Increased training load and the β-adrenergic-receptor system on human lymphocytes

K. SCHALLER, D. MECHAU, H. GROSSE SCHARMANN, M. WEISS, M. BAUM, AND H. LIESEN
Institute of Sports Medicine, University of Paderborn, 33098 Paderborn, Germany

Schaller, K., D. Mechau, H. Grosse Scharmann, M. Weiss, M. Baum and H. Liesen. Increased training load and the β-adrenergic-receptor system on human lymphocytes. J. Appl. Physiol. 87(1): 317–324, 1999.—The influence of increased training on the sympathoadrenergic system was investigated. Moderately trained male subjects (n = 15) increased their training within 10 wk by 60% eight of the subjects increased their training volume, and seven increased their training intensity. Before and after the training, an exhaustive treadmill exercise was carried out. Acute treadmill exercise increased β-adrenergic receptor number on mononuclear lymphocytes, isoproterenol-stimulated cAMP production, and plasma catecholamine concentration. The increase of receptor number can at least partially be explained by a changed lymphocyte composition at rest and after exercise. After training, the exercise-induced increase of β-adrenergic receptor number was significantly blunted, and the exercise-induced increase of isoproterenol-stimulated cAMP production per β-receptor was enhanced. Subjects who experienced increased symptoms of physical discomfort and/or mood changes showed an enhanced cAMP production after training. These findings point to an altered regulation of the receptor and postreceptor mechanisms as an effect of a 10-wk period of hard training.

catecholamines; adenosine 3',5'-cyclic monophosphate; lymphocyte subsets; exercise; overtraining

IT HAS BEEN WELL DOCUMENTED that short-term exhaustive exercise (4, 6, 7, 11, 16, 32) and short-term infusion of adrenergic agonists (26, 29) upregulate the β-adrenergic-receptor number on mononuclear lymphocytes (MNL) and intracellular isoproterenol (Iso)-stimulated production of cAMP of MNL. Less is known about the effects of long-term training on the sympathoadrenergic system. Endurance training as well as long-term infusion of adrenergic agonists seem to downregulate the β-adrenergic-receptor density on MNL with a concomitant reduced cAMP production (11, 12, 20).

The regulation of the sympathoadrenergic system seems to play an important role in training effects and in the development of overtraining (5, 18, 28). Both increased and decreased basal catecholamine concentrations have been shown in overtrained athletes (9, 17), pointing to a dysregulation of the sympathoadrenergic system, possibly depending on the stage of overtraining (9). In different periods of endurance training or high-intensity training in several sports disciplines (11), receptor density and responsiveness may be changed in a different manner. Consequently, receptor regulation and postreceptor mechanisms may be involved in adaptation to increasing training or, on the other hand, may be responsible for symptoms of overload, overreaching, or early phases of overtraining. Therefore, we studied the effect of an acute bout of exercise on β-adrenergic-receptor density on MNL, Iso-stimulated cAMP-production of MNL, and the plasma concentration of catecholamines before and after 10 wk of increased volume of training as well as increased intensity of training.

METHODS

Training Groups and Training Program

Healthy, drug-free, moderately trained male students (n = 15) participated in the study after having given their informed written consent. The subjects were allowed, depending on their personal preferences, to choose one of two training groups to ensure that they would achieve their training program. The two groups had to complete a training program of progressively increasing training load during 10 wk. The high-volume group (HV group; n = 8 subjects, age 26 ± 3 yr, height 182 ± 3 cm, weight 76.6 ± 5.1 kg) increased their training volume by increasing the number of sessions per week as well as by increasing the distance from 30.7 ± 18.3 to 48.2 ± 19.3 km/wk (see Table 1 for details). The intensive group (I group; n = 7 subjects, age 24 ± 2 yr, height 183 ± 8 cm, weight 77.3 ± 8.3 kg) increased their training intensity by introducing more interval training into their training program and adding high-speed races. At the beginning of the training period they ran 22.2 ± 7.5 km/wk below the speed at the anaerobic threshold and 8.9 ± 5.5 km/wk above the speed at the anaerobic threshold; after training they ran 10.7 ± 7.9 km/wk below and 18.7 ± 12.3 km/wk above the speed at the anaerobic threshold (see Table 1 for details).

