Recovery time affects immunoendocrine responses to a second bout of endurance exercise

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The purpose of this study was to examine the effect of different durations of rest between two bouts of exercise on immunoendocrine responses during and after the second bout of exercise. Nine endurance athletes participated in three 25-h trials: 1) complete bed rest (REST), 2) two bouts of exercise separated by 3 h of rest (SHORT), and 3) two bouts of exercise separated by 6 h of rest (LONG). Each cycle ergometer exercise bout lasted 75 min at 75% of maximal O2 uptake. We observed a more pronounced increase in epinephrine, norepinephrine, adrenocorticotropic hormone, and cortisol, but not in growth hormone, and a larger neutrophilia and lymphocytopenia in connection with the second bout of exercise in trial SHORT compared with trial LONG. Lymphocyte activation was unaltered by the difference in rest protocol. In conclusion, a second bout of exercise elicited more pronounced change in neuroendocrine factors and leukocyte counts when preceded by 3 h of rest as opposed to 6 h of rest after the first bout of exercise.

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A PERSUASIVE FUNCTIONAL LINK among the nervous, endocrine, and immune systems has been established through numerous animal and human studies (1, 2, 5, 42), and both cells and signal molecules of the brain, endocrine organs, and lymphoid tissue are closely integrated. Anatomical links between the sympathetic nervous system and lymphoid tissue (23, 42) and the presence of adrenoreceptors and glucocorticosteroid receptors on leukocytes (27, 40), as well as interleukin receptors in the brain (4, 8), underline this meticulous interplay. Furthermore, the communication between different components of these systems is reciprocal (13). Thus a change in activity in a neuroendocrine pathway, such as the hypothalamus-pituitary-adrenal (HPA) axis, elicits a multitude of responses in the immune system (6, 33, 41, 52), and, conversely, activation of immune cells can evoke alterations in the function of several regions of the central nervous system (3, 10, 26, 36).

Regulation to preserve a state of homeostasis is a strong governing principle in the interaction between various cells and soluble factors of the neuroendocrine immune systems (34). Several types of stress, i.e., psychological, physical, and immunological, elicit both specific and nonspecific stress reactions and induce perturbations in the cells and soluble factors in these systems. Depending on the intensity and duration of an exercise stress exposure, these perturbations may represent both short-term effects, such as increased catecholamine secretion, and more long-term effects, such as increased cortisol secretion (35). Thus it is conceivable that, by imposing repetitive stress exposures within a period of a few hours, an interaction effect between the subsequent stress reactions may occur. In other words, incomplete recovery from the first stress challenge could result in both quantitatively and qualitatively different responses to the next challenge.

Recently, we demonstrated that when a session of strenuous endurance exercise is repeated after only 3 h of rest, more pronounced neuroendocrine (38) and leukocyte (39) changes were found during and after the second exercise bout. Interestingly, we observed the augmented neuroendocrine response in connection with the second exercise, despite completely normalized plasma concentrations of all hormones before the second exercise session. This indicates that homeostasis cannot be evaluated just by measuring resting levels of neuroendocrine hormones. The regulating factors and kinetics by which homeostasis is achieved after an acute stress reaction are not fully understood, but time in itself is likely to be an important determinant for reaching complete recovery (34). Repeated physical exercise including the following recovery period may be a useful model to study the effect of various rest inter-
vals on subsequent stress reactions. Such information may improve our understanding of how the human body reacts to repeated stress and how the recovery time may affect the outcome of a following stress exposure. Even clinicians handling patients with various stress dysfunctions may benefit from this knowledge. Furthermore, in professional sports, optimal regeneration and recovery are considered essential to maximize the gain from repeated exercise sessions and to avoid overreaching and performance decrements (19, 20). Because the number of hours for rest between exercise sessions may be limited when an athlete must perform two bouts of exercise per day, it is of great importance to know how many hours it may take to achieve sufficient recovery before resuming the next exercise session.

Therefore, we designed a study that compared neuroendocrine and immune responses during days with two equal bouts of high-intensity endurance exercise, but different periods of rest between the first and second bout. We hypothesized that the neuroendocrine and immune responses elicited by the second bout of exercise are more pronounced when the rest period between the bouts is 3 h compared with 6 h.

