Adrenalectomy in mice does not prevent loss of intestinal lymphocytes after exercise

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Hoffman-Goetz, L., J. Quadrilatero, J. Boudreau, and J. Guan. Adrenalectomy in mice does not prevent loss of intestinal lymphocytes after exercise. J Appl Physiol 96: 2073–2081, 2004; 10.1152/japplphysiol.01262.2003.—Exhaustive exercise is associated with an increase in circulating glucocorticoids (GCs), lymphocyte apoptosis, and a reduction in intestinal lymphocyte number. The present study examined the role of GCs on the numerical changes seen in intestinal lymphocytes after exercise. Female C57BL/6 mice were bilaterally adrenalectomized (ADX; n = 18) or given sham surgery (Sham; n = 18) and assigned to one of three exercise conditions: treadmill running (28 m/min, 90 min, 2° slope) and killed immediately or after 24 h recovery, or not exercised and killed immediately after 90-min exposure to the treadmill environment. Lymphocytes were isolated from the intestines with CD45—cells collected by positive selection using magnetic bead separation columns, and lymphocyte subpopulations were analyzed by flow cytometry for CD45—, CD3—β, CD3γδ, CD8α, CD8β, CD8ε, CD4+, and NK+ phenotypic markers. ADX mice had significantly more intestinal CD45—lymphocytes (P < 0.05) and CD3εβ+ (P < 0.05), CD3γδ+ (P < 0.01), CD8α+ (P < 0.001), and NK+ (P < 0.05) intestinal lymphocytes than Sham mice. There was a significant effect of exercise condition on total intestinal CD45—lymphocytes (P < 0.01) and CD3εβ+ (P < 0.05), CD8α+ (P < 0.001), and CD4+ (P < 0.05) intestinal lymphocytes, with fewer cells at 24 h postexercise compared with the other treatment conditions. There were no surgical × exercise interaction effects on the CD3 and CD8 phenotype numbers. Plasma corticosterone was virtually nil in ADX mice regardless of exercise condition but was significantly elevated in Sham mice immediately postexercise (P < 0.001). The data indicate that ADX does not prevent the loss of lymphocytes from the intestinal mucosa 24 h after strenuous exercise and GCs are not directly causal in the leukopenia of exercise.

treadmill running; adrenal glands; glucocorticoids

NUMEROUS STUDIES HAVE SHOWN that acute exercise is associated with induction of apoptosis or programmed cell death and loss of lymphocytes from blood, thymus, and spleen in humans and laboratory animals (9, 20, 28, 29). Lymphocyte loss and/or apoptosis have been observed in other situations characterized by extreme physiological stress, including burn injury (15), electrical shock (48), and chronic restraint stress (42), and may reflect release of stress hormones, in particular glucocorticoids (GCs). GCs trigger apoptosis in lymphocytes by acting on an intracellular pathway via the mitochondria (2), which results in the accumulation of mitochondrial derived cytochrome c within the cytosol, loss of mitochondrial membrane integrity, externalization of phosphatidylinerine at cell membranes, and DNA fragmentation (27, 45).

The intestinal intraepithelial lymphocytes (IELs) are a unique population of T cells that function as a first line of defense against exogenous pathogens, many of which develop independently of the thymus gland (i.e., T cells with the γδ form of the T cell receptor (TCR)) (35). IELs play an important role in protection from epithelial tumor cells through their function as cytolytic agents (37). Lamina propria lymphocytes (LPLs), also mainly T cells, are derived from the thymus; they constitute primarily the αβ form of the TCR (12) and have some cytolytic activity against tumor cell lines (38). CD8 T lymphocytes, compromising two subpopulations of α- and β-chain phenotypes, have cytolytic functions and are found in both the intraepithelial and lamina propria regions (4). CD4 (helper) T cells and natural killer (NK) cells make up a smaller component of the IEL and LPL compartments.

