Intensive resistance exercise induces lymphocyte apoptosis via cortisol and glucocorticoid receptor-dependent pathways


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Intensive resistance exercise induces lymphocyte apoptosis via cortisol and glucocorticoid receptor-dependent pathways. J Appl Physiol 110: 1226–1232, 2011. First published March 10, 2011; doi:10.1152/japplphysiol.01295.2010.—Intensive endurance exercise is known to induce lymphocyte apoptosis, which might affect immune function. Less is known about the effects of resistance exercise on apoptosis and its underlying mechanisms. In this study, subjects performed an intensive resistance test (IRT) and a moderate resistance test, and lymphocyte apoptosis, apoptosis-related parameters, and underlying mechanisms were investigated. IRT induced a significant increase of lymphocyte apoptosis 3 h after exercise, which was accompanied by a significant decrease of mitochondrial membrane potential, a reduction of Bcl-2, and an upregulation of the CD95 receptor. Blood lactate, IL-6, C-reactive protein, and cortisol increased significantly 3 h after IRT. A significant correlation was observed between the increase of apoptosis and cortisol levels 3 h after IRT. Incubation of freshly isolated lymphocytes in IRT serum indicated an important role of serum correlates for apoptosis induction. Selective incubation of lymphocytes in concentrations of selected serum parameters corresponding to levels found post in IRT serum demonstrated a major role for cortisol in apoptosis induction. This result was confirmed by attenuation of apoptosis after addition of mifepristone before incubation in IRT serum. In summary, resistance exercise induced lymphocyte apoptosis in an intensity-dependent way. Furthermore, cortisol signaling via glucocorticoid receptors might be an important mechanism for lymphocyte apoptosis after resistance exercise.

Strengthen or resistance training RT is an integral part of athletes’ training to develop discipline-specific exercise performance. Furthermore, its popularity as leisure-time activity has constantly increased (4). To stimulate regular adaptation toward specific training goals, progressive and intensive resistance test (IRT) protocols are necessary. Compared with endurance exercise, RT is accompanied by lower oxygen demands and cardiorespiratory drive. Instead, RT is focused on exercising specific muscle groups, and exercise intensity can be modulated by the weights lifted and numbers of repetitions (1). Physiologically, RT is accompanied by strong, local glycogen depletion, excessive lactate production, and acidosis, which are limiting factors of exercise performance. As a consequence of high muscle tension and eccentric contractions, muscular integrity is disturbed, resulting in muscle damage and a low-grade inflammatory reaction (15). Further characteristics of RT include a stress reaction indicated by the activation of the hypothalamic-pituitary-adrenocortical axis, followed by release of cortisol from the adrenal cortex (5).

Accordingly, heavy RT protocols elicit systemic metabolic, inflammatory, and hormonal changes (2). Some of these factors are well known to target lymphocytes in the circulation and have the potential to induce cellular activation and apoptosis (30). Despite the wide area of application and different user groups, little is known about activating or detrimental effects on immune function. Some studies that focused on these aspects were specifically designed to induce muscle damage or were performed with unusual exercise loads, both of which do not reflect RT under practical conditions (34). Increased lymphocyte activation and apoptosis after exercise were demonstrated for endurance exercise of different duration and intensity-like marathon runs, intensive treadmill running, and cycle ergometer exercise. In these studies, apoptosis induction was only observed after exceeding a specific intensity of exercise, whereas no effects were demonstrated after moderate exercise programs (17, 30). In this context, the question about the effect of different exercise modes and the underlying molecular mechanisms of lymphocyte apoptosis emerged.

The mediators of exercise-induced lymphocyte apoptosis have been discussed intensely. It was demonstrated that a change of extra- or intracellular pH levels regulates apoptosis in different cell types (14). In this regard, intensive exercise is followed by an increased lactate production and decreased blood pH, which might affect cell death (10). In addition, recently, Samuvel et al. (24a) demonstrated that lactate acts directly as a signaling molecule affecting inflammatory gene expression in macrophages. Furthermore, intensive exercise induces a systemic inflammatory response accompanied by an increase of acute-phase proteins and cytokines. It was repeatedly demonstrated that intensive exercise increases levels of TNF-α, IL-6, and C-reactive protein (CRP), which are known to promote apoptosis in different cell types (8). Similarly, glucocorticoids (GC), such as cortisol, are well known to trigger lymphocyte apoptosis (22). Evidence for a GC pathway in exercise-induced apoptosis comes from studies showing that corticosterone administration at a level similar to that observed during exercise induced apoptosis in mouse thymocyte culture (13). In addition, pretreatment of rodents with mifepristone (MIF) before an exercise protocol inhibited thymocyte DNA fragmentation (23).

