Anaerobic exercise induces moderate acute phase response

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ABSTRACT

MEYER, T., H. H. W. GABRIEL, M. RÄTZ, H. J. MÜLLER, and W. KINDERMANN. Anaerobic exercise induces moderate acute phase response. *Med. Sci. Sports Exerc.*, Vol. 33, No. 4, 2001, pp. 549–555. **Purpose:** It was intended to compare the immune reaction after single and repeated short bouts of anaerobic exercise. **Methods:** Twelve unspecifically trained male subjects (27 ± 2 yr, 75 ± 2 kg, $\dot{V}O_{2peak}$ 52 ± 2 mL·min^{-1}·kg^{-1}) performed one 60-s all-out test (SMT) on a cycling ergometer and the same test followed by eight 10-s all-out tests every 5 min (AN-TS). These tests and one control day (Co-Day) were applied in randomized order. At rest and 15 min, 2 h, and 24 h after cessation of exercise the following venous blood parameters were determined: concentration of neutrophils and ($CD16^+$-) premacrophages (both flow-cytometrically), interleukin 6 and 8 (IL-6, IL-8), C-reactive protein (CRP) and cortisol. **Results:** Two hours after cessation of exercise the neutrophils increased stronger after AN-TS than after SMT ($P < 0.01$). The peak in the number of premacrophages occurred earlier after SMT (15 min post; $P < 0.01$ to Co-Day) than after AN-TS (2 h post; $P < 0.05$ to Co-Day). IL-6 was elevated at 15 min and 2 h after AN-TS ($P < 0.01$ to SMT and Co-Day) but only slightly 2 h after SMT ($P < 0.01$ to Co-Day). There were no significant changes in IL-8. CRP was the only elevated parameter 24 h postexercise exclusively after AN-TS ($P < 0.05$ to Co-Day). **Conclusion:** Repeated short anaerobic bouts of cycling lead to an acute phase response, which is more pronounced than after a single bout. Athletes should take care in performing such training sessions several times a week because signs of inflammation are detectable even 24 h after cessation of exercise. **Key Words:** ANAEROBIC TRAINING, MONOCYTES, PREMACROPHAGES, CYTOKINES

In several athletic disciplines, training sessions commonly include large amounts of anaerobic work. Single short bouts of anaerobic exercise have been shown to induce a moderate delayed leukocytosis without relevant signs of inflammatory activation similar to infectious disease (8,13). Such signs would be an activation of monocytes, i.e., an increase in the number of $CD16^+$-monocytes (7,21), an increase of interleukin 6 (10), and an increase of the C-reactive protein (CRP (1)). Athletes only seldom perform single bouts of anaerobic work during their regular training. They usually repeat several "intervals." At present, it is unknown which immunological reactions such typical training sessions evolve.

It is a frequent observation that periods of intense training precede susceptibility to infections (4). Some authors speculate on the responsibility of a chronically depressed immune system for this phenomenon (6,12,14,17). Repetitive immune stimulations due to anaerobic training could be a triggering mechanism for a reactive down-regulation of immune defense functions.

Additionally, it has been postulated that the appearance of overtraining and/or overstrain (staleness) is closely coupled to incompletely cured infections. Changes in the functional status of immune cells have been proposed to be involved in the induction of overtraining which is characterized by a lack of improvement or even a deterioration of athletic performance despite continuing training. This assumption seems reasonable because of the well-established link between the immune system and the function of the autonomic nervous system which is thought to be compromised in overtraining. Thus, a chronically or too frequently activated sympathetic system could lead to changes of immune function and, consequently, overtraining (9,11,16,20). This represents another argument in favor of more closely investigating the immune response to common anaerobic training sessions.

Therefore, the aim of this study was to test a typical anaerobic training session on its ability to induce an acute phase response of the immune system. Some efforts were made to exactly describe the degree of anaerobism being induced and the adrenocorticotropic reaction. For purposes of comparison a single short bout of maximal exercise was performed as well as a quiet resting control condition.

TABLE 1. Anthropometric date, heart volume, maximal oxygen uptake, maximal lactate concentration, maximal workload, and workload at the IAT in the incremental graded exercise test (means ± SEM).

