The Effect of Strenuous Exercise, Calorie Deficiency and Sleep Deprivation on White Blood Cells, Plasma Immunoglobulins and Cytokines

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ABSTRACT

Moderate exercise appears to stimulate the immune system, but there is good evidence that intense exercise can cause immune deficiency. In the present study we examined the effect of continuous physical exercise (~35 % of VO_2 max), calorie deficiency and sleep deprivation on the immune system of young men participating in a 5-7 days military training course.

There was a 2-3 fold increase of neutrophils from day 1, the values remained high and decreased slightly at the end of the course. Monocyte counts also increased with a pattern similar to that of neutrophils. Eosinophils decreased to 30 % of control and lymphocyte numbers decreased by 30-40%. All the major subgroups (CD4 T cells , CD8 T cells, B cells, NK cells) were reduced.

Neutrophil function, as tested by measuring chemotaxis, was significantly stimulated during the first days of the course, in particular in the group with the lowest calorie intake. The mitogenic response of lymphocytes to PHA and Con A was variable, ranging from stimulation during one course to no effect in another course.

Serum levels of immunoglobulins decreased significantly during the course. IgG was reduced by 6-7%, IgA by 10-20% and IgM by 20-35%.

We found no changes of interleukin 1 , 2 and 4 during the course, but a (12-20%) reduction ($p<0.01$) of interleukin 6, and an increase ($p<0.01$) of granulocyte-macrophage colony stimulating factor.

Altogether the results from the ranger course present a mixed-up picture. The non-specific phagocyte-related immunity was enhanced. On the other hand, our data indicate that even a moderate physical activity, around the clock, caused significant suppression of a number of parameters reflecting the status of the specific, lymphocyte-related immunity. Still, it is noteworthy that there was no significantly increased infection rate during the course or in the first 4-5 weeks thereafter.

INTRODUCTION

In recent years considerable interest has been directed to the effects of exercise on immune function. As demonstrated in animal experiments [1,2], moderate exercise appears to stimulate the immune system. However, several studies indicate that intense training increases susceptibility to illness. These illnesses range from persistent colds, sore throats to flu-like illnesses and post-viral fatigue syndrome [3]. Clearly the immune system may be a limiting factor in human performance. There are however, distinct individual variations.

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Some athletes can withstand rigorous training without problems, others are very susceptible to colds and infections. The mechanisms for these effects are only partly known. Reduced levels of salivary IgA has been observed in well-trained Nordic skiers [4], and it has been speculated that this might reduce the resistance to infection. Following intensive exercise the in vitro response to T and B cell mitogens is variable [5], but mostly a suppression has been observed [6,7]. This could be due to increased levels of cortisol or catecholamines [8,9], both of which are generally immunosuppressive [10,11]. However, epinephrine may either suppress or stimulate the immune response, depending upon the experimental set-up [12]. Reduced bactericidal activity of neutrophils has also been observed, but otherwise the effect of exercise on neutrophil function is quite variable [13].

In the present work, published previously in Scandinavian Journal of Immunology [14], we have studied young army cadets before and during strenuous exercise lasting for 5-7 days, combined with sleep deprivation and calorie supply deficiency amounting to 35-40 000 kJ/24 h. The intent was to examine whether the severe strain of this ranger course leads to suppression of the immune system and thereby constitutes a health hazard.

MATERIALS AND METHODS

The ranger training course. Well-trained cadets of the Norwegian Military Academy participated in the courses, which lasted for 5-7 days, usually in june, july or august. The mean age of the cadets varied from 22 to 24 years (range 21 to 27), mean height 183 cm and mean weight 78 kg. During the course they slept for only 2-3 h and were exposed to continuous physical activity around the clock of about 35% of their VO_2 max with a calorie consumption of 35,000-40,000 kJ per 24 h. In general the daily intake of food represented less than 3000 kJ, which lead to a weight loss of 4-5 kg, mostly fat, in a 5 day period [9]. The intake of water was free. The results are from 8 different courses (87 cadets), each comprising 8 cadets or more. Most of the data are from 3 courses denoted I,II and III. In course II the cadets were split in group IIA and IIB, with average daily energy intake of 1000 kJ and 6000 kJ, respectively, which implies an energy deficiency of approximately 97 or 85 %. Otherwise they were treated similarly except that group IIB received 3 hours of extra sleep/rest on day 5. The cadets were under medical surveillance throughout the courses. During the courses (two), and 4-5 weeks thereafter, the cadets self-evaluated their health condition daily, based on a detailed standardized questionnaire.