Each subject received an individual training plan referring to his personal ventilatory anaerobic threshold. Every week the subjects had to perform a control test (50-m sprint, 300-m sprint, 45-min run with measurement of the distance covered). Training plans were adapted every 2 wk according to the personal daily training protocol and the results of the weekly control test. After 10 wk of training, the subjects had increased their training by ~60% (see Table 1 for details).

To monitor their physical and mood status, subjects had to answer a questionnaire based on the one described by Lehmann et al. (18): they rated weekly their physical complaints in general, complaints directly after training, and complaints in the morning after the training. In detail, “score A” asked for the following: well behavior (very well to bad = 0–2 points), illness [no symptoms = 0 points or points = number of symptoms like headache, cold, arthralgia, myalgia, fever below (1 point) or above (2 points) 38.5°C multiplied by number of days the symptoms lasted], illness-induced training pause (points = number of failing training sessions), signs of musculoskeletal overload like tendopathia, muscle soreness, complaints concerning joints (points = number of symptoms multiplied by number of days the symptoms lasted). In “score B,” subjects rated in comparison with the week before (0–2 points for unchanged, decrease, or increase) their appetite, resting heart rate in the morning, weight, sleep, performance in general or in test results (50-m sprint, 300-m sprint, 45-min run), and accomplishing daily activities.
Venofix winged infusion set (Braun), blood was collected in evacuated monoject blood collection tubes (leveling-off phenomenon of oxygen uptake) and immediately after exhaustion. Venous blood samples were taken before the treadmill exercise (after a 30-min rest period), immediately after 3 min of recovery after the exercise (Woodway treadmill). The test was performed between 8:00 and 10:00 AM after a day without training to exclude effects of short-term fatigue. The subjects were asked for the feeling after the end of each training session (easier, unchanged, harder). In a similar three-point scale, we asked for the feeling after the end of each training session (well to very exhausted) and in the morning after the training session (regenerated to worn out) on the average that week. Furthermore, the subjects rated their mood in general and mood in the course of the 10-wk period. In this way, by an increasing index of complaints and mood changes we were able to judge in a qualitative experience-based manner whether or not particular subjects tolerated increasing training load.

### Treadmill Exercise Bout and Blood Sampling

At the beginning and the end of the study, the subject’s ventilatory anaerobic threshold was determined according to Wasserman et al. (30) by increasing exhaustive treadmill exercise (Woodway treadmill). The test was performed between 8:00 and 10:00 AM after a day without training to exclude effects of short-term fatigue. The subjects were allowed to have a small breakfast without coffee or tea. Running speed started at 8 km/h at a constant slope of 1%. The speed of 3 km/h was increased every 3 min by 2 km/h until subjective exhaustion (leveling-off phenomenon of oxygen uptake). Minute ventilation, oxygen uptake, and carbon dioxide production were measured with EOS-Sprint (Jaeger). Venous blood samples were collected in vacutainers containing ice-cold EGTA and reduced glutathione (Amersham) for determination of catecholamines and was immediately centrifuged at 4°C (1,000 g, 10 min). An additional 45 ml of blood were collected in a 50-ml Falcon tube (Becton Dickinson) containing 5 ml of 1% Na-EDTA for cell preparation (receptor and cAMP assay), and a further 5 ml were collected in evacuated mononect blood collection tubes with K-EDTA (Sherwood Medical) for the cell counting and fluorescence-activated cell sorter analysis.

### Cell Separation

**β-Adrenergic Receptors**

- Adrenergic receptors were determined on MNL by radioligand assay.

  **Cell separation.** The venous blood anticoagulated with EDTA was diluted 1:1 with NaCl (0.9%), and lymphocytes were isolated by density-gradient centrifugation (Lymphoprep, Nycomed Pharma, Oslo, Norway). After the centrifugation (800 g, 20 min, 25°C), the separated lymphocytes were washed twice (250 g, 10 min, 25°C) with NaCl (0.9%) and resuspended in PBS at a fixed number of 5 × 10⁶ cells/ml.

