Effect of intense wrestling exercise on leucocytes and adhesion molecules in adolescent boys
D Nemet, P J Mills, D M Cooper

Background: In adults, exercise is a powerful and natural stimulator of immune cells and adhesion molecules. Far less is known about exercise responses during childhood and adolescence and whether or not exercise in ‘real life’ activities of healthy adolescents influences immune responses.

Objective: To determine if strenuous exercise leads to significant changes in leucocyte number and adhesion molecule expression in adolescent boys.

Methods: Eleven healthy, high school boys, aged 14–18.5 years, performed a single, typical, 1.5 hour wrestling practice session. Blood was sampled before and after the session. Flow cytometry was used to evaluate changes in immune responses.

Results: The exercise led to significant ($p<0.05$) and robust increases in granulocytes, monocytes, and all lymphocyte subpopulations. The most significant changes were observed for natural killer cells ($p<0.0005$). The number of $T$ cytotoxic and $T$ helper cells expressing $CD62L$ increased significantly ($p<0.002$ and $p<0.0005$ respectively), as did the number of $T$ cytotoxic and $T$ helper cells not expressing $CD62L$ ($p<0.003$ and $p<0.009$ respectively). The density of $CD62L$ on lymphocytes decreased significantly with exercise ($p<0.0005$), whereas $CD11a$ ($p<0.01$) and $CD54$ ($p<0.01$) increased.

Conclusions: The data show that an intense wrestling bout in adolescent boys leads to profound stimulation of the immune system. The role of these common changes in overall immune status and the development of the immune and haemopoietic systems has yet to be determined.

METHODS
Sample population
The study was approved by the institutional review board, University of California, Irvine (UCI) and written informed consent as well as assent were obtained. Eleven healthy adolescent boys participated (table 1). The study was designed to examine responses of peripheral blood mononuclear cells and adhesion molecules to intense exercise in a field setting in which each participant served as his own control. There was no independent, non-exercising control group. No subjects were receiving any drugs at the time of the study.

Measurement of height, weight, and body mass index (BMI)
Standard calibrated scales and stadiometers were used to determine height, weight, and BMI (weight (kg)/height (m)$^2$). As BMI changes with age, we calculated the BMI centile for each child using the recently published standards from the Centers for Disease Control, National Center for Health Statistics. Weight was measured before and at the end of the practice.

Abbreviations: BMI, body mass index; ICAM-1, intercellular adhesion molecule-1 (CD54); LFA-1, leucocyte function associated antigen-1 (CD11a)
Exercise and leucocytes in adolescents

Measurement of cardiorespiratory fitness

On a separate day, within one week of the wrestling practice, each volunteer underwent standard measurements of cardiorespiratory fitness. Each subject performed a ramp-type progressive exercise test on a cycle ergometer in which he exercised to the limit of his tolerance. Vigorous encouragement was given during the high intensity phases of the exercise protocol. Gas exchange was measured breath by breath, and the V_o_2 peak was determined as previously described for children and adolescents.11

Field study

The wrestling practice was held about six weeks after the end of the wrestling season. The field study was designed to mimic real life exercise, such as is encountered in the daily activities of these adolescents. To accomplish this, we arranged a 1.5 hour wrestling practice modelled on typical sessions of this sport. The practice was coached by one of the wrestling team coaches. None of the subjects trained during the day preceding the blood sampling. The subjects were instructed to have a light breakfast on the morning of the test, and the exercise session began at about noon. Subjects were admitted to the General Clinical Research Center (GCRC) at the University of California, Irvine. The study took place at the wrestling facility of the UCI faculty/student recreation centre.