The purpose of the current study was to determine the effect of either an IRT or a moderate resistance test (MRT) on lymphocyte apoptosis and activation. Therefore, we designed a whole body resistance exercise test that consisted of eight
different strength exercises, which were performed at defined intensities. Furthermore, we tried to find out which signaling pathways induce apoptosis. Since RT induced metabolic, inflammatory, and hormonal changes in serum, we tested in an in vitro study if lactate, IL-6, CRP, or cortisol of similar levels to those measured during the IRT affects lymphocyte apoptosis. We hypothesized that RT induces lymphocyte activation and apoptosis in an intensity-dependent manner. Furthermore, we assume that these alterations are induced by serum correlates of RT.

**MATERIALS AND METHODS**

**Subjects.** Fifteen male subjects [age (years) 26.86 ± 1.01, weight (kg) 78.92 ± 2.81, body mass index (kg/m²) 24.33 ± 0.79] were informed about the nature, purpose, and potential risks of the study. All subjects were nonsmokers, nonmedication users, infection free, and cardiopulmonary risk free. Participants’ written, informed consent was obtained according to the Declaration of Helsinki. The Ethical Committee of the Justus-Liebig-University Giessen (Germany) had approved the study. Before inclusion in the study, all subjects were medically screened and approved by a physician as being healthy. None of the subjects were specifically trained in either strength or endurance exercise, which means that they did not perform regular exercise training of more than 3 h/wk. In addition, all subjects refrained from strenuous physical activity 48 h before each exercise protocol.

**Exercise testing.** Subjects were tested for their one repetition maximum (1RM) on eight different devices designed for RT after a short warm-up (16), which consisted of 5–10 repetitions at an estimated 40–50% of maximum. The results were used to control relative work amongst individuals at the same relative intensity.

**Resistance exercise protocols.** RT exercise protocols regularly started at 8:30 AM. After a general warm-up and a specific warm-up session consisting of 15 repetitions at 30% of 1RM on each training devise, the subjects performed a whole body RT program including eight different exercise sets: bench press, latissimus pull-downs, seated rows, shoulder press, leg press, shoulder pull-downs, biceps curls, and leg curls. Resistance exercise intensity for the IRT was defined individually as 75% of the 1RM. In contrast, exercise intensity for the MRT was defined as 60% of the 1RM. The results were used to control relative work amongst individuals at the same relative intensity.

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**Lymphocyte counts.** Blood cell counts, hemoglobin, and hematocrit determinations were performed on plasma anticoagulated with EDTA using a semiautomated hematology analyzer (F-820, Sysmex, Nordrstedt, Germany).

**Cell isolation procedure.** Lymphocytes were prepared by density gradient centrifugation. After centrifugation, the lymphocyte band between the sample layer and the Percoll solution was removed. Cell viability was about 98%, as demonstrated by Trypan blue exclusion, whereas purity was about 95%, as checked by flow cytometry in the forward- and sideward-scatter mode.

**Analysis of apoptosis-related cellular surface markers, activation status, and mitochondrial membrane potential.** Lymphocytes were labeled with Annexin-V FITC and nuclear propidium iodide (PI) uptake for detection of apoptosis and necrosis, respectively. The mitochondrial membrane potential (MMP) was analyzed using the fluochrome stain DiOC₆ (Invitrogen, Darmstadt, Germany). In brief, 10³ cells were stained in a solution with 40 nm DiOC₆ for 30 min. For further detection of lymphocyte phenotype and function, cells were labeled with specific antibodies against CD95, CD69 (ImmuNoTools, Friesoythe, Germany), and CD95 ligand (BioLegend, San Diego, CA). Analyses were performed using an EPICS XL flow cytometer (Beckman Coulter, Krefeld, Germany).