Age	(yr)	26.9 ± 1.5
Height	(cm)	182 ± 2
Body mass	(kg)	75.1 ± 2.2
Body fat	(%)	12.3 ± 1.2
Heart volume$_{relative}$	(mL·kg^{-1})	12.6 ± 0.4
$\dot{V}O_{2max}$	(mL·min^{-1}·kg^{-1})	51.6 ± 1.7
Lactate$_{max}$	(mmol·L^{-1})	11.8 ± 0.9
Workload$_{max}$	(W)	319 ± 13
Workload$_{IAT}$	(W)	214 ± 12

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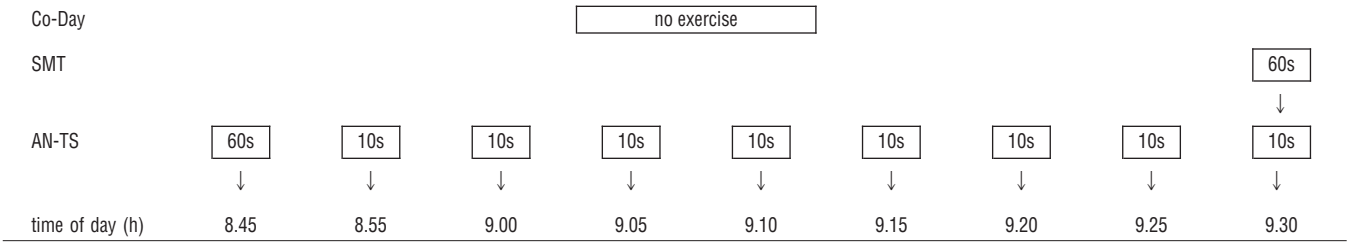
TABLE 2. Overview of the study design; sequence of exercise bouts.



METHODS

Subjects

Twelve male students of physical education took part in the study. They were not specifically endurance-trained but actively involved in various kinds of sports for 6–8 h weekly on average. Before the start of the study, subjects provided written informed consent.

At the first appointment and before the start of the study, all subjects were screened for their health status by a physician: present and past clinical and training history, physical examination, anthropometric measurements including skin folds by caliper method, resting ECG, and maximal incremental graded bicycle ergometry (starting with 100 W, 3 min-stages, lactate measurements to calculate the individual anaerobic threshold = IAT (18)), including ECG and indirect measurements of blood pressure. In addition, one- and two-dimensional echocardiography was conducted and the heart volume calculated (modified Simpson rule (2)). Routine laboratory parameters were taken at that time to exclude actual and chronic diseases. Subjects reporting recurrent infections of any kind were excluded from the study. Anthropometric data are shown in Table 1.

Experimental Design

In random order and within 5–8 d, the participants had three appointments at the institute's lab: two for exercise tests and one as a control day without exercise. They came in at 08:00 a.m. after an overnight fast, and they were instructed to avoid strenuous exercise on the preceding 2 d. One day served as control day (CO-Day) without physical activity. On another day the subjects did one 60-s all-out test (single maximal test, SMT) on a cycle ergometer (Lode Excalibur, Groningen, The Netherlands) in the upright position at 9:30 a.m. On a further day at 8:45 a.m., the same 60-s all-out test was done followed by 10-min rest and subsequently 8 all-out tests for 10 s each with 4:50-min rest in between them (anaerobic training session, AN-TS). So the last 10-s test was done at 9:30 a.m. corresponding to the time of SMT (overview of time schedule in Table 2). On each of the exercise and control days a total of four venous blood samples was drawn by venipuncture of a superficial cubital arm vein at 8:30, 9:45, 11:30 a.m., and at 8:30 a.m. of the following day. The exact list of parameters being determined at each time point is shown in Table 3.

Laboratory Methods

Postexercise blood parameters were corrected for plasma volume changes (3).

Immunophenotyping

Direct immunophenotyping of whole blood leukocytes was done with directly conjugated monoclonal antibodies (mAb) and acquisition of FCM 1.0 list mode data with five-parameter flow-cytometry (FACScan, Becton Dickinson, Heidelberg, Germany). In detail, 20-μL EDTA-blood was incubated for 20 min (room temperature, darkness) with antiCD45-FITC (fluoresceinisothiocyanate; clone: Immu 19.2) + antiCD14-PE (phycoerythrin; clone: RMO52) conjugate for leukocyte subpopulations and antiCD16-FITC (clone: 3G8) + antiCD14-PE (clone: RMO52, all mAb Coulter-Immunotech, Hamburg, Germany) for monocyte subpopulations ($CD14^+CD16^-$ = regular monocytes; $CD14^+CD16^+$ = premacrophages), respectively, in fourfold saturation concentrations (after appropriate preceding experiments). Erythrocytes were lysed and leukocytes fixed using the Lysis solution® (Becton Dickinson). Cells were washed once (200 g, 5 min, room temperature) and diluted in 200-μL phosphate-buffered saline (PBS) for subsequent measurement in the flow cytometer. Within 4 h after blood sampling, list mode files were collected from 15,000 events within a live-gate around lympho-, mono-, and granulocytes in the forward vs sidescatter display. For determination of monocyte subpopulations, a gate was set in the forward vs side scatter. Percentages of $CD16^+$ monocytes were calculated from a CD14/CD16 dot plot.