METHODS

Subjects and design. Nine male, elite endurance athletes (age range 21–27 years, weight 74.7 ± 5.4 kg mean ± SE, maximal \( \text{O}_2 \) uptake 69.1 ± 3.7 ml·min \(^{-1} \) · kg \(^{-1} \) mean ± SE), four triathletes, and five speed-skaters from the national teams participated in the study. All subjects were accustomed to two daily training sessions as part of their exercise schedule, including cycling as one of the training modalities. A medical examination was performed on each subject before entering the study, and all subjects were thoroughly informed about the purposes and procedures of the study before a written consent was obtained. The protocol was approved by the Regional Ethics Committee for Medical Research in Norway.

All subjects participated in three trials, each lasting from 0700 to 0800 the next day (Fig. 1): 1) trial REST with 25 h of bed rest, 2) trial SHORT with two bouts of exercise separated by 3 h of rest (Ex-M: 1100–1215 and Ex-A: 1515–1630), and 3) trial LONG with two bouts of exercise separated by 6 h of rest (Ex-M: 0800–0915 and Ex-A: 1515–1630). The first bout of exercise (Ex-M) took place at different hours in the morning in trials SHORT and LONG in order to integrate 3 h and 6 h of rest and still have the second bout of exercise at the same time in the afternoon (Ex-A) in both trials. All trials were separated by 12–17 days to ensure complete recovery between trials and were randomized in a counterbalanced order with each subject serving as his own control. The triathletes were tested between January and March, and the speed-skaters were tested between April and June, outside the competitive season for both groups. Except for the last 2 days before each trial, when exercise was controlled according to the study protocol, the subjects completed their regular training program without any significant changes.

Pretrial procedures. Approximately 2 wk before the first trial, as well as after the last trial, the subjects performed an incremental exercise test on a cycle ergometer (Lode, Groningen, The Netherlands), starting at a workload of 175 W with a subsequent increase of 25 W every 5 min until they reached a workload of 275 W. The subjects then rested for 10 min before a continuous ramp test was used to estimate maximal \( \text{O}_2 \) uptake, starting at 275 W with a subsequent increase of 25 W every 30 s until volitional exhaustion (i.e., the subject could not sustain the workload for a period of >30 s). A respiratory exchange ratio (RER) >1.1 was used as an additional criterion that maximal \( \text{O}_2 \) uptake had been reached. The results were used to estimate a workload corresponding to 70% of maximal \( \text{O}_2 \) uptake for each subject based on the regression line of \( \text{O}_2 \) uptake vs. workload from the incremental exercise test. There was no difference in maximal \( \text{O}_2 \) uptake between the exercise tests performed before the first trial and after the last trial. No medication or nutritional supplements were allowed the last week before or throughout the study period. Serum ferritin and hemoglobin concentrations were measured 2 wk before the first trial and at the end of each trial. Iron supplementation was given if serum ferritin concentration was below 30 \( \mu \)g/l but discontinued 7 days before each trial. Hemoglobin concentration was measured again in the morning before each trial, and the trial was postponed for 1 wk if the concentration was reduced by more than 1.0 g/dl from the previous trial. If a subject had an episode of illness with fever, the trial was postponed for 1 wk. If a subject had an episode of illness with fever, the trial was postponed for 1 wk.
or malaise, the trial was postponed until he had been without symptoms and medication for at least 5 days. High-intensity exercise was not allowed during the last 2 days before trials, and no exercise was permitted the last day before each trial. A dietary record was obtained for the last 24 h before the first trial, and the subjects were instructed to consume an identical diet the day before each subsequent trial. A standardized meal of cereal and milk was served at 2100 the evening before each trial, and the subjects spent the night at the Olympic Sports Center next to the laboratory.