**Bcl-2 levels.** Detection of Bcl-2 protein content was performed by Western immunoblotting using an enhanced chemiluminescence Western blotting detection system (Amersham Pharmaica Biotech, Piscataway, NJ). Lymphocytes were lysed with cell lysis buffer. Twenty micrograms of each protein sample was subjected to SDS-PAGE, followed by blotting onto an Immobilon polyvinylidene difluoride membrane. Primary antibodies for the detection of Bcl-2 (8C8) and GAPDH (FL-335) (Santa Cruz Biotechnology, Santa Cruz, CA) were used. Western blot data for GAPDH were included as a control for equal protein loading.

**Inflammatory and hormonal parameters.** Serum samples for the hormonal analyses were kept frozen at −20°C until assayed. Concentration of human TNF-α, IL-6, and CRP was detected by high-sensitivity ELISA (Diaclone, Hö lz el, Cologne, Germany). Cortisol concentrations were measured by electrochemiluminescence immunoassay using a Cobas immunoassay system. Since cortisol levels show a distinct circadian rhythm, we included a control session that compared cortisol concentrations with the respective time point of exercise sessions.

**Preparation and culture of lymphocytes.** To analyze the effect of serum parameters on lymphocyte apoptosis, lymphocytes were isolated from nonexercised, healthy controls. Cell viability was ~98%, as demonstrated by trypan blue exclusion, whereas purity was ~95%, as determined by flow cytometry by the forward/sideward-scatter mode. In a first approach, we incubated cells for 24 h in serum taken before exercise (con control serum) and serum taken 3 h after IRT (IRT serum). Serum concentrations in all approaches were 50%. To further elucidate the effect of different serum correlates, cells were cultured for 24 h in medium after addition of L-lactate (16 mmol/l; Sigma, St. Louis, MO), recombinant (r)IL-6 (25 pg/ml; ImmunoTools), rCRP (4 µg/ml; ImmunoTools), or hydrocortisone (30 µg/dl; Sigma). Concentrations were derived from the highest concentrations observed 3 h after IRT. In lactate experiments, L-lactate was first solved in PBS, and pH was adjusted to 7, 4 to mimic puffer capacity of blood. Medium contained RPMI 1640, 5% FCS, 1% l-glutamine, and 1% penicillin/streptomycin, and cells were incubated in a humidified atmosphere with 5% CO₂ at 37°C. To obtain information whether apoptosis was induced by protein molecules, IRT serum was

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**Table 1. IRM and numbers of repetitions for each strength device**

<table>
<thead>
<tr>
<th>Device</th>
<th>Bench Press</th>
<th>Latissimus Pull-Downs</th>
<th>Seated Rows</th>
<th>Shoulder Pull</th>
<th>Leg Press</th>
<th>Triceps Curls</th>
<th>Biceps Curls</th>
<th>Leg Curls</th>
</tr>
</thead>
<tbody>
<tr>
<td>1RM, kg</td>
<td>55.56 ± 5.56</td>
<td>63.17 ± 4.79</td>
<td>62.5 ± 3.95</td>
<td>39.56 ± 3.29</td>
<td>163.05 ± 26.81</td>
<td>23.61 ± 2.20</td>
<td>28.22 ± 3.16</td>
<td>87.22 ± 13.49</td>
</tr>
<tr>
<td>Number of repetitions (means for each series)</td>
<td>10.2 ± 1.5</td>
<td>13.0 ± 2.2</td>
<td>11.2 ± 1.5</td>
<td>8.4 ± 1.8</td>
<td>15.5 ± 3.9</td>
<td>9.2 ± 2.5</td>
<td>8.2 ± 2.2</td>
<td>10.3 ± 1.7</td>
</tr>
</tbody>
</table>

Data are means ± SE. 1RM, one repetition maximum.
Data were analyzed using the SPSS statistical analysis program.

To further elucidate cortisol effects, isolated cells were incubated in IRT serum after addition of MIF (Sigma), a GC receptor (GR) blocker. After each incubation period, cells were washed and labeled with Annexin-V and PI, respectively. Apoptosis was determined by flow cytometry.

**Statistical analysis.** Data are means ± SE, unless indicated otherwise in the figure legends. Differences between pre-exercise values and values at the postexercise time points were compared with repeated measures ANOVA. If significant main effects were observed, post hoc analysis was conducted by using the Bonferroni test. Pearson’s correlation analysis was used to identify any significant relationships. In all cases, P < 0.05 was accepted as being significant. Data were analyzed using the SPSS statistical analysis program.