TABLE 3. Overview of the parameters determined from venous blood samples.

	Total Neutrophils $CD16^+$ Monocytes CRP Cortisol Interleukin 6/8	Total Neutrophils $CD16^+$ Monocytes Cortisol Interluekin 6/8	Total Neutrophils $CD16^+$ Monocytes Interluekin 6/8	Total Neutrophils $CD16^+$ Monocytes Interleukin 6/8 CRP
Time of day (h)	8.30 = rest	9.45 = 15 min post	11.30 = 2 h post	8.30 = 24 h post

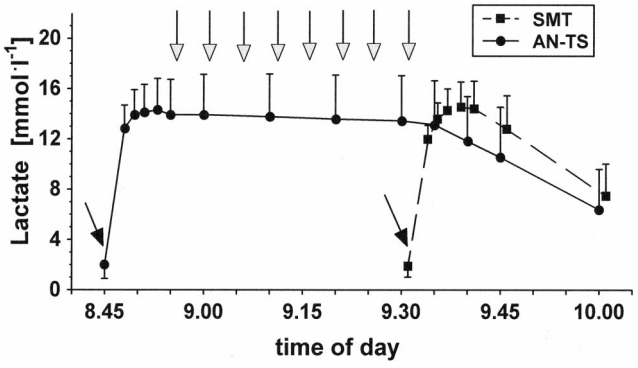


FIGURE 1—Concentrations of lactate in capillary blood. Gray arrows indicate the 10-s tests during AN-TS, black arrows mark the 60-s tests during SMT and AN-TP, respectively.

Cytokines

Plasma samples were taken for determination of interleukin-6 (IL-6) and interleukin-8 (IL-8) concentrations by enzyme-immunoassay (Immunotech, Hamburg, Germany). The sensitivity was 3 pg·mL^{-1}, and 10 pg·mL^{-1} for the IL-6 assay, and the IL-8 assay, respectively. IL-1α, IL-1β, IL-2, TNFα, and GM-CSF up to a concentration of 1 μg·mL^{-1} did not interfere with the IL-6 detection. Intra-assay coefficients of variance were between 1.3 and 6.8% (IL-6), and between 4 and 6% (IL-8). Interassay coefficients of variance were 7.9–14.6% (IL-6), and 3–8% (IL-8), respectively. All samples of the same subject were measured in duplicates in the same assay.

Cortisol

An enzyme-immunoassay (Serono, Freiburg, Germany) served for determination of serum cortisol levels. All samples from each athlete were analyzed in the same assay as duplicates.

Other Parameters

Lactate concentrations were measured enzymatically in capillary whole blood taken from the hyperemized earlobe (LacT, MPR2, Boehringer Mannheim, Germany) at rest and 3, 4.5, 6, 8, 10, 15, and 30 min after cessation of exercise in SMT as well as at rest, 3, 4.5, 6, 8, and 10 min after the first 60-s bout, immediately after each pair of the subsequent 10-s bouts, and 5, 10, 15, and 30 min after completion of the last bout in AN-TS. The pH was determined from capillary blood (ion-selective electrode; Ciba Corning, Fernwald, Germany) at rest, 6, 15 min, and 2 h post exercise. No measurements of lactate concentrations were performed at the Co-Day because in healthy subjects resting values can be expected. C-reactive protein was determined turbidimetrically (Biomed, Oberschleiβheim, Germany).

Data Analysis

The Kolmogoroff-Smirnov test was done to test for normal distribution ($P > 0.2$). As many parameters were not normally distributed, it was decided to uniformly use the Wilcoxon test for pair differences. Therefore, this procedure was applied to comparisons between conditions and between time points of blood sampling. Linear correlational analysis was done by Spearman's rank correlation coefficient. Data are presented as medians (and quartiles for CRP and cortisol). An α-level of $P < 0.05$ was considered significant. Statistical calculations were processed using Statistica software (Stat soft, Tulsa, OK).

RESULTS

SMT led to an increase of blood lactate concentrations to maximal values of 14.6 ± 2.0 mmol·L^{-1} and a reduction of blood pH to 7.16 ± 0.08. During AN-TS the mean lactate values were between 13 and 14.5 mmol·L^{-1}. The blood pH 6 min after cessation of AN-TS was 7.17 ± 0.07. The exact course of lactate concentrations and pH-values is shown in Figures 1 and 2, respectively. No changes of pH were detected on Co-Day.

