Fig. **2**). The ratio followed a downward trend during the exercise period, but the same ratio was reversed for an upward trend during recovery.

P498766.PDF [Page: 8 of 12]

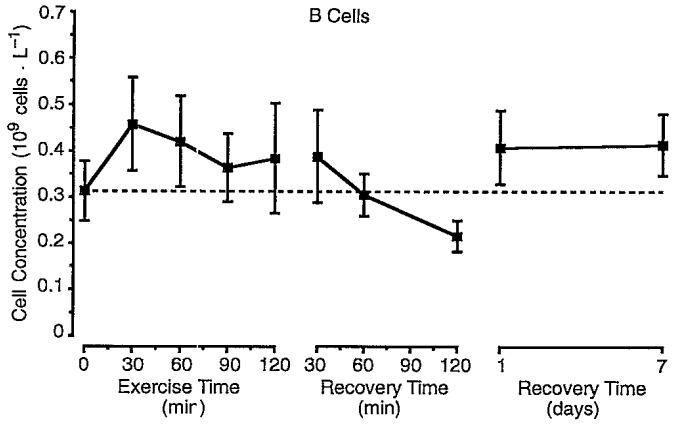

Fig. 3 Peripheral blood CD19⁺ B-cell concentrations during and after exercise. Each point represents the mean ± SEM determined at a given time from the blood samples of 6 exercising subjects. All the exercise-induced changes were determined to be statistically insignificant.

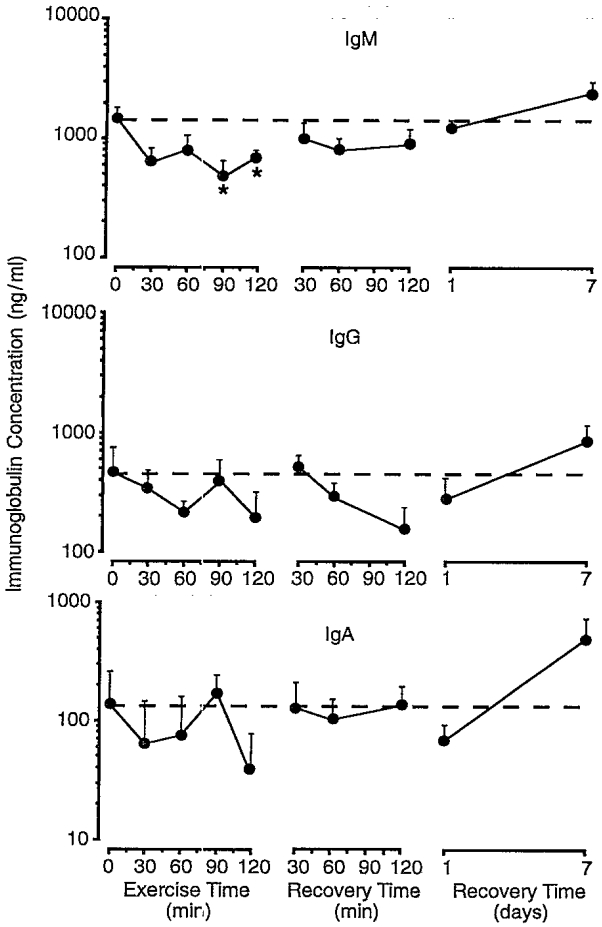

Fig. 4 Immunoglobulin production *in vitro* by peripheral blood lymphocytes during the exercise and recovery periods. The procedures used for the culture of pokeweed mitogen stimulated cells and the determination of the concentrations of immunoglobulin (IgM, IgG and IgA) produced are described in Materials and Methods. Each point represents the mean ± SEM determined at a given time from the blood samples of 6 exercising subjects. Each asterisk denotes a significant difference ($p < 0.05$, Student's t-test) between the mean value and the value at time zero.

CD19⁺ B cells

As with the CD3⁺ T cells (Fig. **2**), peripheral B cells bearing the CD19 marker showed the same trend of increased circulating levels during exercise (Fig. **3**) and decreased cell concentrations after exercise. The apparent changes in B cell concentrations, however, were statistically insignificant.

Immunoglobulin production

Pokeweed mitogen (PWM) was used to stimulate the *in vitro* production of immunoglobulins (IgM, IgG and IgA) by peripheral blood mononuclear cells (PBMC) prepared from blood samples collected from the experimental subjects. It can be seen from Fig. **4** that there was a general trend of depressed immunoglobulin production by PBMC obtained during the exercise period. The depression was quite pronounced for IgM production which fell significantly to 33 and 42 % of normal at 90 and 120 min of exercise, respectively. The apparent depression of IgG and IgA production by lymphocytes obtained during and after exercise, however, was not statistically significant.