Dextran 500 (Pharmacia) was dissolved in water and used as a 6% solution. **Lymphoprep** and **Metrizoate 32.8%** were provided by Nycomed (Oslo). The osmolality of Lymphoprep was increased from 300 to 320 mOsm/kg by adding 60 mg NaCl per 100 ml. Dextran-Metrizoate was made by mixing 10 parts Metrizoate with 25 parts dextran 6 % .

Blood (20-80 ml) was collected in vacutainers, with or without anticoagulant (EDTA), between 06.00 and 07.00 h in the morning. Plasma and serum were sampled from blood to which Trasylol 500 IU/m (Bayer, Leverkusen, Germany) had been added immediately. Leucocytes (EDTA-blood) were separated 5-6 h later and enumerated in an electronic counter. Erythrocytes and platelets were counted microscopically after appropriate dilution with 0.9% NaCl (erythrocytes) or 1% ammonium oxalate (platelets). Reticulocyte counting was done in smears made after staining with brilliant cresyl blue (0.25%).

Smears were made as follows: Two ml EDTA-blood was layered over 2.5 ml Dextran-Metrizoate. The leucocyte-rich plasma was collected when the red cells had sedimented to the bottom, centrifuged (5 min at 600 g), resuspended in a small volume and smears were made. The differential count of these smears corresponds to that in whole blood.

Cell separation. Equal parts of EDTA-blood and 0.9% Nacl were mixed and 30-35 ml of the mixture was layered over 12-15 ml of Lymphoprep and centrifuged for 17 minutes at 600 G. Mononuclear cells (MNC) were collected from the interface between plasma and Lymphoprep, and granulocytes from the bottom fraction [15]. Contaminating erythrocytes in the granulocyte suspension were removed by NH_4Cl (0.83 %) lysis for 7 minutes at room temperature (22-24^o C).

Flow cytometry. MNC were incubated with optimal concentrations of fluorescein- (FITC) or phyco-erythrine (PE) -labeled monoclonal antibodies (Becton Dickinson) directed against the following lymphocyte subsets: T cells (CD3, CD4 and CD8), natural killer cells (CD16) and B cells (CD19). The distribution of lymphocyte subgroups was then determined in a flow cytometer (Argus, Scatron, Drammen, Norway). Irrelevant isotype-matched controls were run for each antibody, and at least 10000 cells were analysed for each sample.

Phytohemagglutin (PHA) and Concavalin A (Con A) cultures. MNC (10^5) were suspended in 200 ml RPMI 1640 containing 5% fetal calf serum (FCS) and 10 mg/ml of PHA or Con A. The cells were cultured for 72 h in a humified atmosphere with 7.5% CO_2 at 37° C. After 48 h 1 mCi of ^{3}H-thymidine was added and 24 h later the cells were harvested onto glass fibre filters with a semiautomatic microculture harvester (Skatron, Lier, Norway). ^{3}H-thymidine incorporation was determined with a liquid scintillation counter.

Chemotaxis. Neutrophil chemotaxis [16] was measured by the ability of cells to migrate towards a chemotactic peptide (n-formyl-methionyl-leucyl-phenylalanine). The net distance migrated by cells in the leading front was measured after incubation for 90 minutes at 37° C.

Immunoglobulins and acute phase proteins. Serum immunoglobulins, a_1-antitrypsin and orosomucoid were measured with a Behring nephelometer, and C- reactive protein (CRP) was measured with a Cobas Bio turbidimeter.

Plasma cytokines. Plasma concentrations of interleukin 1,2 and 6 were determined with radioimmunoassays (RIA) (Amersham, UK). Interleukin 3 and 4, granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) were quantified with Enzyme-linked Immunosorbent Assay (ELISA) kits (Genzyme Corp. Boston).

Statistics. Alterations during the course were analysed with an analysis of variance for repeated measures. Student's t test was used to identify differences by comparing each individuals test value at different time points to their own baseline value.

RESULTS

There were no indications of increased infection rate during the course or the following weeks. Some complaints of rhinorrhea were noted.

Cell numbers

Cell numbers in blood changed significantly during the course (Fig. 1). The highest number of neutrophils, a 3 fold increase, was observed 24 h after start, and the values remained high throughout the course. The majority of these cells were mature polymorphonuclear granulocytes, but there was a slight ($p<0.05$) increase of band forms during the course (Table I).

The monocyte numbers increased by in parallel with granulocytes. Eosinophils decreased to approximately 30% of control, and the lymphocyte numbers decreased by 30-40%. A moderate increase of energy intake (IIB) did not change this pattern (not shown). Flow cytometric measurements showed that all major subgroups (CD3, CD4 and CD8 T cells, B cells, NK cells) were reduced (Fig. 2). The CD4/CD8 ratio increased during the first 24 h (Table II, $p<0.01$), followed by a decrease on day 2 ($p<0.05$). Otherwise the relative proportions of different cell types did not change appreciably, although there was a percentual decrease of all lymphocyte subgroups during the course. Thus the total sum of CD4, CD8, CD16 and CD19 cells, which amounted to 120% at start, decreased to 77 % on day 4 (Table II).