SMT and AN-TS induced an average decrease in plasma volume of 13% and 15%, respectively. On Co-Day, no significant change in plasma volume was detected.

Neutrophils, Monocytes

Two hours after cessation of SMT and AN-TS a neutrophilia was detected, which was significantly larger for AN-TS ($P = 0.003$ and $P = 0.002$ to SMT and Co-Day, respectively; $P < 0.01$ to all other times of blood sampling). On the next day, there were no differences in the number of neutrophils in comparison to Co-Day any more (Fig. 3).

The absolute number of CD 16^{+} monocytes rose faster after SMT than after AN-TS. So 15 min after cessation of exercise in SMT, the CD16^{+} monocytes were significantly higher than resting values ($P = 0.004$) and higher than under both other conditions ($P = 0.003$ for both comparisons). Two hours after exercise there was a significantly higher number of activated monocytes in AN-TS ($P = 0.019$ to Co-Day; NS to SMT; $P = 0.01$ to rest; $P = 0.002$ to 15 min post; NS to 24 h post). In the morning of the next day, average (but not median) values still remained at 1.5

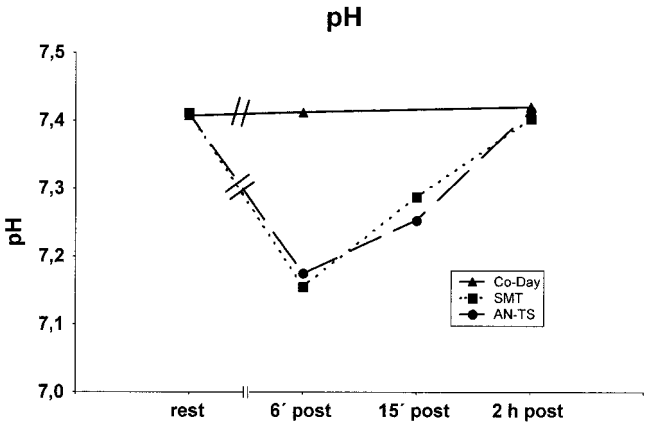


FIGURE 2—pH-values in capillary blood before and after SMT, AN-TP, and Co-Day, respectively (means).

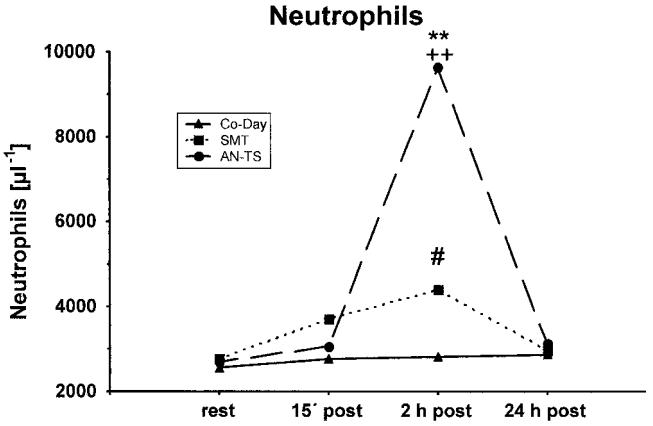


FIGURE 3—Neutrophils before and after SMT, AN-TP, and Co-Day, respectively. + Significant difference between AN-TS and SMT (+ $P < 0.05$; ++ $P < 0.01$); * significant difference between AN-TS and Co-Day (* $P < 0.05$; ** $P < 0.01$); # significant difference between SMT and Co-Day (# $P < 0.05$; ## $P < 0.01$); Wilcoxon test; $N = 12$ (medians).

times the Co-Day concentrations but due to the large variability of individual results no statistically significant differences were detected (Fig. 4). For the percentages of $CD16^+$ cells from the total number of monocytes no differences could be detected between the three conditions.

Interleukin 6 and 8

Fifteen minutes after AN-TS IL-6 increased to about 6 times the resting concentrations. This represented a significant difference to SMT, Co-Day, and the other times of blood sampling ($P = 0.002$ for all comparisons). The slight rise in IL-6 values after SMT at this point in time reached significance in comparison to Co-Day ($P = 0.003$). AN-TS still led to increased concentrations of IL-6 after 2 h ($P = 0.004$ and 0.009 to Co-Day and SMT, respectively; Fig. 5).

The concentrations of IL-8 stayed remarkably constant during the whole measurement period. No changes in IL-8 values could be documented in comparison to control values and within the conditions. Thus, neither SMT nor AN-TS induced any relevant increases in IL-8.