**Trial procedures.** The subjects arrived in the laboratory at 0700 after a 2-min walk, emptied their bladder, had their body weight measured, and were subsequently put to bed. A flexible temperature probe was inserted in the rectum, and the subjects were connected to a temperature, ECG, and heart rate monitor (Siemens SC 6000 P, Siemens Medical Systems). A flexible intravenous catheter (Venflon 1.2 32 mm; BOC Health Care, Helsingborg, Sweden) was inserted into an antecubital vein and kept there for the whole trial.

All exercise bouts were equal in intensity and duration and consisted of a 10-min warm-up period at 50% of the predetermined workload immediately followed by 65 min of predetermined workload and a cadence of 90–100 rpm. The \( O_2 \) uptake was measured for 60 s after 15, 30, 45, 60, and 70 min of exercise. The ambient room temperature was kept at 20°C (18–22°C) and relative humidity at 40% (30–50%). The subjects rested in bed at all hours apart from the exercise sessions and slept in the laboratory from 2300 until 0700 the next morning. The subjects were allowed to read and listen to music during bed rest, but TV watching was restricted to a maximum of 4 h in the evening between 1800 and 2200. No sleep was allowed during the day. During each trial, the subjects were served four standardized meals at the hours prescribed by Hallman et al. (16). Breakfast, lunch, and dinner, four for lunch and supper, making a total of 4,000 kcals per trial. The same type and number of sandwiches were served during each trial. Water was consumed ad libitum during exercise and recovery except for the first 60 min postexercise when \( O_2 \) uptake was measured continuously.

**Sampling protocol and measurements.** During the pretrial test, \( O_2 \) uptake was measured during the last 90 s at each increment using an automated Oxycon Champion System (Erich Jaeger GmbH, Germany) with a nose clip and a flexible intravenous catheter (Venflon 1.2 32 mm; BOC Health Care, Helsingborg, Sweden) was inserted into an antecubital vein and kept there for the whole trial.

The first blood sample was drawn at 0730, and subsequent samples were obtained according to the schedule outlined in Fig. 1. To avoid coagulation, 0.3 ml heparin (100 IE) was injected into the catheter after each sampling. All samples were collected during bed rest, except for the sample taken during the last minute of exercise, which was obtained with the subjects seated on the cycle ergometer. At each sampling, blood was collected on a 3-ml, precooled 143 USP heparinized tube for hemoglobin, hematocrit, neutrophil, and lymphocyte counts; and on a 3-ml heparinized tube for 

**Assessment of T and NK cell activation.** Assessment of T and NK cell activation was carried out as described previously (39). Briefly, T and natural killer (NK) cells were stimulated by a mixture of two monoclonal antibodies (MAbs): anti-CD2 and anti-CD2R (Becton Dickinson, San Jose, CA). After incubation for 4 h at 37°C, the cells were stained with combinations of fluorochrome-conjugated MAbs against CD69 (Becton Dickinson), CD4 (Serotec), CD8 (Becton Dickinson), and CD56 (Becton Dickinson). Flow-Count (Coulter, Miami, FL) fluorospheres were added to each tube as an internal control to assess absolute cell numbers. The samples were analyzed by a FACSscan or FACSCalibur (Becton Dickinson) flow cytometer. The degree of activation was assessed in two ways: 1) as the percentage of the total number of CD4+, CD8+, and CD56+ cells, respectively, that expressed the CD69 molecule, and 2) as the degree of fluorescence from CD69 molecules on the CD4+, CD8+, and CD56+ cells, respectively. The latter was expressed as a ratio of the geometric mean of the CD69 fluorescence of stimulated cells divided by nonstimulated cells. All leukocyte and hormone concentrations were corrected for plasma volume changes relative to the values from the first morning sample, according to the method of Dill and Costill (9).