  **β-Adrenergic-receptor radioligand assay.** Aliquots of 400 μl lymphocytes (5 × 10⁶ cells/ml) were incubated with 100 μl [³H]CGP-12177 (5 final concentrations in the range 0.8–17 nM) and 100 μl PBS. To define the unspecific binding, 100 μl propanol (final concentration 60 × 10⁻⁵ M) were used instead of the buffer. The incubation of 30 min at 37°C was stopped by the addition of 5 ml ice-cold NaCl (0.9%) and rapid filtration over Whatman GF/C fiberglass filters. The filters were washed with 10 ml NaCl. The specific activities of wet-cell-containing filters were determined after adding 3 ml

### Table 1. Training data of subjects in high-volume group and intensive group

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>HV group</td>
<td>3.5 ± 2.1</td>
<td>3.0</td>
<td>3.5</td>
<td>3.2</td>
<td>3.8</td>
<td>3.7</td>
<td>3.5</td>
<td>3.8</td>
<td>4.2</td>
<td>5.0 ± 1.2</td>
</tr>
<tr>
<td>I group</td>
<td>3.0 ± 0</td>
<td>3.0</td>
<td>3.8</td>
<td>3.5</td>
<td>3.7</td>
<td>3.8</td>
<td>4.3</td>
<td>5.0</td>
<td>4.8</td>
<td>4.5 ± 1.6</td>
</tr>
<tr>
<td>Total distance, km/wk</td>
<td>30.7</td>
<td>26.3</td>
<td>30.5</td>
<td>27.2</td>
<td>35.9</td>
<td>38.0</td>
<td>31.4</td>
<td>36.8</td>
<td>43.6</td>
<td>48.2</td>
</tr>
<tr>
<td>HV group</td>
<td>22.2</td>
<td>16.0</td>
<td>20.9</td>
<td>16.3</td>
<td>17.4</td>
<td>16.1</td>
<td>16.1</td>
<td>15.5</td>
<td>13.6</td>
<td>10.7</td>
</tr>
<tr>
<td>I group</td>
<td>8.9</td>
<td>11.5</td>
<td>17.0</td>
<td>15.4</td>
<td>12.1</td>
<td>15.8</td>
<td>17.3</td>
<td>22.9</td>
<td>17.8</td>
<td>18.7</td>
</tr>
<tr>
<td>HV group</td>
<td>32.4 ± 19.6</td>
<td>28.4</td>
<td>32.5</td>
<td>28.1</td>
<td>36.9</td>
<td>38.6</td>
<td>33.7</td>
<td>38.9</td>
<td>44.4</td>
<td>48.9 ± 19.5</td>
</tr>
<tr>
<td>I group</td>
<td>31.0 ± 8.8</td>
<td>27.5</td>
<td>37.9</td>
<td>31.7</td>
<td>30.0</td>
<td>31.9</td>
<td>33.4</td>
<td>38.4</td>
<td>38.4</td>
<td>29.4 ± 17.6</td>
</tr>
</tbody>
</table>

Values are means and means ± SD. HV group, high-volume group; I group, intensive group; VAT, ventilatory anaerobic threshold according to Wasserman et al. (30). For details see text.

### Table 2. Index of complaints and mood changes

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score A</td>
<td>0.93</td>
<td>0.29</td>
<td>1.50</td>
<td>2.93</td>
<td>0.79</td>
<td>2.21</td>
<td>2.07</td>
<td>0.36</td>
<td>1.17</td>
<td>0.75</td>
</tr>
<tr>
<td>HV group</td>
<td>0.83</td>
<td>0.75</td>
<td>0.33</td>
<td>0.17</td>
<td>0.5</td>
<td>1.5</td>
<td>1.25</td>
<td>1.75</td>
<td>3.25</td>
<td>3.67</td>
</tr>
<tr>
<td>Score B</td>
<td>2.21</td>
<td>1.57</td>
<td>0.43</td>
<td>3.17</td>
<td>2.14</td>
<td>2.0</td>
<td>2.14</td>
<td>2.14</td>
<td>4.17</td>
<td>3.25</td>
</tr>
<tr>
<td>HV group</td>
<td>1.92</td>
<td>1.92</td>
<td>2.75</td>
<td>1.58</td>
<td>3.67</td>
<td>5.0</td>
<td>5.92</td>
<td>4.67</td>
<td>6.25</td>
<td>4.50</td>
</tr>
<tr>
<td>Sum score A + score B</td>
<td>3.14 ± 1.25</td>
<td>1.86</td>
<td>1.93</td>
<td>6.1</td>
<td>2.93</td>
<td>4.21</td>
<td>4.21</td>
<td>2.5</td>
<td>5.34</td>
<td>4.0 ± 1.93</td>
</tr>
<tr>
<td>HV group</td>
<td>2.75 ± 1.61</td>
<td>2.67</td>
<td>3.08</td>
<td>1.75</td>
<td>4.17</td>
<td>6.5</td>
<td>7.17</td>
<td>6.42</td>
<td>9.5</td>
<td>8.17</td>
</tr>
</tbody>
</table>