**RESULTS**

**Cardiovascular, metabolic responses, and cellular responses to the resistance tests.** The results of 1RM testing differed depending on the size of the specific muscle group used at the different devices. Lowest values were reached at triceps curls 23.61 ± 2.20 kg, whereas highest weights were lifted in the leg press 163.05 ± 26.81 kg. The number of repetitions during the IRT session was between 8.2 ± 2.2 for the biceps curls and 15.5 ± 3.9 for the leg press (Table 1).

Both exercise programs induced a substantial increase in heart rate with maximum increases after leg press from 67 ± 5.5 to 152 ± 6.2 beats/min for the IRT and 74 ± 4.5 to 138 ± 4.3 beats/min for the MRT, respectively. Similarly, blood lactate increased significantly after both IRT and MRT. During IRT, lactate increased continuously from the baseline of about 1.2 ± 0.3 mmol/l to 13.8 ± 1.9 mmol/l after the leg press. Lactate values then declined slightly to 10.6 ± 2.3 mmol/l to the end of the IRT. During MRT, highest lactate values were again measured after the leg press (8.1 ± 1.2 mmol/l). At every time point, heart rate and lactate values were significantly lower during MRT compared with IRT (Table 2).

Both resistance tests induced a leukocytosis, which peaked 3 h after exercise. After IRT, leukocyte counts increased from 4,900 ± 400 to 7,300 ± 1,600 cells/µl, whereas after MRT, the increase was significantly lower (from 4,800 ± 400 to 5,900 ± 400 cells/µl). Twenty-four hours after exercise, leukocyte counts returned to baseline levels. Leukocytosis was mainly induced by a significant increase of granulocytes. In contrast, lymphocyte numbers decreased significantly from 1,800 ± 500 to 1,400 ± 600 cells/µl 3 h after the IRT and returned to pre-exercise levels 24 h after exercise. No significant changes of lymphocyte numbers were observed after the MRT (Table 3).

**Apoptosis and related markers.** IRT induced a significant increase of Annexin-V-positive lymphocytes from 16.76 ± 4.23% to 22.46 ± 5.34% 3 h after exercise. Twenty-four hours after exercise, lymphocyte apoptosis returned to pre-exercise levels. Likewise, MMP decreased significantly to 60% of baseline values at the same point of time and returned to pre-exercise values 24 h after exercise. After MRT, no significant changes occurred in either lymphocyte apoptosis or in MMP (Fig. 1, A and B).

As we focused on apoptosis-related surface markers, IRT significantly increased the percentage of CD95-positive cells (8.89 ± 0.46% to 12.42 ± 2.82%; Fig. 1C), whereas no changes were observed in Fas ligand expression (data not shown). In contrast, after MRT, we observed no changes in death receptor expression. Expression of activation marker CD69 did not change after either IRT or MRT (data not shown).

Lymphocyte Bcl-2 content was significantly affected by IRT. Three hours after exercise, Bcl-2 expression decreased significantly to 72.69 ± 5.98% when baseline was set to 100%. Twenty-four hours after exercise, there was a further decrease to 40.70 ± 12.23% compared with baseline values. MRT did not affect cellular Bcl-2 content (Fig. 2, A and B).

**Inflammatory and hormonal parameters.** IRT induced a significant increase of IL-6 (from 0.7 ± 0.4 to 5.4 ± 1.7 pg/ml) and CRP (from 0.7 ± 0.2 to 2.0 ± 0.3 µg/ml) 3 h after exercise, whereas no changes in TNF-α were observed. No significant changes of any of these parameters were measured after MRT (Table 4).