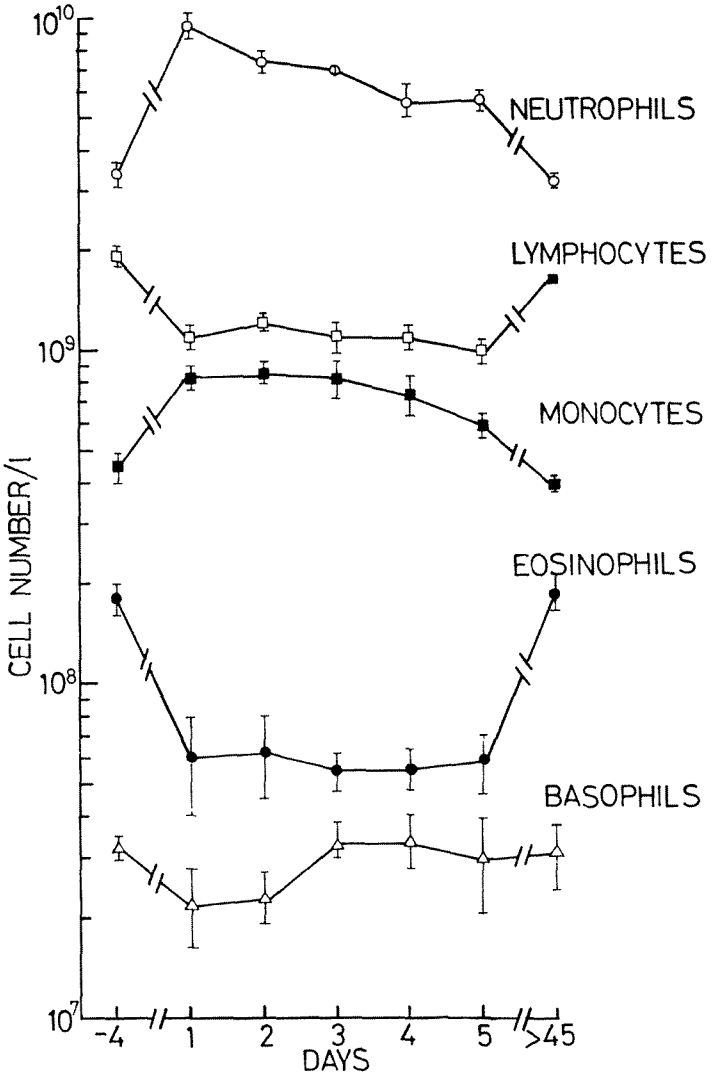

Fig. 1. Cell numbers (per liter) in blood during the training course. The number of days from start are indicated on the abscissa. Mean values (±SE) from five separate courses, each comprising 8-10 individuals.

Table I. Number of band forms of granulocytes in blood

Control	Day 1	Day 2	Day 3	Day 6
49 ± 19	263 ± 71	118 ± 24	204 ± 38	130 ± 59

The table gives the number of band forms per ml blood on different days of the ranger course. Mean values (± SE) from 10 individuals.