**Statistical analyses.** For comparison of the three trials, an ANOVA procedure for repeated measures with a two-factor analysis was used to test for main effects (trial and time) and interaction effect (trial × time), including all three trials (REST, SHORT, and LONG). Where significant effects were found, a separate ANOVA procedure testing trial SHORT vs. trial LONG was performed. Nine measurements of hormones, neutrophils, and total lymphocyte and four measurements of CD4+, CD8+, and CD56+ cells counts from 1500 to 0730 the next morning were included in the statistical analysis. *F*-values for the trial-by-time interaction are reported, unless otherwise specified. Degrees of freedom were adjusted according to the Huynh-Feldt method. Additionally, Student's *t*-test with Bonferroni corrections for multiple comparisons was used for pre- and posttrial comparisons and for comparisons at the same time point in the different trials. Correlations were performed on pooled data from the three exercise trials using the Pearson correlation coefficient. Results are presented as means ± SE or *r* value unless otherwise noted. Exact *P* values are generally given, and *P* values <0.05 were considered significant.
RESULTS

Catecholamines, cortisol, and ACTH. There was a more pronounced increase in E and NE in trial SHORT compared with trial LONG during the second exercise and recovery period (1500–0730, \( F_{8,64} = 7.31, P < 0.001; \) and \( F_{8,64} = 2.30, P = 0.033, \) respectively; Fig. 2, A and B), but no difference in plasma catecholamine concentrations between the two trials after 15 min of recovery. There was no difference in the urinary excretion of E and NE between trials SHORT and LONG during the same period (E: 55.9 ± 8.4 μmol vs. 53.9 ± 6.2 μmol; NE: 387.6 ± 56.3 μmol vs. 333.9 ± 25.0 μmol, respectively). There were more pronounced increases in ACTH and cortisol in trial SHORT compared with trial LONG during the second exercise and recovery period (\( F_{8,64} = 2.33, P = 0.029; \) and \( F_{8,64} = 2.70; P = 0.013, \) respectively; Fig. 3, A and B).

Testosterone and GH. Testosterone concentration was decreased before the start of the second bout of exercise in both trials SHORT and LONG compared with REST (14.7 ± 0.9 and 15.7 ± 0.8 nmol/l vs. 19.1 ± 1.3 nmol/l, respectively, both \( P < 0.05; \) Fig. 4B). A similar decreased level of testosterone was observed in both trials SHORT and LONG compared with REST after the second bout of exercise, but there was no difference in the magnitude of change between trial SHORT and LONG (\( F_{8,64} = 0.44, P = 0.89; \) Fig. 4B). GH increased 53-fold in trial SHORT and 46-fold in trial LONG during the second bout of exercise (Fig. 4A), but there was no difference in the GH response between trials SHORT and LONG during the combined exercise and recovery period (\( F_{8,64} = 1.24, P = 0.29. \))

Leukocyte counts before second exercise. At the start of the second bout of exercise (1500), concentrations of neutrophils were higher in trial SHORT compared with trial LONG (6.9 ± 0.5 × 10⁹/l vs. 4.8 ± 0.3 × 10⁹/l, \( P = 0.002). \) Conversely, concentrations of lymphocytes were lower in trial SHORT compared with trial LONG (1.7 ± 0.1 × 10⁹/l vs. 2.0 ± 0.1 × 10⁹/l, \( P = 0.02; \) Fig. 5, A and B) with concurrently lower CD4+ and CD8+...
counts in trial SHORT compared with trial LONG (CD4+ cells: 532 ± 47 × 10^6/l vs. 774 ± 86 × 10^6/l, P = 0.016; CD8+ cells: 289 ± 35 × 10^6/l vs. 419 ± 53 × 10^6/l, respectively, P = 0.012; Fig. 6A). No difference between trials was seen in concentrations of CD56+ cells.

**Leukocyte counts during second exercise.** During the second bout of exercise, there were higher concentrations of neutrophils in trial SHORT compared with trial LONG (trial effect: F_{1,2} = 21.6, P = 0.002; Fig. 5A), but there was no difference between the magnitude of increase in the two trials (F_{1,2} = 4.6, P = 0.065). There was no difference between trial SHORT and LONG with regard to the magnitude of change in lymphocytes from pre- to postexercise (F_{1,2} = 0.8, P = 0.41; Fig. 5B). The increase in lymphocyte counts correlated with the increase in E (r = 0.44, P ≤ 0.05). Among the lymphocyte subsets, there were lower concentrations of CD4+ cells in trial SHORT compared with trial LONG during the second bout of exercise (trial effect: F_{1,2} = 8.1; P = 0.02; Fig. 6A), but there was no difference in the magnitude of increase in CD4+ cells between the two trials (F_{1,2} = 3.1, P = 0.09). Among the CD8+ and CD56+ cells, there were slightly larger increases in trial SHORT compared with LONG (F_{1,2} = 5.4, P = 0.049 and F_{1,2} = 5.7, P = 0.042, respectively; Fig. 6B and C).