There is only limited research on the impact of exercise on intestinal IELs and LPLs. We have previously shown that 90 min of heavy exercise is associated with reduced numbers of CD3+ lymphocytes in mouse intestine (19), a phenomenon similar to the blood lymphocytopenia observed after heavy duration exercise (41). Because exercise triggered a sharp increase in the secretion of corticosterone, we hypothesized that the loss of intestinal lymphocytes was due to apoptosis mediated through GC as demonstrated by an increase in the expression of phosphatidylserine (annexin V). In this present study, we further characterize the contribution of GCs on the intestinal lymphocyte numerical response to exercise stress. Because the GC response can be abrogated by excision of the adrenal glands, we examined the effect of acute exercise on the number and phenotype distribution of intestinal lymphocytes from mice after adrenalectomy (ADX). We show in this study that ADX, which eliminates systemic GCs, fails to buffer the reduction in the total number of T lymphocytes in the mouse intestine after acute exercise.

MATERIALS AND METHODS

Animals

Thirty-six female C57BL/6 mice, purchased from Harlan Sprague Dawley (Indianapolis, IN, colony 217), age 4–5 wk and weighing ~14 g at arrival, were housed in groups of three under temperature- and humidity-controlled conditions with a 12:12-h reversed light-dark cycle. Mice were given food (Laboratory Rodent Chow, PMI Feeds, Richmond, IN) and tap water ad libitum and allowed to acclimate to our vivarium for at least 1 wk before use in the experiments. All procedures involving mice concurred with guidelines established by the Canadian Council on Animal Care and were approved by the university IRB.

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Adrenalectomy

Mice were randomly assigned to one of two surgical conditions: bilateral ADX (n = 18) or sham surgery (Sham; n = 18). Mice were anesthetized by intraperitoneal injection of a ketamine-xylazine mixture (128 mg/kg and 8.5 mg/kg, respectively); buprenorphine (Temgesic, 0.003 mg/100 g body wt) was administered subcutaneously as an analgesic. A 0.5-cm skin incision was made in the back, the skin on both sides of the incision was moved to the side, and a 2-mm muscle incision was made on the top of each adrenal gland. The entire adrenal gland was removed with a pair of sterile fine curved forceps. The incision was closed with surgical staples, and 0.8 ml of 0.9% sterile saline solution was injected subcutaneously to restore body fluid. The mice were placed into individual clean cages and recovered under a heat lamp. After recovery, the mice were maintained by providing food and drinking water containing 0.9% sodium chloride ad libitum. Control mice (Sham) underwent the same surgical procedure as the ADX group, except their adrenal glands were not excised. Experiments were performed 2–3 wk after ADX.

Exercise Protocol

Within ADX and sham conditions, mice were randomly assigned to one of three exercise groups (6 mice per surgical × exercise condition): Immediate, which involved 90 min of treadmill running at 20 m/min at a 2° slope and death immediately after completion of the exercise bout; 24 h Post, which involved treadmill running at 20 m/min at a 2° slope and death 24 h after cessation of the exercise bout; and Nonexercised, which involved 90 min of exposure to treadmill laneway noise and vibration without actual running. The Immediate and 24 h Post groups were preconditioned to treadmill running by three prior exposures to the treadmill (Ommi-max metabolic small rodent treadmill, Columbus, OH) at 15 m/min for 10 min on day 3; 20 m/min for 15 min on day 2, and 24 m/min for 20 min on day 1 before the experiment.

Tissue Collection

Cardiac blood. Mice were killed by an overdose of sodium pentobarbital sodium (0.06–0.08 ml). On a negative toe pinch response, skin at the midventral position of the body was grasped and a 2 cm incision was made across the chest. The rib cage was exposed and cut along the sides to expose the heart. Blood was collected immediately into clean eppendorf tubes, mixed, and the procedure was repeated with fresh HBSS for 10 min. The skin at the abdominal cavity was cut to expose the gastrointestinal tract. The entire bowel was removed and the procedure was repeated with fresh HBSS for 10 min. The skin at the abdominal cavity was cut to expose the gastrointestinal tract. The entire bowel was removed and the procedure was repeated with fresh HBSS for 10 min. The skin at the abdominal cavity was cut to expose the gastrointestinal tract. The entire bowel was removed and the procedure was repeated with fresh HBSS for 10 min.