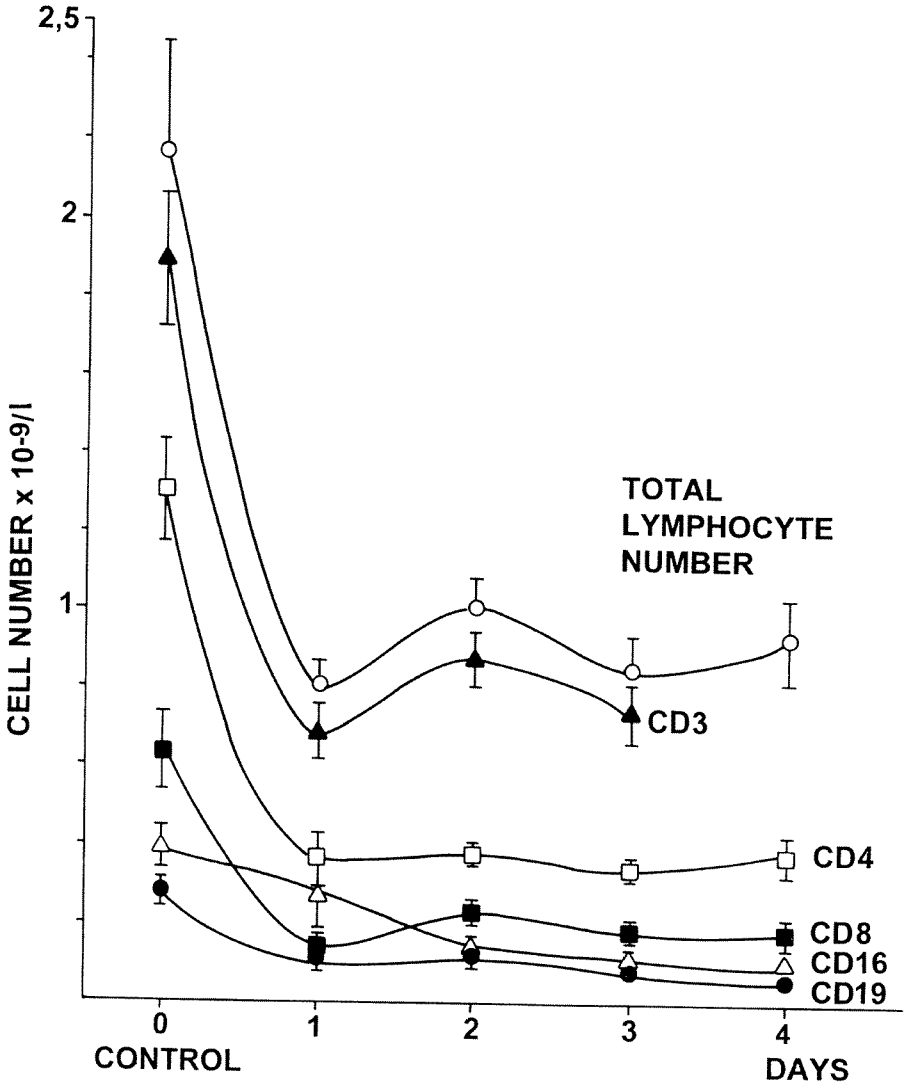

Fig. 2. The number of lymphocytes (per liter) in different subgroups from course I, as determined by flow cytometry. The antibody against CD8 cells was PE-labeled; the other antibodies were FITC-labeled. Mean values (± SE) from 10 cadets.

Table II. Percentages of lymphocyte subsets during course I

	CD4	CD8	CD19	CD16	CD3	CD4/CD8
Contr	60	29	13	18	88	2.1
Day 1	42*	17*	14	29	87	2.7*
Day 2	37*	22*	12	13	87	1.7*
Day 3	42*	22*	9*	10*	87	2.1
Day 4	40*	19*	7*	11*		2,5

The percentages of lymphocyte subsets in blood as determined by flow cytometry. Mean values from 10 cadets. CD16 values varied considerably, in particular during the first two days, with SE up 30% of the mean, as compared to £13% (mostly below 10%) for other subtypes. *denotes values that are significantly different ($p<0.05$) from control.

Erythrocyte numbers (Table III) decreased by 15-20% during the course, and there was a corresponding decrease of hemoglobin values, and a gradual increase of reticulocytes. Platelet numbers were not affected (Table III). All blood cell counts were normal 1-2 months after the course.

Table III. Hemoglobin, erythrocytes reticulocytes and platelets during the training course

	Control	Day 1	Day 2	Day 3	Day 4	Day 5
Hgb g/100 ml	15.3 ± 0.6	15.2 ± 0.6	14.5 ± 0.3	13.7 ± 0.3	13.5 ± 0.3	12.9 ± 0.6
Eryth 1×10^{-12}	5.3 ± 0.1	5.0 ± 0.2	4.7 ± 0.1	4.3 ± 0.1	4.3 ± 0.2	4.3 ± 0.2
Retic (%)	0.32 ± 0.1	0.57 ± 0.1	0.79 ± 0.1	0.99 ± 0.3	1.60 ± 0.6	0.91 ± 0.2
Plat 1×10^{-9}	235 ± 14	303 ± 29	259 ± 29	271 ± 30	273 ± 30	270 ± 10

Average values (± SE) from 11 individuals (5 for reticulocytes).

Cell function

Neutrophil chemotaxis was stimulated ($p<0.01$) on days 1 and 2 and 7 after start of the course (Fig. 3), in group IIA (1000 kJ/day), which confirmed the results observed during course I (not shown). Stimulation was observed also in the energy-supplemented group (IIB, 6000 kJ/day, $p<0.01$, day 1), but less than in the low calorie group ($p<0.01$, day 7).

The mitogenic response by MNC to PHA and Con A (Fig. 5) was not consistent, varying from significant stimulation (course II) on day 1 and 2 ($p<0.01$), to no change of response during course III.