Cortisol levels increased significantly 3 h after the IRT from 13.3 ± 1.6 to 22.4 ± 1.5 µg/dl. In the sedentary control group, control levels were measured at parallel time points to the resistance tests. Here, we found the expected diurnal decrease of cortisol during the morning hours. Consequently, there was a significant difference between cortisol levels at the 3-h time

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**Table 2. Heart rates and lactate values before, during, and after the IRT and the MRT**

<table>
<thead>
<tr>
<th>Device</th>
<th>Pre</th>
<th>Bench Press</th>
<th>Latissimus Pull-Downs</th>
<th>Seated Rows</th>
<th>Shoulder Pull</th>
<th>Leg Press</th>
<th>Triceps Curls</th>
<th>Biceps Curls</th>
<th>Leg Curls</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRT Heart rate, beats/min</td>
<td>67 ± 5.5</td>
<td>109 ± 5.5†</td>
<td>126 ± 8.6†</td>
<td>133 ± 3.9†</td>
<td>122 ± 6.3†</td>
<td>152 ± 6.2†</td>
<td>130 ± 6.9†</td>
<td>123 ± 4.6†</td>
<td>136 ± 6.5†</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>1.2 ± 0.3</td>
<td>6.6 ± 0.3†</td>
<td>8.5 ± 1.1†</td>
<td>9.2 ± 0.8†</td>
<td>9.2 ± 1.3†</td>
<td>13.8 ± 1.9†</td>
<td>11.9 ± 2.3†</td>
<td>10.1 ± 2.5†</td>
<td>10.6 ± 2.3†</td>
</tr>
<tr>
<td>MRT Heart rate, beats/min</td>
<td>74 ± 4.5*</td>
<td>96 ± 6.5*</td>
<td>108 ± 8.5*</td>
<td>128 ± 5.5*</td>
<td>110 ± 4.6*</td>
<td>138 ± 4.3*</td>
<td>119 ± 4.4*</td>
<td>117 ± 6.5*</td>
<td>115 ± 5.4*</td>
</tr>
<tr>
<td>Lactate values, mmol/l</td>
<td>1.2 ± 0.3*</td>
<td>3.8 ± 0.4*</td>
<td>5.1 ± 0.4*</td>
<td>5.8 ± 0.2*</td>
<td>6.1 ± 0.4*</td>
<td>8.1 ± 1.2*</td>
<td>7.0 ± 0.9*</td>
<td>5.5 ± 0.6*</td>
<td>6.1 ± 0.7*</td>
</tr>
</tbody>
</table>

Data are means ± SE. Both parameters were taken after 3 series at each strength device. IRT and MRT, intensive and moderate, respectively, resistance test. *Significantly different from Pre, P < 0.05; †significantly different from MRT at same point of time, P < 0.05.

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**Table 3. Absolute numbers of leukocytes, lymphocytes, and granulocytes before, immediately after, 3 h after, and 24 h after the IRT and the MRT**

<table>
<thead>
<tr>
<th>Device</th>
<th>Pre</th>
<th>Post</th>
<th>3 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td>4,900 ± 400</td>
<td>6,100 ± 300</td>
<td>7,300 ± 1,600†</td>
<td>5,100 ± 500</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1,800 ± 500</td>
<td>1,500 ± 300</td>
<td>1,400 ± 600*</td>
<td>1,700 ± 400</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>2,900 ± 400</td>
<td>3,700 ± 850</td>
<td>5,400 ± 1,300†</td>
<td>3,000 ± 490</td>
</tr>
<tr>
<td>MRT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td>4,800 ± 400</td>
<td>4,700 ± 300</td>
<td>5,900 ± 400*</td>
<td>4,600 ± 500</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1,800 ± 500</td>
<td>1,800 ± 500</td>
<td>1,900 ± 600</td>
<td>1,800 ± 500</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>3,000 ± 350</td>
<td>3,100 ± 430</td>
<td>3,800 ± 900*</td>
<td>2,900 ± 450</td>
</tr>
</tbody>
</table>

Data are means ± SE. *Significantly different from Pre, P < 0.05; †significantly different from MRT at same point of time, P < 0.05.
point in the control session and the IRT session. In contrast, MRT did not significantly affect cortisol levels (Table 5). In addition, a significant correlation was obtained between plasma cortisol levels and rate of apoptotic lymphocytes 3 h after exercise ($r = 0.63; P < 0.05$; Fig. 3).