Isolation of intestine. The skin at the abdominal cavity was cut to expose the gastrointestinal tract. The entire bowel was removed and intestinal lymphocytes were isolated via a modification of the methods of LeFrançois (24) and Todd et al. (44). Briefly, tissue was excised and flushed with PBS to remove fecal matter; visible fat and Peyer’s patches were dissected out, and the bowel was cut open longitudinally and separated into 0.5-cm pieces. The samples were gently washed in PBS four times by inversion, discarding the supernatant each time. HBSS (containing 1 mM EDTA and 5% FBS; Ca²⁺ and Mg²⁺ free) was added to each sample and incubated at 37°C in a shaking water bath for 20 min. Samples were vortexed, supernatant was collected, and the procedure was repeated with fresh HBSS for 10 min. The supernatants were combined for each sample, centrifuged at 450 g at 4°C for 5 min, decanted, and resuspended in cold RPMI-1640 solution (containing 2.5% FBS, 0.5% HEPES, and 0.2% penicillin). Cell suspensions were passed over a nylon wool column, and then cells were collected, centrifuged at 450 g for 5 min at 4°C, resuspended in buffer (0.5% BSA/PBS containing 2 mM EDTA), and centrifuged again at 450 g for 5 min. Cells were stained with Turk’s solution and counted manually by microscopy using a hemocytometer.

Intestinal lymphocyte isolation. CD45 positive cells (leukocyte fraction) were isolated by use of a magnetic separation system (mini-MACS system, Miltenyi Biotec, Auburn, CA). Cell pellets were suspended in 90 μl of buffer and 10 μl MACS CD45 microbeads per 10⁷ total cells, incubated at 6–12°C for 15 min, washed, centrifuged, and resuspended in buffer. Cell suspensions were separated by passing over a separation column with a preseparation filter in the magnetic field using a positive selection protocol.

Antibody Staining Procedure

Intestinal leukocytes were stained with FITC-conjugated monoclonal antibodies (MAbs) against CD3ε (clone: GL3), CD8β (clone: H57–597), CD4 (clone: GK1.5), Pan-NK (clone: DX5), and PE-conjugated MABs against CD3εβ (clone: 53–5.8) and CD8ε (clone: 53–6.7) with all antibodies obtained from PharMingen (Mississauga, ON, Canada). Briefly, 5 × 10⁶ cells suspended in 50 μl of PBS were initially incubated 5 min with 0.5 μl of Fe Block [CD16/FcγIII/CD32 FcγIl (clone: 2.4G2), PharMingen], before specific cell surface phenotype staining to inhibit nonspecific binding via Fe receptors. Cell suspensions were incubated with MAb for 45 min in the dark at 4°C and washed with 500 μl PBS, and cells were read on a flow cytometer (Epics XL Flow Cytometer, Beckman Coulter, Hialeah, FL) first by gating on the CD45 population using a FITC-conjugated MAB (clone: YW62.3; Cedarlane Laboratories, Hornby, ON, Canada).

Corticosterone

Plasma samples were analyzed for corticosterone via a commercially available [125I]radiimmunoassay kit designed for rat and mouse plasma samples (IDS, Bolton, UK) according to the manufacturer’s protocol, with a 1:10 dilution and extraction under methylene chloride. Radioactivity was counted with a Gamma 5500 counter (Beckman Coulter).

Statistical Analysis

Results are given as group means ± SE unless otherwise stated. All data were tested for normal distribution to satisfy the assumptions of analysis of variance. Total number of intestinal lymphocytes, percent of intestinal lymphocyte phenotypes, and plasma corticosterone were analyzed by a two-way analysis of variance (Nonexercised, Immediate, 24 h Post) and surgical treatment (ADX, Sham) as the independent factors. In cases of significant main or interaction effects, Tukey’s honestly significant difference post hoc test was performed to identify statistical differences. In all cases, P < 0.05 was accepted as being significantly different from chance alone.

RESULTS

Total Intestinal Lymphocytes

Figure 1A shows the significant main effect of ADX on total intestinal (intraepithelial and lamina propria) lymphocytes from mice [F(1,30) = 5.828, P < 0.05], with ADX mice having ~30% more intestinal lymphocytes relative to Sham mice. Figure 1B shows the influence of the acute exercise bout on the total number of intestinal lymphocytes recovered. In the 24 h Post group, there were significantly fewer intestinal lymphocytes compared with the Nonexercised and Immediate exercise groups [F(2,30) = 8.175, P < 0.001]. The number of intestinal lymphocytes at 24 h postexercise was ~50% of that obtained from the other exercise conditions. There was no significant interaction between surgical and exercise condition, i.e., the same pattern of a reduction in lymphocyte numbers occurred in both ADX and Sham mice 24 h postexercise compared with Nonexercised mice (Fig. 1C).