Score A represents signs of physical discomfort and illness, and score B represents changes in well behavior and mood state. For details see text.
of a scintillation cocktail (Rotiscint Eco Plus, Carl Roth, Karlsruhe, Germany) in a scintillation counter [Packard Tri-Carb 4530 (35634) United Technologies Packard].

Data analysis. Data were analyzed according to the method of Scatchard (22).

cAMP

As an indicator for responsiveness of the β-adrenergic receptors, the basal production of cAMP by MNL as well as the cAMP production after the stimulation by Iso were determined. The cAMP assay was performed in duplicate, following the protocol as described previously (25). The venous blood was prepared in the same way as for the β-adrenergic-receptor assay. After centrifugation (800 g, 20 min) and washing twice (250 g, 10 min, 25°C) in NaCl (0.9%), the cells were resuspended in theophylline buffer (120 mM NaCl, 5 mM KCl, 1.2 mM MgSO4 ·7 H2O, 15 mM NaOOCH3 ·3H2O, 1 mM Na2-EDTA, 10 mM glucose, 10 mM HEPES, 1 mM ascorbic acid, 0.1% human serum albumin, and 1.6 µg/ml theophylline) at a fixed number of 2 × 10^6 cells/ml. Aliquots (500 ± 1) of isolated mononuclear cells were stimulated by addition of 50 µl Iso (1 and 10 µM final concentrations) and incubated for 2 min. The basal cAMP production was determined by incubation without Iso. The incubation was stopped by centrifugation (7 min, 400 g, 25°C). For complete destruction of the cells, the pellet was resuspended in 500 µl of lysing solution (50 mmol/l Na acetate + 1.6 µg/ml theophylline), incubated 15 min at 8°C, and finally boiled in a water bath for 15 min. After centrifugation (20 min, 1,000 g), the cAMP-containing supernatant was collected and frozen at −20°C until detection with a commercially available radioimmunoassay (NEN-DuPont, Dreieich, Germany).

The results for the stimulation of the two concentrations of Iso were similar. Therefore, results are only shown for the final concentration of 1 µl Iso. The cAMP production was calculated by subtracting the cAMP concentration of non-stimulated cells (basal production) from the concentration of stimulated cells. To get information about the receptor function, we also calculated the cAMP production per β-adrenergic receptor as follows: cAMP production per 10^6 cells/β-receptor number per cell resulting in cAMP production per β-receptor × 10^9.

Catecholamines

Free and sulfoconjugated catecholamines (epinephrine, norepinephrine) in plasma were determined by high-performance liquid chromatography separation as described elsewhere (31).

Flow-Cytometric Analysis of Lymphocytes

White blood cells were counted by a blood analyzer (Sysmex CC180, TOA Medical Electronics). Lymphocytes were analyzed in whole blood by monoclonal antibodies (Becton Dickinson, Heidelberg, Germany) conjugated with FITC or phycoerythrin (PE). One hundred microliters of venous blood were incubated with 10 µl antibody solution for 15 min at room temperature in the dark, and then 500 µl lysing solution (Becton Dickinson) were added. After 10 min the incubation was stopped by addition of 2 ml PBS and centrifugation (250 g, 5 min, 25°C); the cells were washed with 2 ml PBS. The pellet was resuspended in sheath fluid (Becton Dickinson), and the immunofluorescence was measured with a flow cytometer (FACScan, Becton Dickinson). An analysis of the relative proportion of each subset was obtained by electronic gating of lymphocytes based on forward and sideward scatter parameters and on simultaneous staining of leukocytes (CD45, FITC labeled) and monocytes (CD14, PE labeled), resulting in a nonlymphocyte contamination of <5% in the gate. The gate contained at least 95% of all lymphocytes. The absolute cell number in each subset was calculated on the basis of total lymphocytes. The lymphocyte subsets were identified by the following antibodies: CD3 (FITC) for T cells; CD4 (FITC) for helper/inducer T cells, CD3/CD16/CD56 (PE) for natural killer (NK) cells.