**In vitro approaches.** Next, we investigated whether serum taken after the IRT was able to induce lymphocyte apoptosis under in vitro conditions. Incubation of lymphocytes in serum taken 3 h after exercise significantly increased lymphocyte apoptosis compared with serum taken before IRT, suggesting that serum correlates of RT induced apoptosis. In addition, IRT serum increased expression of CD95 receptor slightly from 20.82 ± 1.24% to 23.01 ± 1.03% ($P < 0.05$ ($n = 8$)). Further experiments for a differential analysis of single RT correlates were performed in culture medium. Neither incubation in culture medium alone nor incubation after addition of exercise levels

![](image_url)
of l-lactate (16 mmol/l), rIL-6 (25 pg/ml), or rCRP (4 µg/ml) significantly affected lymphocyte apoptosis. In contrast, incubation of cells with IRT doses of hydrocortisone (30 µg/dl) comparable with post-IRT levels significantly increased lymphocyte apoptosis. Control experiments demonstrated that concentrations of cortisol remained unchanged after heat treatment. Concentrations of cortisol were not significantly affected by heat (data not shown). Finally, addition of MIF to cells incubated in IRT serum attenuated increase of apoptosis, indicating a role of GR in apoptosis induction after IRT (Fig. 4).

DISCUSSION

The current study demonstrated that increased lymphocyte apoptosis after intensive resistance exercise was accompanied by a decreased MMP, reduced Bcl-2 content, and upregulated CD95Fas receptors. Despite a strong increase of lactate and a low-grade inflammatory response after the IRT, increase of plasma cortisol seemed to be the main apoptosis mediator. This conclusion can be drawn from the in vitro experiments indicating the apoptosis-inducing potential of hydrocortisone and a reduction of apoptosis after MIF treatment of cells incubated in IRT serum. In addition, a cortisol effect is supported by a significant correlation of plasma cortisol level and percentage of apoptosis.

Supporting our hypothesis, lymphocyte apoptosis does not specifically occur after endurance exercise but also after RT. Similarly to endurance exercise, apoptosis induction strongly depended on intensity of RT, and apoptosis was accompanied by a decrease of MMP and increased CD95 surface expression. Increased expression of CD95 was also observed by Mooren et al. (19) after marathon run and treadmill running, indicating activation-induced cell death. However, we were unable to demonstrate activation, since CD69 expression was not affected.

With respect to molecular signaling, apoptosis was accompanied by a significant reduction in cellular Bcl-2 content 3 h after exercise. Bcl-2 is an antiapoptotic protein distributed in intracellular membranes, and it appears to contribute to the maintenance of the mitochondrial integrity by preventing the release of proapoptotic proteins, such as cytochrome C, into the cytosol (12). Therefore, we assume that Bcl-2 reduction has chronologically occurred prior to loss of mitochondrial potential. We speculate that a decrease of Bcl-2 was the reason for MMP reduction, because reduction of cellular Bcl-2 was maintained up to 24 h after IRT when apoptosis and MMP returned to baseline levels. This assumption is based on previous studies, which demonstrated that Bcl-2 reduction increased sensitivity to apoptosis but cannot induce apoptosis without additional external stressors (3). However, we cannot exclude that a decrease of MMP and Bcl-2 expression was the result of proportional shifts of lymphocyte subsets.

Exercise-induced modulation of Bcl-2 in peripheral lymphocytes was demonstrated previously (29). Although the mechanisms are not clearly known, it is supposed that cellular stressors, such as increased production of reactive oxygen species, during exercise are responsible for modulation of this protein. This may apply to our RT protocol as well, since some studies detected an increase of oxidative stress markers after RT (7).

Discussing potential mediators of apoptosis, we demonstrated a major role of serum parameters by means of increased apoptosis after incubation of freshly isolated cells in serum...
taken 3 h after the IRT. With the use of lactate in the “in vitro” assays, we considered on the one hand that lactate molecules themselves possess signaling function. On the other hand, we considered that acidosis is a causative agent of lymphocyte apoptosis. Despite buffering, in some samples, pH fell below 6.8 after 24 h incubation. However, we did not observe any effect on lymphocyte apoptosis. These results are supported by studies that observed a strong increase of apoptosis after marathon runs, which are known to be accompanied by very slight increases of blood lactate (20). In addition, IRT concentrations of selected inflammatory mediators did not affect apoptosis. These findings are supported by other studies that did not find correlations between CRP and IL-6 and percentage of apoptotic cells (21, 24). Only one study by Neubauer et al. (21) found an association between IL-6 concentration and the number of necrotic cells immediately after an Ironman triathlon. Referring to this study, we measured numbers of necrotic cells by PI labeling and found no changes after RT (data not shown). Therefore, we share the assumption of Neubauer et al. (21) that in this study, lymphocytes partly undergo an unregulated cell death after an overshooting inflammatory response to an Ironman triathlon, since IL-6 increased up to 50-fold.