Figure 2A shows that ADX was associated with significantly more CD3-positive intestinal lymphocytes with the αβ-TCR...
there was a significant main effect of the exercise on the total number of CD3γδ T cells \( F_{(2,30)} = 3.646, P < 0.05 \) (Fig. 3B). There was no difference in response to exercise as a function of the surgical treatment (i.e., no significant surgical \( \times \) exercise interaction; Fig. 3C).

Figure 4 shows the effect of surgery and exercise on CD8α T intestinal lymphocytes. There was a significant effect \( F_{(1,30)} = 13.234, P < 0.001 \) of ADX on the number of intestinal CD8α T cells recovered from mice (Fig. 4A). ADX was associated with more CD8α T cells compared with the Sham surgical treatment. Exercise condition also significantly \( F_{(1,30)} = 11.758, P < 0.001 \) influenced the total number of intestinal CD8α T cells isolated from mice (Fig. 4B). There were fewer intestinal CD8α T cells recovered at 24 h postexercise relative to the Nonexercised and Immediate exercise conditions \( (P < 0.001) \). The interaction between surgery and exercise is shown in Fig. 4C (not significant). A representative...
single parameter flow cytometry histogram depicting the CD8α population for the ADX and Sham conditions at 24 h postexercise is also given in Fig. 5. In contrast with the intestinal CD8α T cell response, no significant main or interaction effects for the CD8β T cell population were observed either for surgical or for exercise condition (Fig. 6).

ADX did not influence the CD4 cells isolated from the intestine (ADX: 2.7 ± 0.4 × 10⁶ cells vs. Sham: 2.2 ± 0.2 × 10⁶ cells) (Fig. 7A). There was a significant effect of the exercise on the number of CD4 T cells isolated [F(2,30) = 3.68, P < 0.05], with more CD4 T lymphocytes in the Nonexercised (2.9 ± 0.5 × 10⁶ cells) and Immediate (2.9 ± 0.4 × 10⁶ cells) groups compared with mice at 24 h postexercise (1.5 ± 0.2 × 10⁶ cells) (Fig. 7B). There was no significant interaction between surgical treatment and exercise condition for CD4 lymphocytes (Fig. 7C).

NK cells normally make up a very small proportion of murine intestinal leukocytes, and the numbers were absolutely less than the other subsets. Nevertheless, there was still a significant effect of the ADX (Fig. 8A) on NK cell number [F(1,30) = 4.127, P < 0.05]. ADX mice had more NK cells recovered (1.7 ± 0.3 × 10⁶ cells) than Sham mice (0.9 ± 0.3 × 10⁶ cells) (Fig. 8A). There was a significant effect of exercise on the number of NK cells isolated [F(2,30) = 4.127, P < 0.05], with more NK cells recovered in the Nonexercised (1.9 ± 0.4 × 10⁶ cells) and Immediate (1.8 ± 0.3 × 10⁶ cells) groups compared with mice at 24 h postexercise (1.3 ± 0.2 × 10⁶ cells) (Fig. 8B). There was no significant interaction between surgical treatment and exercise condition for NK lymphocytes (Fig. 8C).
There was, however, no significant effect of exercise (Fig. 8B) on NK cell numbers from mouse bowel. Similarly, there was no exercise by surgical treatment interaction effect for NK cell numbers (Fig. 8C).

Phenotype Percentages

Table 1 gives the percentages of the various lymphocyte phenotypes from ADX, Sham, and the various exercise conditions for the mice. ADX mice had a higher percentage of intestinal lymphocytes expressing the CD3γδ T, CD3αβ T, CD4 T, or NK cell phenotypes. The percentages of intestinal CD8α T and CD8β T cells recovered from mice 24 h postexercise were significantly lower (50.6 ± 4.1%) and significantly higher (30.9 ± 5.7%), respectively, than the percentages found in the Nonexercised mice (64.9 ± 2.9% and 20.0 ± 1.4%, respectively; *P* < 0.05 in both cases).