Statistics

All values are shown as means ± SD. Statistical calculations were performed using the statistical software package SPSS (SPSS Software, Munich, Germany). The statistical analysis was carried out by ANOVA. When appropriate, matched pairs were compared post hoc by using the nonparametric Wilcoxon test. Differences between the two groups were determined post hoc by the Mann-Whitney U-test. The significance levels were set at P < 0.05.

RESULTS

The training had no effects on maximal oxygen uptake [HV group: 55.8 ± 5.1 ml·min⁻¹·kg⁻¹ before training (BT) compared with 57.0 ± 3.3 ml·min⁻¹·kg⁻¹ after training (AT); I group: 59.5 ± 6.0 ml·min⁻¹·kg⁻¹ BT compared with 59.6 ± 6.8 ml·min⁻¹·kg⁻¹ AT] and on respiratory minute ventilation (HV group l/min: 141.2 ± 18.6 l/min BT compared with 144.1 ± 13.7 AT, I group: 156.5 ± 14.3 BT compared with 154.9 ± 13.6 l/min AT). The type of training (HV group: pure endurance training; I group: high-intensity training) had no effects on the results of the weekly test for the evaluation of performance (50-m sprint time, 300-m sprint time, 45-min running distance), nor did test results change significantly within the 10-wk period (data not shown). Furthermore, we did not find a significant influence of the training type on the other determined parameters. Therefore, the results were pooled and are shown for the whole training group. Compared with the whole group, four subjects of HV group and three subjects of I group showed a higher incidence of infections as well as longer lasting exhaustion, mood and sleep disturbances, increased anger, and reduced force for a duration of >1 wk, especially at the end of the 10-wk period; their index for psychophysiological scores increased above the median values of the whole group in the last 2 wk of training (mean ± SD of whole group; see Table 2). Two persons did not answer their questionnaire. The remaining six subjects had no or only small changes in their indexes. Nevertheless, we found statistically significant differences between these subgroups of seven vs. six subjects only in the behavior of the Iso-stimulated cAMP production. Thus first the results are shown for the whole training group.

Effects of Acute Exercise

The treadmill exercise increased significantly the β-adrenergic-receptor density (P < 0.01 BT, P < 0.05 AT) (Fig. 1), the plasma catecholamine concentration (P < 0.001 BT and AT) (Fig. 2), and the cAMP produc-
As an index of the β-adrenergic receptor function, we calculated the Iso-stimulated cAMP production per receptor. Before training, the Iso-stimulated cAMP production per β-adrenergic receptor remained unchanged by exercise. After training, the Iso-stimulated cAMP production per β-adrenergic receptor increased significantly by exercise (P = 0.05) (Fig. 3).

Exhaustive treadmill exercise caused a significant leukocytosis (P < 0.01 BT and AT) and lymphocytosis (P < 0.01 BT and AT) (Table 3). Because lymphocyte subsets differ in their β-adrenergic-receptor density, we also had to consider the lymphocyte subset composition. Acute physical stress increased significantly the absolute numbers of lymphocytes (P < 0.01 BT and AT) and lymphocyte subsets (P < 0.01 BT and AT) (Table 3). The relative numbers of CD3⁺/CD16⁻/CD56⁺ (NK) cells were significantly increased by treadmill exercise (P < 0.05 BT, P < 0.01 AT), whereas the relative numbers of CD3⁺ cells (P < 0.01 BT and AT) and CD4⁺ cells (P < 0.01 BT and AT) decreased after stress (Table 3).

Influence of Training on the Effects of Exhaustive Treadmill Exercise

After the training phase the exercise-induced increase of β-adrenergic-receptor number was significantly lower (P < 0.05) compared with pretraining values (Fig. 1), and the exercise-induced increase of catecholamines (Fig. 2) was slightly blunted after training (no significance). After training, we observed a slightly enhanced increase of the cAMP production (Fig. 3). Before training, we did not determine an exercise-induced increase in cAMP production per β-adrenergic receptor; after training we observed a significant (P < 0.01) increase (Fig. 3).