NK cells

The exercise protocol was found to induce a significant elevation of circulating NK cells (CD16⁺) during the entire exercise period (Fig. **5**). This increase in cell number was also accompanied by a concomitant increase in NK cytotoxic activity

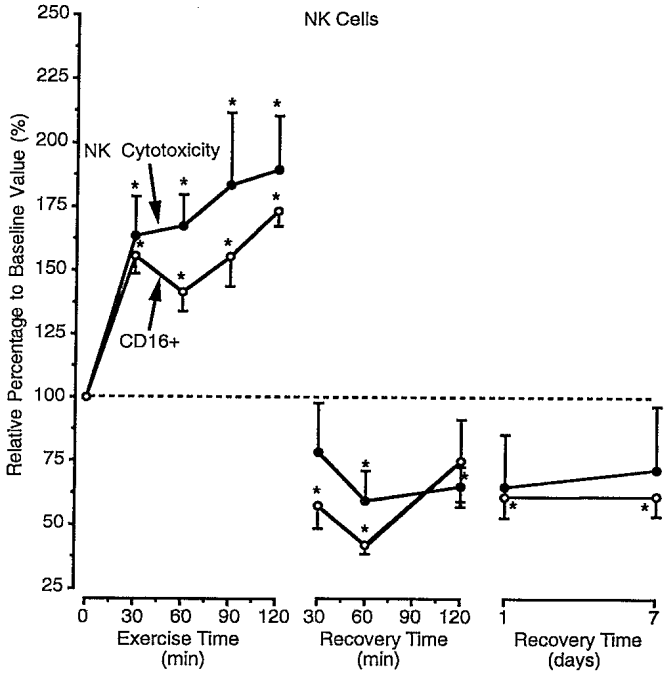

Fig. 5 Exercise-induced changes in circulating NK cell response. NK cells were quantitated by flow cytometry using anti-CD16 monoclonal antibodies and the NK cytotoxic activity was assayed using ^{51}Cr-labelled K562 tumor targets as described in Materials and Methods. Each point represents the mean ± SEM determined at a given time from the blood samples of 6 exercising subjects. Each asterisk denotes a significant difference ($p < 0.05$, Student's t-test) between the mean value and the value at time zero.

P498766.PDF [Page: 9 of 12]

against target cells of a human tumor cell line (K562). A sharp decline in NK cell count and cytotoxicity was evident by 30 min after the cessation of exercise. The most pronounced depression occurred at 60 min post-exercise with NK cell count and cytotoxic activity declining to 60 and 40 %, respectively, below baseline. The NK cell count depression of about 40 % below normal was also found to be very persistent, lasting for at least 7 days post-exercise.

Discussion

The present study examined the impact of strenuous exercise on the immune system as determined by changes in a number of immunologic parameters, both phenotypic and functional. Our results demonstrated that 120 min of treadmill exercise at 65 % VO_2max significantly altered the distribution and function of some lymphocyte subsets, both during the exercise period itself and during the post-exercise recovery period. The changes induced during the post-exercise period were usually in the opposite direction and of different duration, compared to those invariably observed during the exercise period. Unlike most other studies confined to examining primarily pre- and post-exercise changes (1,7,24), our present experimental design addressed a complete analysis of exercise-induced immunological changes by conducting multiple blood sampling to establish a full kinetics curve for each parameter assayed. This approach, we believe, provides more meaningful information to afford a better understanding of the intricate relationship between exercise and immune functions.