There were no significant interaction effects between surgical condition and exercise treatment for any of the lymphocyte phenotype percentages with the exception of CD8β and NK cells (Table 1). ADX-24 h Post mice expressed a higher percentage of NK cells relative to Sham-24 h Post mice 

\[ F_{(2,30)} = 3.59, \ P < 0.05 \]

Sham-24 h Post mice had a higher percentage of CD8α T cells (27.6 ± 3.9%) than the Nonexercised mice (19.1 ± 1.2%). There were no effects of surgical treatment on the percentage of intestinal CD3αβ T, CD4 T, and NK cells recovered.

The effects of the exercise intervention on the phenotype percentages are also shown in Table 1. There were no significant differences by exercise condition on the percentage of intestinal lymphocytes expressing the CD3γδ T, CD3αβ T, CD4 T, or NK cell phenotypes. The percentages of intestinal CD8α T and CD8β T cells recovered from mice 24 h postexercise were significantly lower (50.6 ± 4.1%) and significantly higher (30.9 ± 5.7%), respectively, than the percentages found in the Nonexercised mice (64.9 ± 2.9% and 20.0 ± 1.4%, respectively; *P* < 0.05 in both cases).

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0.2 × 10⁶ cells). There was, however, no significant effect of exercise (Fig. 8B) on NK cell numbers from mouse bowel. Similarly, there was no exercise by surgical treatment interaction effect for NK cell numbers (Fig. 8C).

Fig. 7. CD4 cell count (× 10⁶) by surgical and exercise condition. The effect of adrenalectomy was not significant (A). *P*Significantly (*P* < 0.05) different compared with Nonexercised and Immediate groups (B). The interaction between surgical and exercise condition was not significant (C). All values are presented as means ± SE. Differences were determined by Tukey’s post hoc test.
significant effect of exercise, with plasma corticosterone concentrations higher in mice sampled immediately after exercise compared with all other groups. The corticosterone responses by surgical and exercise condition are shown in Table 2.

**DISCUSSION**

This study demonstrates that excision of the adrenal glands, with a consequent absence of circulating GC hormones, does not prevent the loss of lymphocytes from the gastrointestinal tract. The main effect of exercise (B) and the interaction between surgical and exercise condition (C) were not significant. All values are presented as means ± SE. Differences were determined by Tukey’s post hoc test.

**Table 1. Percent T lymphocyte and NK phenotypes in ADX and Sham mice given treadmill exercise**

<table>
<thead>
<tr>
<th>Phenotype (%)</th>
<th>Exercise Condition</th>
<th>Sham</th>
<th>ADX</th>
<th>Total by Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3αβ</td>
<td>Nonexercised</td>
<td>36.5±3.2</td>
<td>41.7±3.5</td>
<td>39.1±2.4</td>
</tr>
<tr>
<td></td>
<td>Immediate</td>
<td>39.8±1.8</td>
<td>37.2±1.7</td>
<td>38.5±1.2</td>
</tr>
<tr>
<td></td>
<td>24 h Post</td>
<td>47.1±4.4</td>
<td>41.1±1.9</td>
<td>44.1±2.5</td>
</tr>
<tr>
<td></td>
<td>Total by surgery</td>
<td>41.1±2.1</td>
<td>40.0±1.4</td>
<td>40.6±2.2</td>
</tr>
<tr>
<td>CD3γδ</td>
<td>Nonexercised</td>
<td>29.1±1.5</td>
<td>34.9±3.8</td>
<td>32.4±2.1</td>
</tr>
<tr>
<td></td>
<td>Immediate</td>
<td>27.2±2.7</td>
<td>35.5±5.8</td>
<td>31.4±3.3</td>
</tr>
<tr>
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<td>24 h Post</td>
<td>26.3±2.1</td>
<td>33.3±4.1</td>
<td>29.8±2.4</td>
</tr>
<tr>
<td></td>
<td>Total by surgery</td>
<td>27.8±1.2</td>
<td>34.6±2.5</td>
<td>31.2±2.6</td>
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<tr>
<td>CD8α</td>
<td>Nonexercised</td>
<td>58.2±3.1</td>
<td>71.5±3.1</td>
<td>64.9±2.9</td>
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<tr>
<td></td>
<td>Immediate</td>
<td>64.2±3.5</td>
<td>70.3±3.9</td>
<td>67.3±2.6</td>
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<td></td>
<td>24 h Post</td>
<td>40.9±5.7</td>
<td>60.3±2.0</td>
<td>50.6±4.1b</td>
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<tr>
<td></td>
<td>Total by surgery</td>
<td>54.8±3.3</td>
<td>67.4±2.1e</td>
<td>60.9±3.8</td>
</tr>
<tr>
<td>CD8β</td>
<td>Nonexercised</td>
<td>19.4±1.1</td>
<td>20.5±2.6</td>
<td>20.0±1.4</td>
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<td></td>
<td>Immediate</td>
<td>20.6±0.9</td>
<td>17.9±2.5</td>
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<tr>
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<td>24 h Post</td>
<td>42.8±9.1d</td>
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<td></td>
<td>Total by surgery</td>
<td>27.6±3.9</td>
<td>19.1±1.2c</td>
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<td>CD4</td>
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<td>Immediate</td>
<td>12.3±1.7</td>
<td>8.4±1.9</td>
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<td>24 h Post</td>
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<td>9.6±2.1</td>
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<td>Total by surgery</td>
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<td>NK</td>
<td>Nonexercised</td>
<td>5.9±1.2</td>
<td>5.0±1.1</td>
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<td></td>
<td>Immediate</td>
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<td>4.2±0.6</td>
<td>3.9±0.4</td>
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<tr>
<td></td>
<td>24 h Post</td>
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<td>8.9±3.0d</td>
<td>5.7±1.7</td>
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<tr>
<td></td>
<td>Total by surgery</td>
<td>4.0±0.6</td>
<td>6.0±1.1</td>
<td>5.0±1.1</td>
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</table>