The training period did not modify the effects of an acute bout of exercise on the absolute lymphocyte subset counts and the lymphocyte composition (Table 3).

DISCUSSION

Although within an increasing number of weekly training sessions I group dramatically reduced the percentage of moderate-training intensity and HV group performed exclusively long-distance training sessions...
with an intensity below the ventilatory anaerobic threshold, ANOVA revealed no training-induced differences between the two groups in any determined parameter. Therefore, only results of all subjects as a whole group are discussed.

Effects of Acute Exhaustive Treadmill Exercise

In agreement with earlier investigations (2, 4, 7, 16, 32), we determined a significant increase in β-adrenergic-receptor number, plasma catecholamine concentration, and cAMP production after acute exhaustive treadmill exercise. Werle et al. (32) observed an increase of receptor density on isolated CD4⁺ and CD8⁺ cells after strenuous short-term exercise. More recent investigations suggest that the increased receptor number after exercise may be at least partially an effect of the different redistribution of lymphocyte subsets after exercise. Maisel and co-workers (19) explain the exercise-induced increase of β-adrenergic-receptor density of mixed lymphocytes by the redistribution of lymphocytes subsets in the circulation that differ in their receptor number. Fujii et al. (6) reported that dynamic exercise induces the translocation of β-adrenergic receptors from the inside of lymphocytes to the outside.

Table 3. Absolute cell numbers of granulocytes, lymphocytes, and some lymphocyte subsets and lymphocytes subset composition in percentage of lymphocytes

<table>
<thead>
<tr>
<th>Before Training</th>
<th>After Training</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
</tr>
<tr>
<td>Granulocytes, cells/µl</td>
<td>3,218 ± 575</td>
</tr>
<tr>
<td>Lymphocytes, cells/µl</td>
<td>1,358 ± 289</td>
</tr>
<tr>
<td>CD3⁺, cells/µl</td>
<td>920 ± 313</td>
</tr>
<tr>
<td>CD4⁺, cells/µl</td>
<td>528 ± 223</td>
</tr>
<tr>
<td>CD3⁺/CD16⁺/CD56⁺, cells/µl</td>
<td>205 ± 139</td>
</tr>
<tr>
<td>CD3⁺, %lymphocytes</td>
<td>67 ± 7</td>
</tr>
<tr>
<td>CD4⁺, %lymphocytes</td>
<td>37 ± 11</td>
</tr>
<tr>
<td>CD3⁺/CD16⁺/CD56⁺, %lymphocytes</td>
<td>15 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SD. P values are values at rest compared with values after exhaustive exercise (Exhaust). No differences between values before training and after training could be observed.
resulting in a higher receptor number and function determined by Iso-stimulated cAMP production. With respect to the redistribution of lymphocytes, they concluded that redistribution effects can only partially explain the increase in receptor number. According to Van Tuits et al. (29), who reported a recruitment of younger cells with a different receptor density after acute exercise, both redistribution effects and changes in receptor number are involved in the upregulation of β-adrenergic-receptor density after a single bout of exercise.

Because of the very large amount of blood necessary for the assay, we did not determine receptors on separated lymphocyte subpopulations in our investigation. NK cells, which are known to have a very high receptor density (14), increase in the most remarkable way after exercise. CD8+ cells and B cells are also known to possess a high receptor number (15, 29, 19). CD8+ and NK cells increase during exercise (14, 15, 29, 19), whereas B cells are mainly unaffected by acute exercise (24) and thus will not contribute in a major way to shifts in receptor number due to acute exercise. The CD8+ marker is coexpressed on some NK cells (23). Schedlowski and co-workers (23) found an increase of NK (CD16+, CD56+) and CD8+ cells after 20-min infusion of epinephrine: the increase of the CD8+ cells could be explained by the increase of NK cells coexpressing the CD8+ marker (23). In our investigation the percentage of CD3+ and CD4+ decreased by acute exercise; thus there could have been only a minor increase in CD8+ cells. Such a small increase can be explained by the pronounced increase of NK cells that we observed, part of which coexpressing the CD8+ marker. Thus the increased receptor number after acute exercise in our study could have been an effect of the differential lymphocyte mobilization. It remains to be elucidated whether this cell mobilization is an effect of simple redistribution or, according to Van Tuits et al. (29), depends on the appearance of younger cells with a different receptor number.