It has been well-established that physical exercise causes a change in the peripheral redistribution of leukocytes and leukocyte subpopulations (22,30,33,34,44). However, in most studies where pre-and post-exercise blood samples were compared (7,37,45,47), the kinetics of leukocyte subset changes cannot be fully appreciated. In this study, the multiple-time-point analysis of the leukocyte subsets during and after exercise rendered it possible to follow the time-dependent changes. Our results demonstrated that exercising at an aerobic capacity of 65 % VO_2max induced a profound leukocytosis, such that after 1 h and 2 h of exercise, the number of circulating leukocytes had increased to 1.6 and 2.6 times their resting level, respectively. These changes are consistent with those reported for running exercise of comparable durations (30). Granulocytosis, the principal component of the observed leukocyte response, developed at a rate which approximately doubled every 60 min, and the cessation of exercise slowed down the granulocyte up-surge to a near plateau, which was then maintained for about 1 – 2 h during recovery. The kinetics of exercise-induced monocyte changes have been studied much less frequently (30). In this study, the distribution of monocytes during and after exercise was found to follow a remarkably similar trend to that of the granulocytes, both in terms of the kinetics and the magnitude of change. In contrast to granulocytes and monocytes, lymphocytes displayed a distinctly different distribution pattern after the onset and cessation of exercise. The exercise regimen induced the attainment of practically peak lymphocytosis after only 30 min of exercise, and more or less the same lymphocyte level was maintained for the rest of the exercise period. Unlike the accompanying granulocytosis and monocytosis which were sustained for about 2 h post-exercise, lymphocytosis disappeared within 30 min post-exercise. The retrieval of lymphocytes from the circulation continued upon the cessation of exercise, culminating in the development of a moderate lymphopenia for 1 to 2 h in the recovery period. Thus, a full kinetics analysis of exercise-induced changes in leukocyte subset counts allows a much better understanding of the time-dependent variations in cell concentrations during and after the exercise period.

Exercise-induced changes in T cell subpopulations have been extensively studied because of the pivotal immunomodulatory role of $CD4^+$ helper and $CD8^+$ suppressor T cells (5). Although the CD4/CD8 ratio has been frequently used as a convenient index representing the relative distribution of each T cell subpopulation, the ratio, however, could be misleading if it is not appropriately established. The potential pitfall stems from the fact that a small proportion of NK cells also expresses CD8 at low surface densities, recognized by their low fluorescence intensity staining with CD8-specific monoclonal antibodies – the $CD8^{dim+}$ cells (25,29,35,40). In contrast, $CD8^+$ cells representing the suppressor/cytotoxic T cell subset are predominantly expressing high-density CD8 surface antigen – the $CD8^{bright+}$ cells (21,25,29,35). In this study, only $CD8^{bright+}$ cells were included in the determination of the CD4/CD8 ratio, thus primarily reflecting the relationship between the helper/suppressor T-cell subpopulations.

Under the experimental conditions of this study, the CD4/CD8 ratio was primarily depressed during the exercise period. This observaton is in agreement with previous studies which also demonstrated decreased CD4/CD8 ratios after 60 or 120 min of exercise (37,42,46). However, unlike other reports which accounted for the depression of the CD4/CD8 ratio by a decrease in $CD4^+$ and an increase in $CD8^+$ cell concentrations (37,42), our present results demonstrated that *both* $CD4^+$ and $CD8^{bright+}$ T cell subsets were increased during exercise; the resulting decrease in the ratio was due to a smaller increase in $CD4^+$ than $CD8^{bright+}$ cells. It has also been reported by Fry et al. (15) that there is a concomitant increase in $CD4^+$ and $CD8^+$ cells immediately after an intermittent incremental exercise intensity test (3-min exercise bouts interspersed with 2-min recovery intervals) resulting in a decrease in the CD4/CD8 ratio, attributable to a relatively higher increase in CD8-bearing NK cells than $CD8^+$ T cells. Results of this study demonstrated that despite the elevation of $CD3^+$ pan T cells as well as $CD4^+$ and $CD8^+$ T-cell subpopulations during exercise, there was an overall time-dependent post-exercise depression of T cells for at least 2 h, an observation seldom reported by other investigators because of the lack of a similar kinetics analysis. We have previously shown that 1 h of exercise at 60 % VO_2max mediates a depression of $CD3^+$ T cells by 36 % (37) and in this study, 2 h of exercise were found to depress $CD3^+$ T-cell concentration further to 58 %. Thus, it appears that the longer the duration of physical exertion, the greater the depression of circulating T cells.

It is intriguing to note that while the T cell ($CD3^+$, $CD4^+$, $CD8^{bright+}$) counts were decreasing during the 2-h post-exercise period, the CD4/CD8 ratios showed an increasing trend, giving the appearance of a compensation for the downregulation of $CD4^+$ and $CD8^+$ T cells during recovery. To our knowledge, the present finding of post-exercise T cell downregulation and concomitant CD4/CD8 ratio up-trend has not been previously reported. We believe any proposed mechanism(s) to account for post-exercise changes in immune functions must take into consideration the possible presence of kinetic patterns of T cell

P498766.PDF [Page: 10 of 12]

subsets and CD4/CD8 ratios similar to that reported in this study.