Immunoglobulins and acute phase proteins

Serum IgG was slightly reduced (6-7%, $p<0.05$) on days 3 and 6 (course III, Table IV), IgA decreased by 10-20 % from day 1 ($p<0.05$) and IgM by 20-35 % ($p<0.01$). The same effect ($p<0.01$) of stressful training on IgM was observed in course II (EDTA-plasma), and group IIA (1000 kJ/day) and IIB (6000 kJ/day) did not differ. A slight reduction ($p<0.05$) of orosomucoid was observed on days 3 and 6, whereas a_1-antitrypsin and CRP were not significantly affected (Table IV).

Table IV. Immunoglobulins and acute phase reactants during the training course

		Control	Day 1	Day 2	Day 3	Day 4	Day 5
IgG	g/l	13.5 ± 0.6	13.7 ± 0.8	13.5 ± 0.9	13.7 ± 0.3	12.5 ± 0.8*	12.6 ± 0.8*
IgM	g/l	1.7 ± 0.2	1.3 ± 0.2*	1.2 ± 0.2*	4.3 ± 0.1	1.1 ± 0.1*	1.2 ± 0.2*
IgA	g/l	2.7 ± 0.3	2.4 ± 0.2*	2.3 ± 0.2*	0.99 ± 0.3	2.2 ± 0.2*	2.3 ± 0.2*
Orosomuc	g/l	0.9 ± 0.07	0.9 ± 0.04	0.9 ± 0.05	271 ± 30	0.8 ± 0.1*	0.8 ± 0.1*
CRP	mg/l	0.9 ± 0.07	10.8 ± 0.8	18.4 ± 4.7	0.9 ± 0.07	11.8 ± 3.7	13.0 ± 2.5
Alpha-1 antitr	g/l	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	0.9 ± 0.07	2.3 ± 0.1	2.4 ± 0.1

The table shows average values (± SE) in sera from 10 individuals (course III). Control values are pooled data obtained 7 and 2 days before start of the course, and * indicates values that are significantly different ($p<0.05$ or better) from control. For C-reactive protein 10 mg/l was the minimum detection level. Three out of 10 cadets had increased CRP values on days 2 and 3.

Cytokines

Plasma levels of interleukin 1 (1a and 1b) and interleukin 2 did not change. There was a 12-20 % reduction of interleukin 6 ($p<0.05$) on days 4-7 (Fig. 5). There was no difference between the two groups with different energy intake.

Plasma concentrations of interleukin 3 and G-CSF remained below detectable levels in the observation period. A significant increase of GM-CSF ($p<0.01$) was found on days 1,2 and 7 (Table V).

Table V. Concentration of GM-CSF in plasma

Control	Day 1	Day 2	Day 7
2.8 ± 0.5	9.5 ± 1.5	10 ± 1.3	8 ± 1

Plasma concentration (pg/ml) of granulocyte-macrophage colony stimulating factor during the training course. There was no difference between the group IIA (1000 kJ/24 h) and group IIB (6000 kJ/24 h) and the data were pooled. Mean values (± SE) from 16 individuals.

DISCUSSION

The immediate effect of physical exercise of short duration is an increase of blood granulocytes, monocytes, lymphocytes and lymphocyte subgroups [17-20]. The response to prolonged physical activity (Fig. 1) was different in some respects. Neutrophil and monocyte numbers remained at elevated levels, whereas there was a lasting decrease of eosinophils and lymphocytes (Fig. 1) and lymphocyte subsets (Fig. 2) from day 1. Short term exercise has yielded different results, and a typical finding is a decrease of CD4 T cells and an increase of NK cells [21,22].

The mechanism for the changes of blood cells counts (Figs. 1 & 2) are not clear. In previous studies [8,9] a significant increase of cortisol, epinephrine, norepinephrine and dopamine plasma levels was found during the training course. Thus, a combined effect of corticosteroids and catecholamines may account for the observed changes. The leucocytosis-inducing effect of epinephrine was demonstrated [23] already in 1904. After an initial increase of granulocytes and lymphocytes, there may be a secondary decrease of lymphocytes [24,25]. Catecholamines may cause an increase of cells by inducing a wash-out of cells from different organs [26,27].