C-Reactive Protein

Twenty-four hours after AN-TS CRP-concentrations were significantly higher than under both of the other experimental conditions and significantly higher than the respective resting values ($P = 0.02$; Fig. 6). No such differences were detected for SMT. There was no correlation between the CRP increase and any of the other measured parameters. The three highest values for CRP 24 h after exercise were 5.9, 5.7, and 4.6 $ng \cdot mL^{-1}$, respectively. CRP at 24 h post exercise correlated highly with IL-6 at 15 min post exercise in AN-TS ($r = 0.79$, $P < 0.01$) but not in SMT.

Cortisol

Cortisol showed the expected circadian rhythm on the Co-day (decline between 8:00 and 9:45 a.m.). After SMT, there was a slight increase compared to the Co-Day ($P = 0.002$) without a difference to resting values. After AN-TS concentrations reached 3.5 times the Co-Day values on average ($P = 0.002$) and resting values were exceeded ($P = 0.003$; Fig. 7). The neutrophil concentration at 2 h post exercise correlated significantly with the cortisol increase at 15'p in AN-TS only (SMT: $r = 0.48$, $P = 0.11$; AN-TS: $r = 0.63$, $P = 0.028$).

DISCUSSION

The main finding of this study was a marked difference in the pattern of the neutrophils', $CD16^+$-monocytes', and IL-6's reaction between single and repeated bouts of anaerobic work (AN-TS). AN-TS induced a long-lasting lactic acidosis, which led to an increase in cortisol and IL-6 15 min after cessation of exercise. This was followed by a neutrophilia 2 h later and increased concentrations of the C-reactive protein 24 h later. Thus, in humans, repeated anaerobic work induces cellular and humoral changes more suggestive of an acute phase reaction than single bouts of anaerobic exercise. This is important for the athletic population as well as for physicians being involved in their care because it may indicate a diminished loading capacity for at least 24 h after such training sessions. It can be assumed that the amount of anaerobic training has to be limited in order to avoid too frequent stimulations of the immune system and prevent overtraining (20).

Increased concentrations of IL-6 and CRP are well-established markers of the acute phase reaction (7,10), which is usually preceded by a release of stress hormones. These criteria were fulfilled at least partly by AN-TS but hardly by SMT. IL-6 and CRP (although only measured at rest and 24 h after exercise) went up after AN-TS, and on the basis of the documented acidosis and lactate concentrations, it can be firmly assumed that both training sessions were accompanied by an increase in catecholamine concentrations. Furthermore, it was already documented that a single maximal

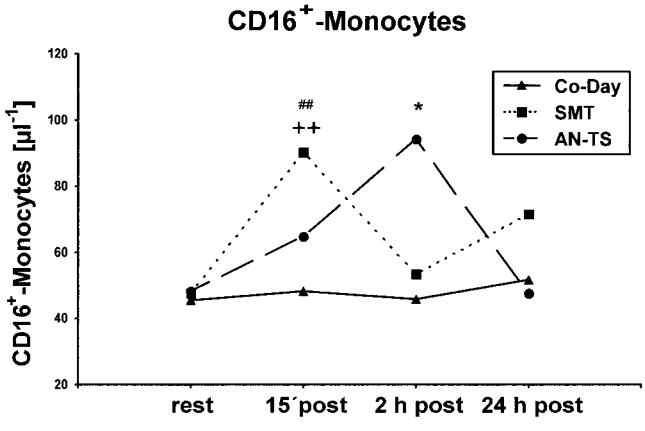


FIGURE 4—Total concentrations of $CD16^+$-monocytes before and after SMT, AN-TP, and Co-Day, respectively. + Significant difference between AN-TS and SMT (+ $P < 0.05$; ++ $P < 0.01$); * significant difference between AN-TS and Co-Day (* $P < 0.05$; ** $P < 0.01$); # significant difference between SMT and Co-Day (# $P < 0.05$; ## $P < 0.01$); Wilcoxon test; $N = 12$ (medians).

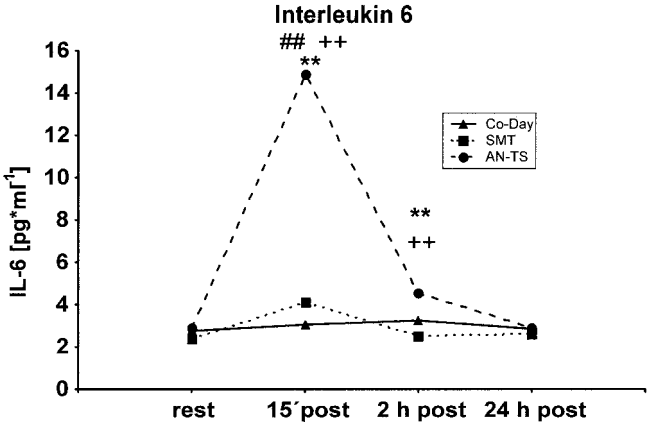


FIGURE 5—Interleukin-6 (IL-6) before and after SMT, AN-TP, and Co-Day, respectively. + Significant difference between AN-TS and SMT (+ $P < 0.05$; ++ $P < 0.01$); * significant difference between AN-TS and Co-Day (* $P < 0.05$; ** $P < 0.01$); # significant difference between SMT and Co-Day (# $P < 0.05$; ## $P < 0.01$); Wilcoxon test; $N = 12$ (medians).