The effects of exercise on B cells and associated immunoglobulin/antibody productions have not been studied as extensively as T cells. Bicycle exercise for 1 h (42,52) and 2 h (37), respectively, has been shown to induce no change in circulating B cell levels by comparing pre- and post-exercise measurements. In this study, we observed an apparent but statistically insignificant increase in peripheral B cell concentrations during and after exercise. Cycle ergometer exercise for 1 or 2 h has also been shown to induce no appreciable effect on circulating B cell counts (37,42). Therefore, it is reasonable to conclude that, unlike T cells and NK cells, B cell counts are least, if at all affected by exercise.

During the exercise period, despite the fact that B cell concentrations remained essentially unchanged, immunoglobulin production appeared to follow a depressed trend and by 90 min of exercise, IgM production was significantly below normal. In other words, normal B cell concentrations failed to maintain normal immunoglobulin production, at least for IgM. Hedfors et al. (19) also reported a similar observation of decreased immunoglobulin production despite a proportionate increase of B cells after a brief (15 min) exercise bout, leading them to suggest the possibility of some form of B cell regulation to account for the apparent paradox. Since the polyclonal stimulation of immunoglobulin production by pokeweed mitogen involves the regulation of the B cell response by T cells, a change in the helper/suppressor ratio may alter B cell functions. Thus, perhaps the depressed CD4/CD8 ratio during exercise impairs immunoglobulin (e.g., IgM) production despite a normal B-cell concentration, whereas a reversal (increase) of the same ratio restores immunoglobulin production, despite the downward trend of B cell concentration following exercise.

Exercise has been known to have an immediate effect on circulating NK cell count and activity (26,38,39). However, despite the many reports on exercise-induced changes in NK response, very few studies were designed to follow the NK changes, in number and function, using a multiple blood-sampling approach during and up to 7 d after exercise. In this study, a multipoint analysis, at 30-min intervals during exercise, demonstrated a significant increase in both $CD16^+$ cell concentration and NK cytotoxic activity within 30 min of exercise and the increased NK response was maintained for the entire duration of the exercise bout. Although the reason for the surge in NK cell concentration and activity during exercise is unknown, it has been suggested that an increase in NK cell function provides added host defence capability during exercise stress (37). It appears that NK cells, capable of destroying infected or transformed targets without MHC restriction, may compensate for possible immune vulnerability due to a transient decrease in the CD4/CD8 ratio prevailing during exercise.

The cessation of exercise was found to induce a reversal of NK cell distribution and activity in a downward direction. The largest decrease in NK parameters occurred 1 h post-exercise and the decrease was still significantly different from normal by 2 h into recovery. Similar observations have also been reported among cyclists after a 2-h bicycle ergometer session (27). In this study where peripheral T cells were also monitored, it became apparent that NK cell depression coincided with T cell depression, giving the appearance of a double jeopardy of high immune vulnerablity during this post-exercise period.

A post-exercise decrease in NK cell response has previously been reported to return to normal within 2 h after a moderate exercise bout of up to 1 h (26,46). In our study, where subjects performed strenuous treadmill exercise for as long as 120 min, NK cell count remained depressed by about 40 % for as long as 7 days after exercise. This observation raises the concern of potential immune compromise created by highly demanding physical exertion commonly experienced by some professionals (e.g., competitive athletes and military personnel) and supports the contention that exhaustive exercise and overtraining could induce a state of immunosuppression and a concomitant increased susceptibility to infection (13,14,18,43,44).

In conclusion, strenuous exercise exerts demonstrable effects on the immune system. Physical activity triggers the redistribution of circulating lymphoid cells characterized by an elevation of NK and T cell counts during exercise, accompanied by a concomitant depression of the CD4/CD8 ratio. Although a trend of B cell elevation and depressed immunoglobulin depression was observed, the changes, however, were mostly not statistically significant, suggesting a very moderate, if any effect of exercise on B cells. The observation of post-exercise immune impairment, especially the persistent depression of the NK cell count for 7 days, warrants further investigations to explore the underlying mechanisms.

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Corresponding Author

Pang Shek, Ph.D.
Operational Medicine Division
Defence and Civil Institute of Environmental Medicine
1133 Sheppard Avenue West
North York, Ontario M3M 3B9
Canada