The increase of neutrophils may not only be due to a redistribution of cells within the vascular system. The increased number of band forms (Table I) indicates an increased influx of cells from the bone marrow, probably caused by cortisol [28]. Short term exercise mostly leads to elevated numbers of lymphocytes in blood [29]. The lymphopenia and eosinopenia during more long-lasting strenuous exercise (Figs. 1 & 2) can probably also be ascribed to an effect of cortisol. Many studies have shown that cortisol depresses the lymphocyte number [30,31]. It appears that cortisol causes a selective depletion of the recirculating portion of the intravascular lymphocyte pool [30,32]. The first step in lymphocyte migration from the blood stream into the lymph nodes involves a specific binding between the lymphocytes and the endothelial cells lining nodal postcapillary venules. In a recent work [33] it was shown that the expression of adhesion proteins (L-selectin) on circulating lymphocytes was decreased during the first 8 hours following prednisolone administration. Furthermore, other investigators have shown that steroids cause a redistribution of cells to other organs, such as the bone marrow [34]. By large the number of different lymphocyte subtypes decreased approximately to the same extent during the course (Fig. 2). It is noteworthy that the subtypes with different markers (CD4, CD8, CD16 and CD19) amounted to 120 % at start (Table II), indicating that some cells had more than one marker. However, on day 4 only 77 % of the cells expressed these markers, suggesting that a significant fraction of the surface markers had been lost in response to the multifactorial strain. An alternate explanation is that the selection of cells in blood changes during the course. It has otherwise been speculated that the eosinopenia, like lymphopenia, also somehow is related to alteration of cell adherence [35].

Neutrophil chemotaxis was significantly enhanced during the course (Fig. 3), in particular on days 1 and 2. This can hardly be attributed to increased GM-CSF levels (Table V), since GM-CSF injections may inhibit

chemotaxis when separated cells are tested in vitro [36,37]. Still, in some respects GM-CSF is an activator [36,38,39] of granulocytes (phagocytosis, antibody-dependent cytolysis).

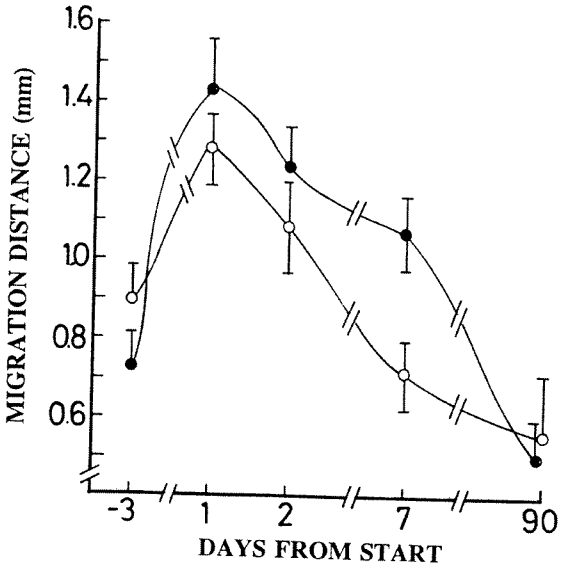

Fig. 3. Neutrophil chemotaxis with cells from the low (IIA, 1000 kJ/24 h, closed circles) and higher calorie group (IIB, 6000 kJ/24 h, open circles). Both groups had a caloric deficiency of more than 80%. Control samples were taken 3 days before, and 90 days after the start of the course. Mean values (±SE) from 8 individuals in each group.

The mitogenic response of lymphocytes to PHA and Con A was not consistent. Stimulation was observed during the first two days of one training course, whereas no effect was found in another course (Fig. 4). A varying physical activity in different courses may also account for these results. A particular problem here is that the differential distribution of the mononuclear cells changed during the course, with an increase of monocyte/lymphocyte ratio. A striking variability has also been found in response to short-term (1-3 h) exercise. Mostly this exercise suppresses the cell proliferation [40], but stimulation has also been observed [1, 29,40]. In long-lasting (~ 60 days) ranger courses the mitogenic response was almost consistently suppressed [41]. However, the results from a consecutive course indicated that the suppression could partly be prevented by increasing the food intake [42]. Based on previous studies [43-45] one should expect suppressed lymphocyte function due to increased levels of cortisol and epinephrine [8,9] found during the present training courses. A short-term immunosuppression has been observed after epinephrine injections [10], but altogether rather conflicting results have been found [12, 29]. Further, it has been shown that glucocorticoid inhibits the synthesis of interleukin 2 [11], a vital regulator in the immune system. However, the complexity of changes in response to strenuous exercise [8,9] makes it difficult to predict the effect on blood cell function based on values of a few parameters.

The reduced immunoglobulin levels (Table IV) during the course, may suggest impaired immune function. No such effect on immunoglobulins was observed during long-lasting (~60 days) endurance exercise [42] with daily energy expenditure of 16-18000 kJ and a significant caloric deficit. In general, heavy short term exercise tends to be associated with increased levels of immunoglobulins (5,46). A decrease of immunoglobulins (IgA and IgG more than IgM) was found following 45 and 75 km runs [47]. However , these results were not reproduced in another marathon run, presumably due to lower intensity as a results of high air temperature. Altogether, many studies have yielded contradictory results [reviews, 5,29,46].