60-s test of the same type as presented in this study, namely SMT, leads to a large secretion of adrenaline and noradrenaline (8).

An important cellular reaction to the anaerobic stress was a $CD16^+$-monocytosis, which occurred earlier after SMT than after AN-TS. This points to different mechanisms being responsible for the monocytosis. It cannot be conclusively decided from our data if a real activation or merely a release of cells being located at the vessel walls (i.e., marginal pool) is responsible for the detected increase in $CD16^+$-cells. Catecholamines can lead to such changes on a short-term basis only. Therefore, they are not responsible for the delayed $CD16^+$-monocytosis we observed after AN-TS. Exogenously elevated levels of cortisol (e.g. by injection) usually induce a decrease in $CD16^+$-monocytes (22), and an activation of the endothelium would also rather make the premacrophages stick to the vessel walls. Therefore, stronger mechanisms must override these attenuating influences. It has to be assumed that either a larger number of monocytes expresses CD16 after AN-TS or there is a more pronounced appearance from other pools like the bone marrow. Tracer studies may have the potential to elucidate the source of premacrophages. We assume that the additional premacrophages after SMT primarily stem from the marginal pool (8), whereas the elevation after AN-TS represents a "real" activation of the monocyte system or a differentiation of cells from the bone marrow. The percentage of these activated cells in relation to the total number of monocytes did not change under any of the tested conditions as it would be expected in acute infections.

Activated monocytes are one of the sources of IL-6 that stimulate the production and release of CRP by hepatocytes (1, 21). The hypothesis that longer durations of elevated $CD16^+$-monocytes lead to higher values of CRP due to prolonged IL-6 elevations seems justified. This is in accordance with our observation that the increase in $CD16^+$ cells after a single bout of anaerobic work is temporary only, whereas it appears later after AN-TS. It cannot be decided from our data if the latter is a long-lasting $CD16^+$-monocytosis. But it seems tempting that the integral (area under the curve) for the number of activated monocytes is bigger than after SMT or Co-Day. In other words, more premacrophages would have the opportunity to produce and excrete IL-6 over a longer period of time. On the other hand, the earlier peak of IL-6 points to additional sources for this cytokine during the first min after exercise.

No increase of IL-8 followed the $CD16^+$-monocytosis in both exercise conditions (22). IL-8 is secreted by monocytes (among other cells of the immune system), and, thus, its concentration could theoretically correlate with their total number in the circulation (22). In this study, however, the time pattern of blood sampling might be responsible for the lack of an IL-8 elevation after AN-TS. Cytokine concentrations in peripheral blood reflect the "spill over" of the local tissue production, which means that a low local cytokine production of IL-8 is not necessarily seen in peripheral blood. This might be an explanation for the absence of an IL-8 increase following AN-TS.

It is well known that single bouts of 1-min all-out cycling exercise cause a moderate delayed neutrophilia. A sampling time 2 h after exercise is appropriate (8,15) to detect it. The SMT induced the expected neutrophilia, but the increase in the concentration of neutrophils was much smaller than after AN-TS. This observation corresponds well to the differential reaction of cortisol which was significantly higher 15 min after AN-TS than after SMT. Cortisol has been shown to be mainly responsible for the delayed leukocytosis, which is due to a neutrophilia (review: 1). Fry et al. (5) were able to show similar increases in cortisol and concentrations of leukocyte subpopulations within 5 min after 15 high-intensity 1-min bouts on a treadmill in seven subjects. These authors did not measure further humoral parameters.