Effects of the Training Period

The plasma catecholamine concentration was not significantly affected by training in our investigation. We only observed a slightly blunted exercise-induced increase of catecholamines after training. Previous investigations reported an increased norepinephrine excretion in stale swimmers (9), or a reduced catecholamine excretion of resting subjects after intensified training, that was considered as an effect of hypothalamic dysfunction (18). Hooper and co-workers (9) assumed a dual catecholamine response to overtraining depending on the stage of overtraining, and Urhausen and co-workers (27) suggested that the type of overtraining (addisonoid/basedowoian) might influence the catecholamine reaction. Our results reporting only a minor change in plasma catecholamine concentration after 10 wk of intensified training suggest that there is no clear border between the catecholamine reaction to training and the catecholamine reaction to an early stage of overreaching.

We observed a blunted β-adrenergic-receptor upregulation after exercise as an effect of the 10 wk of increasing training load. In agreement with investigations on endurance-trained athletes (3, 11) and long-term infusion of adrenergic agonists (1), this downregulation of β-adrenergic receptors seems to be an adaptation to training as a consequence of the frequently enhanced catecholamine concentration during the training sessions. In contrast to the reaction to acute exercise, there is a real change in receptor number as an effect of long-term training: The composition of lymphocytes remained unchanged after training, but the increase of receptor number after strenuous exercise was lower after training.

In contrast to the downregulation of β-adrenergic receptors, the cells showed a sensitized reaction to adrenergic stimulation. The exercise-induced increase of the cAMP production was slightly enhanced after the training period. This may be due to the fact that after the training period the seven subjects with higher psychophysiological indexes, who experienced increased signs of discomfort, delayed recovery, susceptibility to infections, and other symptoms of overload, had a significantly increased Iso-stimulated cAMP production compared with pretraining values. Figure 4 differentiates between the above-mentioned seven subjects with (“maladapted”) and the six subjects without changes in their physical and mood state (“well adapted”). The latter subjects showed identical values and reactions to exercise before and after the training period. Previous investigations describe a downregulation of β-adrenergic receptors of MNL, stimulatory G protein (Gs), and cAMP production of lymphocytes after endurance training (11, 20, 21) or after long-term infusion of adrenergic agonists (12).

In contrast to the above-mentioned authors, at the end of the training period, we observed in the group as a whole a higher cAMP production and cAMP production per receptor, whereas receptors were downregulated.
Because NK cells and CD8\(^+\) cells have a high receptor number, this might have been an effect of increased percentage of NK or CD8\(^+\) cells (14, 29). Nevertheless, relative and absolute lymphocyte numbers remained unchanged by training. Moreover, the cAMP production per receptor, a parameter including the decreased receptor number and the increased cAMP production, was also higher after training. Consequently, the enhanced exercise-induced increase of cAMP production can be considered as enhanced receptor function. Hammond et al. (8) observed in pigs after 7–9 wk of running training a decreased receptor density with a concomitant increase of Gs\(\_\alpha\) indicating an increased receptor function. Similarly, in our investigation we too found a reduced β-adrenergic receptor upregulation with sensitized postreceptor mechanisms but only in those seven athletes with increased score indexes of illness and vegetative and mood changes.

In conclusion, we found that 10 wk of increased training sensitizes the postreceptor system in MNL, which might affect the cell function. Because NK cells possess a high receptor density (14) and play an important role in antiviral defense, and because β-adrenergic stimulation seems to suppress immune function (33), altered sensitivity to adrenergic stimuli might partially explain the increased susceptibility to infections described for overtraining (5, 17).

In summary, after acute exercise we observed an increase of plasma catecholamine concentration, β-adrenergic-receptor density and cAMP production. The increased receptor number after a single bout of exercise could at least partially be explained by redistribution effects of lymphocyte subsets. In contrast, the 10 wk of a high training load led to a blunted receptor reaction to acute exercise with a concomitant increased receptor sensitivity and cell function during acute exercise; these effects were independent of the type of training. Our results point to a central role of the sympathetic regulation in the physiological reaction to acute exercise and training. Cellular mechanisms show the most sensitive reaction. Further investigations on overtraining should include the β-adrenergic receptor system and the postreceptor function.

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