Immune dysfunction has been observed in chronically undernourished subjects [48]. Starvation itself for 5-10 days does not affect immunoglobulin levels [49-51] and an increase has been observed in obese subjects during fasting [52]. However, it remains to be shown whether reduced immunoglobulins (Table IV) is due to a synergistic effect of exercise and starvation. It is also necessary to consider whether a predominant catabolic state of the cadets may affect immmunoglobulin production. However, the decrease of IgM was observed

repeatedly already after 24 h, before they were exposed to maximal metabolic stress. Hormonal changes [review 53] during the course may affect immunoglobulins. It has been reported that high doses of methylprednisolone [54] reduced serum IgG and IgA (but not IgM), and it is possible that increased levels of cortisol during the course [9] have a similar effect. This issue may be further evaluated by measuring serum immunoglobulins in group a well-fed of cadets, since cortisol increases less among participant on a isocaloric diet [9], whereas extra sleep (3 h/24 h) has no such effect [9]. Catecholamines increased consistently during the course, unaffected by extra sleep or energy intake [9], but it is difficult to evaluate their effect on immunoglobulin synthesis. As regards the primary antibody response available reports have yielded conflicting results [12], ranging from inhibition [55,56] to stimulation[57].

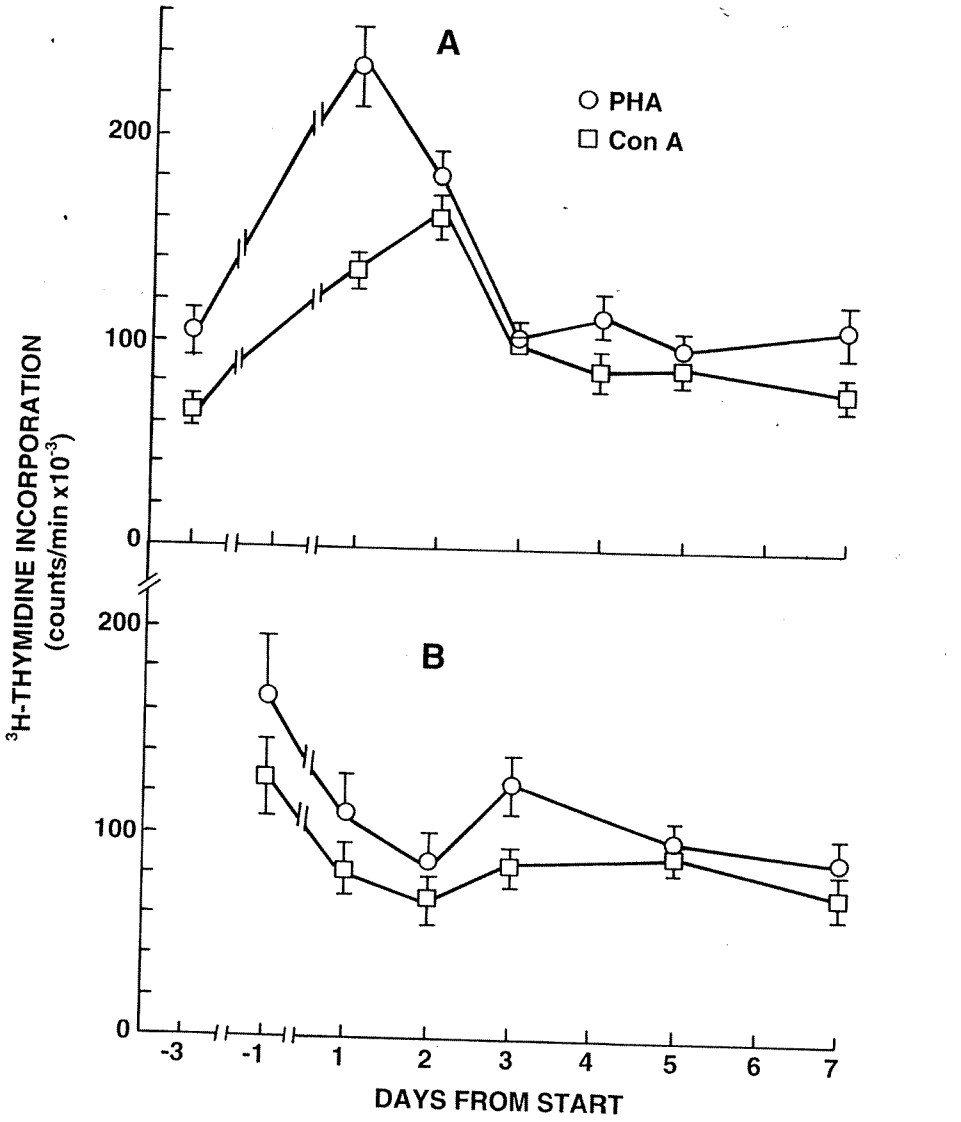

Fig. 4. The response of mononuclear cells to mitogens (PHA and ConA). The cells were cultured 2 days and then ^{3}H-thymidine was added, and the uptake was measured on day 3. Stimulation was observed during course II (A), whereas there was no change of response during course III (B).