It is beyond the limits of the study design to elucidate the detailed mechanisms being responsible for all observed cellular changes after repeated bouts of anaerobic work. The absolute fall in pH was not different between SMT and AN-TS but the duration of lactate concentrations above 10

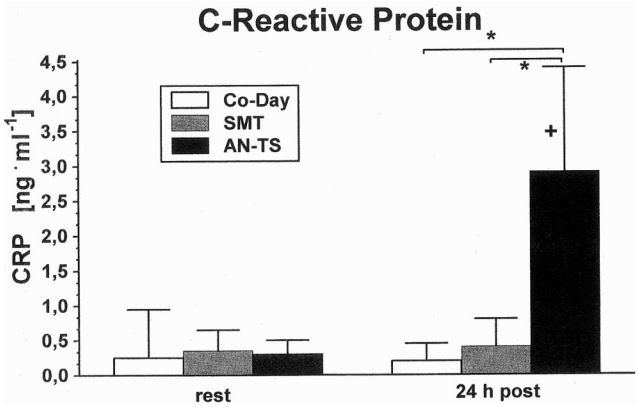


FIGURE 6—CRP before and after SMT, AN-TP, and Co-Day, respectively. * Significant difference between conditions (AN-TS, SMT, and Co-Day), $P < 0.05$; + Significant difference between resting values; Wilcoxon test; $N = 12$ (medians and quartiles).

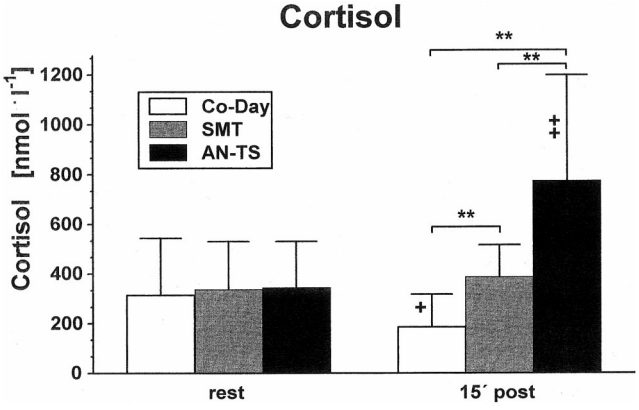


FIGURE 7—Cortisol before and after SMT, AN-TP, and Co-Day, respectively. ** Significant difference between conditions (AN-TS, SMT, Co-Day), $P < 0.01$; + Significant difference between resting values ($P < 0.05$; ++ $P < 0.01$); Wilcoxon test; $N = 12$ (medians and quartiles).

mmol·L^{-1} in AN-TS exceeded 1 h, whereas in SMT after 15 min, a relatively steep decline in lactate concentrations was noted. pH values were not measured simultaneously with lactate concentrations over the whole duration of AN-TS but a constantly lowered pH over 1 h can be safely assumed. *In vitro*, it was shown earlier that lactic acidosis induces an acute phase response in neutrophils. Especially an induction of the fMLP-induced oxidative burst could be documented (personal unpublished observations). The assumption of a similar activation in monocytes seems warranted.

One of our findings implicates a clinically important discovery. At least three of the tested subjects showed concentrations of the C-reactive protein approaching or even exceeding the common cut-off value for acute infections which is set at 5 mg·L^{-1} for adults (19). This observation leads to the recommendation of taking into account the training history from the past 24 h when interpreting CRP values slightly above this threshold. Interval training alone (if including a high number of anaerobic bouts) might produce CRP concentrations in this range.

We created a laboratory test to simulate anaerobic interval training. The Wingate test represents a well-investigated model for a single high intense exercise bout but an appropriate model for repeated anaerobic work was still lacking. From our point of view AN-TS might be regarded as an easily administered tool to study effects of anaerobic work not only in immunological research. The lactate concentrations measured and the resulting pH values clearly show that the aim of inducing a lactic acidosis of considerable duration was reached without exceeding the physical limits of our not specifically trained subjects. Therefore, the test seems to represent a good simulation of anaerobic interval training sessions commonly performed by athletes. The lactate concentrations measured were comparable to those reported by Fry et al. (5), who used 15 1-min intervals on a treadmill at 120% of maximal velocity from an incremental test with breaks of 2 min. However, their protocol was dependent on pretesting results, which makes its administration more costly.

In conclusion, indications for an acute phase reaction after repeated bouts of anaerobic exercise could be documented. Cellular and humoral parameters show a pattern suggestive of an activation of monocytes. Even 24 h after the anaerobic training session, an increase in CRP concentrations was observed. These changes were more pronounced than after a single 60-s all-out test and partly showed a different pattern. The results have important clinical implications because athletes commonly perform training sessions similar to the tested conditions. It is recommended to take care in performing strenuous training within 24 h after repeated anaerobic workouts, which induce lactic acidoses of long duration.