**Leukocyte counts during recovery.** There were no differences between trials SHORT and LONG in the concentrations of neutrophils during the recovery period from 1630 to 0730 (trial effect: F_{1,24} = 0.9, P = 0.35; Fig. 5A). The 0- to 2-h postexercise increase in neutrophil counts correlated with the increase in E (r = 0.66, P = 0.001), GH (r = 0.54, P ≤ 0.005), and cortisol (r = 0.48, P ≤ 0.01) during exercise. The concentrations of lymphocytes fell to lower levels in trial SHORT compared with trial LONG (trial effect: F_{1,24} = 11.6, P < 0.01; Fig. 5B). The 0- to 2-h postexercise decrease in lymphocyte counts correlated with the exercise-induced increase in E (r = 0.57, P ≤ 0.005), GH (r = 0.47, P ≤ 0.01), and cortisol (r = 0.50, P ≤ 0.01). After 4 h of recovery, lymphocyte counts in both trials were at baseline values, whereas the neutrophil counts were still elevated. At the same time point, the concentrations of CD4+ cells were lower in trial SHORT compared with trial LONG (P = 0.02, Fig. 6A), but among the CD8+ and CD56+ cells there were no differences between the two trials (P = 0.16 and P = 0.91, respectively; Fig. 6, B and C). At the end of the three trials (0730 next morning), there was no difference between the concentrations of any leukocyte subset, and no differences in cell counts were observed from the beginning to the end of each trial.

**Lymphocyte activation.** There was a decrease in the percentage of CD56+ cells expressing the CD69 activation marker (CD56+ CD69+ cells) in both trials SHORT (56%) and LONG (37%) during exercise (time effect: P = 0.030 and P = 0.045, respectively), but no
change in CD4+ CD69+ cells and CD8+ CD69+ cells was seen (Table 1). However, there was no statistical difference in the magnitude of change in percentage of CD56+ CD69+ cells between trials SHORT and LONG ($P = 0.387$). From pre- to postexercise, we also observed a decrease in CD69 fluorescence intensity ratio (CD69 FIR; Table 1) for CD8+ cells and CD56+ cells in both trial SHORT (17 and 26%, respectively) and trial LONG (17 and 24%, time effect: $F = 16.6$ and $F = 16.5$, respectively, both $P < 0.01$), but there were no difference between these trials with regard to the magnitude of change in the fluorescence intensity of these cells ($P = 0.82$ and $P = 0.81$, respectively).

**DISCUSSION**

The present study demonstrated that when a second bout of exercise was performed after 3 h compared with 6 h of rest, increases in E, NE, ACTH, and cortisol were augmented. Furthermore, a more pronounced neutrophilia during the second bout and lymphocytopenia after the second bout were observed in the trial with the shortest rest period. To our knowledge, this is the first study showing that recovery time as such is a significant factor in achieving homeostasis between repeated sessions of endurance exercise.

It is well documented that strenuous exercise recruits both neutrophils and lymphocytes into the circulation. This exercise-induced accumulation of leukocytes is believed to be governed by several factors, including maturity of the cell density and affinity of β-adrenoceptors, as well as adhesion molecules on the surface of endothelial cells and lymphocytes (2, 6, 12, 32, 35). However, during recovery, lymphocyte concentrations rapidly decrease, resulting in lymphocytopenia if exercise intensity and duration are of sufficient magnitude. A contrasting pattern of cell kinetics is seen among neutrophils after exercise, as they continue to accumulate in the circulatory compartment resulting in a second period of neutrophilia (18, 25). This exercise-induced neutrophilia is a result of cell recruitment from margined pools of the intravascular bed, as well as from sequestered pools in the lungs, spleen, and other lymphoid organs (51). In the present study, the changes in neutrophil and lymphocyte concentrations during exercise and recovery followed the patterns of cell trafficking described above (Fig. 5). However, a novel finding of this study was the more pronounced neutrophilia and lymphocytopenia during and after the second bout of exercise in the short rest trial compared with the long rest trial. This could indicate an augmented stress reaction to some of the circulating leukocytes in the short rest trial, and thus a concurrent change in some of the internal functions of these cells may be expected. However, during the second bout of exercise in the short rest trial, we were not able to demonstrate a correspondingly more pronounced decrease in lymphocyte responsiveness measured by the expression of the early activation marker CD69 on any of the lymphocyte subsets (Table 1). Even though we found normalized CD69 expression on the lymphocytes after 4 h of recovery (data not shown), we may have missed a possible difference between the short and long rest trials by not measuring during the immediate postexercise period when lymphocytopenia was most evident.