Our data strongly suggest a major role of cortisol in mediating apoptosis after IRT. On the one hand, IRT concentrations of hydrocortisone induced lymphocyte apoptosis. On the other hand, attenuation of apoptosis by adding MIF into media demonstrated that GR are involved in apoptosis signaling. It was often speculated that an increase of cortisol contributes to postexercise lymphopenia by affecting lymphocyte migration patterns or by increasing lymphocyte apoptosis. An increase of cortisol was observed in other studies following RT sessions of high total work. Kraemer et al. (2010) and Smilios et al. (28) found increased cortisol concentrations in young and old men following intensive resistance exercise. Other studies, which did not observe an increase of cortisol, often did not include a control session in their research design (9). Therefore, we included a control session that compared cortisol concentrations with the respective time point of exercise sessions. Here, we observed a significant decrease during the morning hours, probably due to the circadian rhythm.

Discussing the molecular signaling, we find that it is well known that cortisol has the potential to induce apoptosis via GR. Prior to activation by interaction with its ligand, GR are associated with heat shock protein 90 (hsp90). Upon hormone binding, a conformational change occurs where hsp90 dissociates from the receptor molecule and allows the receptor hormone complex to migrate into the nucleus. Here, it can transactivate genes that are involved in apoptosis (33). Therefore, GC-induced apoptosis does not directly proceed via the extrinsic or the intrinsic pathway of apoptosis. However, the increase of CD95 expression simultaneously to apoptosis induction supports the meaning of the extrinsic apoptosis pathway. A CD95-dependent way for apoptosis induction by GC was shown for monocytes (25). Whether these pathways interact during exercise remains speculative. Our results support a connection between Bcl-2 reduction and cortisol-induced apoptosis. These findings are in agreement with other studies that demonstrated that overexpression of Bcl-2 protected from GC-induced cell death (14, 18). The importance of Bcl-2 is further underscored by the analysis of Bcl-2-deficient mice, which display fulminant lymphoid apoptosis in vivo and enhanced cell death of thymocytes in vitro after GC treatment (31). This underpins our suggestion that levels of Bcl-2 seem to determine the sensitivity to GC-induced apoptosis during exercise.

**Perspective and significance.** Turning to an applied context, it can be speculated that the immunologic impact of RT depends on its relation to circadian rhythms of cortisol levels. Similar to cortisol levels, T cells show a clear circadian rhythm. This rhythm seems to be strongly affected by cortisol (7). It was demonstrated that T cell subpopulations, which are sensitive to cortisol-induced apoptosis, are redistributed into bone marrow. An uncommon morning increase of cortisol due to RT might disturb the regular circadian interplay between cells and hormones and therefore, induce apoptosis in cells that are more sensitive to apoptosis caused by external stressors. In addition, Hayes et al. (10) recently presented evidence for a diurnal variation of cortisol responses to RT. Accordingly, the cortisol response to RT is higher in the morning hours compared with the training response in the late afternoon (26). In this context, it is important to distinguish between different lymphocyte subsets, which is a limitation of the study. Actually, it is not known if apoptotic lymphocytes are senescent or dysregulated immune cells or naive or functional cells (27). Therefore, we can only speculate about immunologic impacts or long-term effects of lymphocyte apoptosis.

In summary, we demonstrated that intensive RT induced lymphocyte apoptosis, similar to endurance exercise. In this relation, RT seems to increase lymphocyte sensitivity to cortisol-induced apoptosis by a decrease of Bcl-2 levels. Within this framework, we assume that daytime resistance exercise performance affected cortisol-induced apoptosis, since RT induced a counter-regulated cortisol pattern during the morning hours. The relevance of these processes for immune competence has to be confirmed in future studies using suitable infection models. Further research should attempt to characterize the effect of diurnal variation of exercise-induced apoptosis and the effects of regular training to blunt these effects.

**ACKNOWLEDGMENTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**

RESISTANCE EXERCISE AND APOPTOSIS