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REFERENCES

1. BAUMANN, H., and J. GAULDIE. The acute phase response. *Immunol. Today* 15:74–80, 1994.
2. DICKHUTH, H. H., A. URHAUSEN, M. HUONKER, et al. Die echokardiographische Herzgrößenbestimmung in der Sportmedizin (Determination of heart volume in sports medicine using echocardiography). *Dtsch. Z. Sportmed.* 41:4–12, 1990.
3. DILL, D. B., and D. L. COSTILL. Calculation of percentage changes in volume of blood, plasma, and red cells in dehydration. *J. Appl. Physiol.* 37:247–248, 1974.
4. FITZGERALD, L. Overtraining increases the susceptibility to infection. *Int. J. Sports Med.* 12:S5–S8, 1991.
5. FRY, R. W., A. R. MORTON, G. P. M. CRAWFORD, and D. KEAST. Cell numbers and in vitro responses of leucocytes and lymphocyte subpopulations following maximal exercise and interval training sessions of different intensities. *Eur. J. Appl. Physiol.* 64:218–227, 1992.
6. FRY, R. W., A. R. MORTON, P. GARCIA-WEBB, G. P. M. CRAWFORD, and D. KEAST. Biological responses to overload training in endurance sports. *Eur. J. Appl. Physiol.* 64:335–344, 1992.
7. GABRIEL, H., and W. KINDERMANN. The acute immune response to exercise: What does it mean? *Int. J. Sports Med.* 18:S28–S45, 1997.
8. GABRIEL, H., A. URHAUSEN, and W. KINDERMANN. Mobilization of circulating leucocyte and lymphocyte subpopulations during and after short, anaerobic exercise. *Eur. J. Appl. Physiol.* 65:164–170, 1992.
9. GABRIEL, H., A. URHAUSEN, G. VALET, U. HEIDELBACH, and W. KINDERMANN. Overtraining and immune system: a longitudinal study in endurance athletes. *Med. Sci. Sports Exerc.* 30:1151–1157, 1998.
10. HAAHR, P. M., B. K. PEDERSEN, A. FOMSGAARD, et al. Effect of physical exercise on in vitro production of interleukin 1, interleukin 6, tumor necrosis factor-alpha, interleukin 2 a, tumor necrosis factor-alpha, interleukin 2 and interferon-gamma. *Int. J. Sports Med.* 12:223–227, 1991.
11. KUIPERS, H., and H. A. KEIZER. Overtraining in elite athletes. *Sports Med.* 6:79–92, 1988.
12. NIEMAN, D. C., L. S. BERK, M. SIMPSON-WESTERBERG, et al. Effects of long-endurance running on immune system parameters and

lymphocyte function in experienced marathoners. *Int. J. Sports Med.* 10:317–323, 1989.
13. NIEMAN, D. C., D. A. HENSON, R. JOHNSON, L. LEBECK, J. M. DAVIS, and S. L. NEHLSEN-CANNARELLA. Effects of brief, heavy exertion on circulating lymphocyte subpopulations and proliferative response. *Med. Sci. Sports Exerc.* 24:1339–1345, 1992.
14. NIEMAN, D. C., L. M. JOHANSSEN, J. W. LEE, and K. ARABATZIS. Infectious episodes in runners before and after the Los Angeles Marathon. *J. Sports Med. Phys. Fitness* 30:316–328, 1990.
15. NIEMAN, D. C., S. L. NEHLSEN-CANNARELLA, K. M. DONOHUE, et al. The effects of acute moderate exercise on leukocyte and lymphocyte subpopulations. *Med. Sci. Sports Exerc.* 23:578–585, 1991.
16. PEDERSEN, B. K., and H. BRUUNSGAARD. How physical exercise influences the establishment of infections. *Sports Med.* 19:393–400, 1995.
17. SHEPHARD, R. J., T. J. VERDE, S. G. THOMAS, and P. SHEK. Physical activity and the immune system. *Can. J. Sport Sci.* 16:169–185, 1991.
18. STEGMANN, H., W. KINDERMANN, and A. SCHNABEL. Lactate kinetics and individual anaerobic threshold. *Int. J. Sports Med.* 2:160–165, 1981.
19. THOMAS, L. *Labor und Diagnose (Laboratory and Diagnosis)*. Marburg: Medizinische Verlagsgesellschaft, 1995, p. 781.
20. URHAUSEN, A., H. GABRIEL, and W. KINDERMANN. Blood hormones as markers of training stress and overtraining. *Sports Med.* 20: 251–276, 1995.
21. ZIEGLER-HEITBROCK, H. W. L. Heterogeneity of human blood monocytes: the CD14+CD16+ subpopulation. *Immunol. Today* 17:424–428, 1996.
22. ZIEGLER-HEITBROCK, H. W. L., M. STROBEL, D. KIEPER, et al. Differential expression of cytokines in human blood monocyte subpopulations. *Blood* 79:503–511, 1992.