Values are means ± SE for 6 animals per group. ADX, adrenalectomized mice; Sham, sham group given sham surgery; Immediate, mouse group with death immediately after exercise; 24 h Post mouse group with death 24 h after exercise. Differences were determined by Tukey’s post hoc test. *Significant (P < 0.05) vs. Sham group regardless of exercise condition; **significant (P < 0.01) vs. Nonexercised and Immediate group regardless of surgical condition; ***significant (P < 0.001) vs. Sham group regardless of exercise condition; ****significant (P < 0.001) vs. all other groups (interaction effect); *****significant (P < 0.05) vs. Nonexercised and Immediate group regardless of surgical condition; ******significant (P < 0.05) vs. Sham 24 h Post group (interaction effect).

**Table 2. Plasma corticosterone concentrations in ADX and Sham mice given treadmill exercise**

<table>
<thead>
<tr>
<th>Surgical Condition/E</th>
<th>Plasma Corticosterone Concentration, ng/ml</th>
<th>Number of Mice</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonexercised</td>
<td>3.6±1.7</td>
<td>6</td>
</tr>
<tr>
<td>Immediate</td>
<td>3.8±0.7</td>
<td>6</td>
</tr>
<tr>
<td>24 h Post</td>
<td>1.9±1.1</td>
<td>6</td>
</tr>
<tr>
<td>SHAM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonexercised</td>
<td>28.1±3.3</td>
<td>6</td>
</tr>
<tr>
<td>Immediate</td>
<td>54.4±6.8*</td>
<td>6</td>
</tr>
<tr>
<td>24 h Post</td>
<td>23.3±6.4</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Differences were determined by Tukey’s post hoc test. Main effects of surgical condition and exercise significant at P < 0.001 (see text for details). *Sham mice, immediate postexercise significantly (P < 0.01) different from all other groups.
mucosa after physical stress in mice. Mice given a 90-min session of treadmill exercise had a marked reduction 24 h later in the cellularity and phenotype distribution of T lymphocyte subpopulations resident in the gastrointestinal tract. That ADX failed to buffer the loss of lymphocytes (either total or by phenotype) 24-h postexercise stress agrees with findings of others that ADX does not affect 12-h restraint-stress-induced reduction of lymphocytes from other lymphoid tissues (49).