Among the interleukins, a significant (15-20%) reduction was observed for interleukin 6 (Fig. 5). Il-6 is a multifunctional cytokine that stimulates B and T cells by different mechanisms, and also acts in synergy with colony stimulating factors. This decrease of Il-6, although small, may impair immune function.

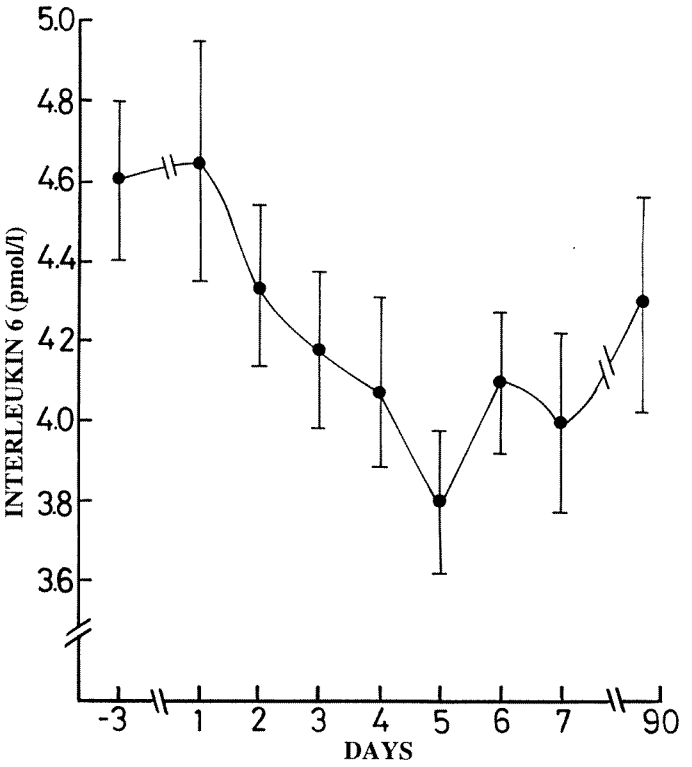

Fig. 5. Concentration of interleukin 6 in plasma from course II (A + B) , determined with radioimmuno-assay. Mean values (±SE) from 15 individuals.

The decrease of Hgb values (Table III) from day 2 ($p<0.05$) is in agreement with previous studies, with a decrease already on day 1 [58]. There was a corresponding decrease of erythrocytes, which coincided in time with an increase of reticulocytes (Table III). These changes can hardly be totally due to the amount of blood (30-40 ml) taken per day. Reduced Hgb, immunoglobulin or Il-6 levels could be explained by plasma expansion due to excessive water intake. However this may not seem likely since alpha-1-antitrypsin remained constant (Table III), and total serum protein was also constant and decreased only slightly (6-7%) from day 4 [58]. Reduced haptoglobin values [58] rather suggests that the drop in Hgb is caused by mechanical damage of red cells.

the intensity, energy supply and the duration of the exposure, and is affected by a large number of molecular participants [59]. The response to acute exercise is transient and quite variable and may also differ in trained and untrained subjects [6]. In our study we found some indication of impaired immune function without any increased infection rate, possibly due to a relatively short duration of the course. The infection rate was increased in a more long-lasting (62 days) ranger course, with daily energy expenditure of 16800 kJ and energy intake of about 6000 kJ (41). The majority of these infections involved cellulitis of the lower extremities, possibly due to injuries (blisters) and environmental factors (contaminated water), together with suppressed immune function. However, the prevalence of infections was lower when the caloric intake was increased [42]. This improved resistance is also consistent with the effects on cellular immune function [42]. Increased infection rates were observed in a even more long-lasting ranger course (30 weeks), with marked periodicity for viral infections of the respiratory system [60]. In any case, in studies of this type it appears important to define the actual physical stress program properly. This may be fairly easily achieved for energy supply and duration. However, conceivably it is more difficult to maintain a constant intensity level for courses arranged at different times. Still, our data indicate that even moderate physical exercise may be suppress the immune system within a few days, when the activity is continuous, around the clock. However, it remains to elucidate more thoroughly the role of energy and sleep deprivation in this multifactorial stress situation.

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