The current view holds that neuroendocrine factors mediate changes in leukocyte subpopulations in relation to exercise (33, 35), and it has been demonstrated that the immediate exercise effect on several lymphocyte subpopulations is mimicked by intravenous infusion of E (45, 50). The correlation between increases in E and lymphocyte concentrations during exercise observed in the present study supports this view. In keeping with this, it should be expected that the more pronounced increase in catecholamines in the short rest trial of the present study would elicit a larger increase in lymphocytes compared with the long rest trial, and not the same magnitude of change as found in the two trials. However, cortisol has been shown to reduce the number of circulating lymphocytes and in-

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Lymphocyte activation measured as the number of CD4+ T-cells, CD8+ T-cells, and CD56+ natural killer cells that have detectable amounts of the early activation marker (antigen) CD69. The percentage (%) of CD4+CD69+ cells, CD8+CD69+ cells, and CD56+CD69+ cells is an estimate of the proportion of CD69 expression in the total population of CD4+, CD8+, and CD56+, respectively. CD69 FIR (fluorescence intensity ratio) is an estimate of the amount of CD69 markers found on each cell among the CD4+, CD8+, and CD56+ lymphocytes. See METHODS section for more details. Pre-ExA, preexercise activation; post-ExA, postexercise activation; SHORT, 2 bouts of exercise separated by 3 h of rest; LONG, 2 bouts of exercise separated by 6 h of rest.
duce lymphocytopenia (11, 48), a view also supported by the correlation between the exercise-induced increase in cortisol and the postexercise decrease in lymphocyte counts observed in the present study. Consequently, the higher levels of cortisol observed at the end of the short rest trial (Fig. 3) may have counteracted the effect of increased catecholamine levels, resulting in equal changes in total lymphocyte concentrations in the two trials. Nevertheless, the CD56+ cells, being the most catecholamine-responsive subgroup of lymphocytes (24), did show a larger increase from pre- to postexercise in the short rest compared with the long rest trial, and this change correlated with the exercise-induced increase in E.

In the postexercise period, catecholamines were quickly removed from the blood, whereas cortisol remained elevated for several hours (Fig. 2). The changes in both of these hormones were associated with the postexercise lymphocytopenia and may explain in part the exit of lymphocytes from the circulation. However, we suggest that the more pronounced lymphocytopenia during the recovery period in the short compared with the long rest trial mainly is a result of concomitantly higher concentrations of cortisol during recovery. This is further substantiated by the increased ACTH response found in the short rest compared with the long rest trial (Fig. 3A). The more pronounced increase in ACTH in the short rest trial also supports our hypothesis that upon a reactivation of the HPA axis by the second bout of exercise, the duration of rest between the first and second exercise becomes a significant determinant for the magnitude of the corresponding stress response. In contrast to the difference in cortisol response between the short and long rest trial, we observed similar testosterone responses both during and after the second bout of exercise. In accordance with earlier investigations (35), this does not suggest a mechanistic role for testosterone in exercise-induced leukocyte responses, but recent observations indicate that low levels of testosterone can modulate other aspects of immune function such as the cytokine response (14).