Stress activates the hypothalamic-pituitary-adrenal axis with a concomitant release of GCs from the adrenal cortex. In vitro exposure to GCs induces thymocyte apoptosis (18, 26), and blocking GC receptors, inhibiting GC biosynthesis, and ADX reduce lymphocyte cell death (6, 15, 43). Furthermore, surgical removal of the adrenal glands is associated with less DNA fragmentation and lower nitric oxide production of splenic macrophages (5). Thus, if GCs were the primary factor in the depletion of intestinal lymphocytes 24 h after exercise, it would be expected that ADX-24 h Post would have a lower lymphocyte loss than Sham-24 h Post relative to their respective nonexercised controls. This was not the case and suggests that other mechanisms contribute to the loss of cellularity in ADX mice 24 h after a physical exercise challenge.

During exercise, splanchic blood flow decreases proportional to the rise in heart rate (31). Ardevol et al. (1) found that in the rat gastrointestinal blood flow (17 ml/min) decreases during intense exercise (8 ml/min) but returns to basal levels during the initial recovery period. Therefore, an alternative explanation for the lymphocyte loss observed in the gastrointestinal tract is that cardiovascular dynamics with exercise indirectly affect this lymphocyte pool. However, it remains to be determined whether alterations in blood flow would affect the lymphocyte pool in the gastrointestinal tract at 24 h postexercise because at this time point cardiac output, and consequently splanchic blood flow, would be expected to return to preexercise levels.

Another factor that could contribute to the loss of intestinal lymphocytes with heavy exercise is ischemia-reperfusion injury. Noda et al. (34) found that occlusion of the rat superior mesenteric artery (to simulate ischemia) induced apoptosis of intestinal epithelial cells, which was further exacerbated after reperfusion. Similarly, CooperSmith et al. (10) found that apoptosis of mouse intestinal epithelial cells was maximal at 24 h after ischemia-reperfusion injury. Apoptosis of intestinal epithelial cells after ischemia-reperfusion injury can be inhibited by overexpression of the antiapoptotic protein Bcl-2 and provision of antioxidants (10, 22). It is reasonable to assume that the mechanisms responsible for ischemia-reperfusion-induced apoptosis in intestinal epithelial cells may also directly induce apoptosis of lymphocytes. A loss of intestinal epithelial cells via apoptosis after ischemia-reperfusion could indirectly induce intestinal lymphocyte apoptosis though growth factor withdrawal because cytokines such as IL-7 and IL-15 are produced by intestinal epithelial cells (23, 36) and these cytokines provide essential survival signals as well as prevent apoptosis of intraepithelial lymphocytes (50). Whether the loss of intestinal lymphocytes represents a direct effect of ischemia and reperfusion on intestinal lymphocytes or an indirect effect due to cytokine withdrawal as a result of atrophy of epithelial cells remains to be determined.

Heavy exercise increases oxidative respiration and the generation of reactive oxygen species (ROS) (32). Oxidative stress during exercise induces apoptosis of lymphocytes and the loss of cellularity of lymphoid tissues by generating ROS with consequent damage directly to DNA (33) or indirectly by rapid consumption of antioxidant defenses resulting in membrane lipid peroxidation (3). ROS production may also play a role in cell loss during exercise by affecting lymphocyte migration (39). The oxidative stress pathway is further supported by the findings that supplementation with dietary antioxidants protected against exercise-induced apoptosis and DNA damage of human and rodent lymphocytes (17, 25). Although we did not measure whole body or cellular respiration in this study, previous work (19) showed that, at the same exercise workload in C57BL/6 mice, there was a significant increase in plasma 8-isoprostanes (a biomarker of oxidative stress) immediately after exhaustive treadmill exercise relative to noneexercised animals. Whether ADX-exercised mice generate equivalent 8-isoprostanes as sham-exercised mice is not known. However, exposure to GCs increases the production of intracellular ROS (8), and it is reasonable to assume that basal intracellular ROS production in intestinal lymphocytes might be lower in the GC-depleted, ADX animals. Because ADX and Sham mice did not differ in the loss of intestinal lymphocytes 24 h after the exhaustive exercise bout, this finding suggests that differences in intracellular ROS production by GCs do not mediate the observed intestinal lymphocyte depletion. Although intracellular ROS via GC signaling may differ between surgical groups, it is unlikely that ROS production via increased oxidative metabolism due to exercise would differ at the active muscle. This raises the possibility that spillover of ROS from the working muscle may be a mechanism for lymphocyte cell loss during exercise.