According to the established kinetics of neutrophils after exercise (35), it is not surprising to find an increased concentration of neutrophils immediately before the second bout in both trials, with the largest neutrophilia in the short rest trial (39). GH has been found to mediate recruitment of neutrophils into the circulation (17), whereas catecholamines do not seem to play a significant role in this neutrophil mobilization (45). We observed no correlation between the exercise-induced increase in neutrophils and simultaneous changes in GH, catecholamine, or cortisol concentrations. However, the observed association between the postexercise changes in neutrophil and lymphocyte counts and the exercise-induced increases in these hormones adds support to the contention that GH, E, and cortisol play an important role in the stress-induced interaction between the neuroendocrine and immune systems (23).

Several previous investigations have demonstrated that prolonged strenuous exercise strongly activates the neuroendocrine system (35), and this study shows that repeating such exercise within only a few hours results in magnified neuroendocrine stress responses. Given the close interaction between the neuroendocrine and immune systems, it is conceivable that performing daily repeated exercise sessions and thus imposing substantial physical, psychological, and metabolic stress could lead to both adaptive and maladaptive alterations in the immune system. The number of hours of rest, as well as the replenishment of lost calories and fluids after exercise, may represent crucial factors that could bring about increased tolerance or susceptibility to various challenges to the immune system and thereby influence the subsequent clinical outcome. With regard to the biological significance of our findings, it has been shown that cortisol mobilizes sequestrated neutrophils to the circulatory compartment and inhibits migration of neutrophils out of the circulatory compartment (7, 47). This may reduce the initial response of the innate immune system to challenges like infection, inflammation, and trauma in extravascular tissue with subsequently impaired microbial killing capacity, anti-inflammatory action, and tissue regeneration. Another biological aspect of exercise-induced neutrophilia is the activation of these cells toward increased oxidative burst and degranulation with subsequent release of reactive oxygen species and increased oxidative stress (37, 44, 46). There is disagreement on the “cut-off point” between beneficial and detrimental effects of such activation, but a larger increase in the exercise-induced neutrophilia in the short rest trial may represent a negative effect on neutrophil-linked aspects of innate immunity.

Postexercise lymphocytopenia is a well-documented finding linked to both intensity and duration of the prior exercise and the associated cortisol response (18, 25, 30, 35). It has been argued that a reduction in the number of circulating lymphocytes (mostly T cells and NK cells), along with a potential decrease in proliferation rate (28, 30, 49) and cytotoxic capacity in the blood (29, 43), could indicate reduced host protection against several microbes. In the present investigation, we demonstrated a suppression of mitogen-induced activation of CD56+ (NK) cells after a second bout of exercise. Together with reduced IgA levels in saliva of the upper respiratory tract (15, 22), these alterations may be of clinical importance to elite athletes, assuming they represent a temporary immune impairment during the immediate recovery period after repeated sessions of strenuous exercise (21, 31). Furthermore, increased levels of corticosteroids have been linked to alterations in the immune system, including increased apoptosis in T lymphocytes, decreased phagocytosis and antigen presentation in macrophages, and a shift in cytokine secretion from Th1 to Th2 profile in T helper cells (35, 40, 52). A reduced competence in resisting bacterial and viral infections may be one of the consequences of such changes. Thus it is not surprising, considering the prolonged increased levels of cortisol found in the trials.
with two exercise sessions, that certain functions of the immune system might be negatively affected by repeated exercise sessions on the same day. Moreover, catecholamines have also been shown to induce potentially immune suppressive effects. Reduced adherence to the endothelial wall with subsequently reduced diapedesis and chemotaxis among lymphocytes has been observed as a result of increased catecholamine exposure, even within the physiological range observed during exercise (6, 7, 42).

In conclusion, a second bout of strenuous endurance exercise resulted in more pronounced increases in catecholamines, ACTH, and cortisol, as well as an augmented neutrophilia and postexercise lymphocytopenia when the previous bout of exercise was performed only 3 h as opposed to 6 h earlier. These observations indicate that certain aspects of neuroendocrine-immune interaction and function may be increasingly affected if repeated bouts of exercise are performed with reduced recovery time between the sessions. The findings could have practical implications regarding the training and recovery schedule for athletes and possibly the management of repeated exposure to other nonexercise stressors.

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