Intestinal lymphocytes (especially CD3γδ and CD8αα T cells) are a distinctive population of lymphocytes that function in protection against epithelial tumors, pathogens, allergens, and antigens. Normally, the lymphocyte pool at the gastrointestinal mucosa is maintained at equilibrium between activation during antigen exposure and apoptosis once the challenge is removed (46). Imbalances due to excessive proliferation or insufficient apoptosis could lead to inflammatory bowel disease (7), whereas excessive apoptosis or insufficient proliferation could lead to reduced barrier functions (14). We observed a reduction by 50% in the intestinal lymphocyte cell numbers after a single episode of heavy treadmill exercise in mice. Of note is the loss of CD8αα T cells, which include the CD8αα homodimer phenotype, unique to the intestinal mucosa (16). Das and colleagues (11) reported that CD8αα T cells suppress intestinal inflammation. In contrast to the CD8αα T population, which was affected by treadmill exercise, there was no apparent effect on CD8ββ T cells. Several studies (30, 47) indicate that, although all subsets of intraepithelial intestinal lymphocytes are susceptible to GC-induced apoptosis, some subsets (CD8αα and CD3γδ) are more resistant because of higher expression of intracellular Bcl-2 (an antiapoptosis protein). This raises the possibility of differential regulation of intestinal lymphocyte subtypes to those apoptosis-inducing factors associated with heavy exercise.

The presence or lack of GCs may alter the morphology of the intestinal epithelium. Bilateral ADX in rats led to partial atrophy and disorganization of the epithelium with increased villus apoptosis (13); however, it has also been noted that GC administration in vitro increases intestinal epithelial cell apop-
tosis and inhibits cellular proliferation (21). Although intestinal epithelial cells produce cytokines that regulate apoptosis and cell proliferation of intestinal lymphocytes (50), our experiments demonstrate significantly higher lymphocyte counts for most phenotypes in ADX mice regardless of exercise condition. These findings suggest that basal GC secretion, irrespective of exercise condition, may contribute to intestinal lymphocyte loss either directly or indirectly via loss of intestinal epithelial cells. This latter indirect effect remains to be determined because we did not measure intestinal epithelial cell loss. Regardless of the effect of GC (or lack or GC in the case of ADX mice) on the intestinal epithelial cells, GCs are not the primary mechanism for intestinal lymphoid cell loss 24 h after acute treadmill running because a similar pattern of lower cell numbers was observed in both ADX and sham-treated mice.

There are limitations with this study. Although ADX eliminates GCs, it reduces epinephrine production as well. Catecholamines have apoptosis-inducing effects on lymphocytes (40, 41), and it is possible that the higher lymphocyte numbers observed in ADX compared with Sham mice were due to overall lower epinephrine production. After ablation of the adrenal glands, circulating and tissue epinephrine levels may still be high because of extra-adrenal synthesis of epinephrine by methylation of norepinephrine via tissue phenylethanolamine N-methyltransferase (PMNT) (51). We did not measure plasma epinephrine levels in the ADX mice, and it is possible that the reduction in intestinal lymphocyte numbers 24 h postexercise was due to extra-adrenal epinephrine effects on apoptosis. Whether PMNT induction occurs in mouse intestine postexercise was due to extra-adrenal epinephrine effects on apoptosis. Whether PMNT induction occurs in mouse intestine after heavy, dynamic exercise.

The physiological consequences of the overall reduction in intestinal lymphocyte numbers with intense exercise and the apparent greater susceptibility of CD8α relative to CD8β T to contribute to the cell loss remain to be determined. It is likely that the physiological implications will depend, in part, on the time course for reconstitution of this intestinal lymphocyte pool and the environmental exposures during this “open window” period. It is still unclear whether loss of intestinal lymphocytes during exercise represents a direct effect of lymphocyte apoptosis inducing triggers that accompany exercise, an indirect effect due to growth factor withdrawal as a result of atrophy of epithelial cells by these same triggers, or a consequence of cardiovascular readjustments. This study suggests that at least one of the apoptosis-inducing triggers, GCs, does not directly mediate the loss of lymphocytes in the gastrointestinal tract after heavy, dynamic exercise.

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REFERENCES

ADRENALECTOMY, LYMPHOCYTES, AND EXERCISE