Wrestling practice involves both aerobic and anaerobic exercise and both concentric and eccentric types of movement. The practice consisted of:

- Warm up (20 minutes): jogging, “stretch” exercise with sports specific calisthenics such as push ups and sit ups.
- Technique drills (20 minutes): the subjects performed typical wrestling skills including take downs, escapes, pin combinations, and pin counters. The technique drills involved high intensity exercise of short duration (6–10 seconds).
- Situation wrestling (15 minutes): the subjects were paired or placed in groups of three. Specific wrestling positions were assigned, and subjects wrestled from the given situation practicing a specific move and its counters. This involved exercise at maximal effort in bursts of 15–20 seconds.
- “Iron man” (15 minutes): wrestlers were placed in groups of five or six with one wrestler in each group designated the “iron man”. The “iron man” continuously wrestled facing a new partner every 30 seconds. Designation of “iron man” rotated after about three or four minutes. Each wrestler was the “iron man” at least once during this drill.
- Live wrestling (10 minutes): each wrestler was paired with a partner of similar weight and ability. Each pair wrestled a full six minute match.

As frequent blood sampling would have been unfeasible in the context of a vigorous wrestling exercise, we sampled blood twice, before and immediately after the exercise training session, by standard phlebotomy. The mean (SD) time interval between the end of the training session and phlebotomy was 41 (4) seconds (27–70). Samples were maintained at room temperature (23°C) and transported to the flow cytometry laboratory. Heart rate was measured by individual palpation at baseline and at three time points (20, 50, and 80 minutes) during the practice. As is typically the case in high school wrestling practices, subjects were permitted free access to water and encouraged to drink when thirsty and rest briefly when excessively fatigued.

Serum measurements

Lactate

Lactate was measured with the use of a YSI lactate analyser (YSI 1500; Yellow Springs, Ohio, USA). The in-assay coefficient of variation was 2.8%, the interassay coefficient of variation was 3.5%, and the sensitivity was 2 g/l.

Flow cytometry

Whole blood was preserved with EDTA and maintained at room temperature (23°C). As previously described,12 flow cytometry (FACSCalibur; Becton Dickinson, San Jose, California, USA) using CellQuest software was used to quantify leucocytes and lymphocyte subsets and CD62L and CD54 expression. A complete blood count analysis was performed using a Coulter STKS CBC counter. Whole blood was stained with monoclonal antibodies conjugated to various fluorochromes (Becton-Dickinson and PharMingen). The lysing reagent was FACS Brand Lysing Solution (Becton-Dickinson) which results in simultaneous lysis of red blood cells and partial fixation of leucocytes. Fluorescence compensation was performed using CalIBRITE beads (Becton-Dickinson) and FACSComp software. Optimal amounts of antibodies were used, and 8000–15 000 events were analysed per tube. Isotypic controls were used for each assay to determine non-specific staining. In addition to determining CD62L and CD54 expression, we determined CD62L, CD54, and CD11a density on mixed lymphocytes. For density determinations, flow cytometric estimation of antibodies bound/cell was performed using Quantibrite PE beads (Becton-Dickinson). The number of antibodies bound to the specific cell or microbead population provides a good approximation of antigen density expressed on the cell. The Quantibrite PE beads were run at the same instrument settings as the assay, and the FL2 (PE) axis was converted into the number of PE molecules bound/cell.

Statistical analysis

Paired t tests were used to determine changes after exercise. α was set at 0.05. Correlation and linear regression analyses were computed between changes in lactate, leucocytes, and adhesion molecules as well as with BMI and indexes of fitness. Data are presented as mean (SEM). Figure 1 shows the mean (SEM) of the percentage changes found in each subject. This is not identical with the percentage change that would be calculated using the mean values of the whole group before and after exercise.

RESULTS

Height, weight, BMI, and fitness level

Table 1 shows the subject characteristics. Weight had decreased significantly after the practice (75.4 (2.9) to 74.7 (2.9) kg, p<0.018).

Cardiorespiratory effects of the wrestling practice

All 11 subjects completed the 1.5 h practice. Mean baseline heart rate was 74 (2) beats/min. Mean heart rate at three measurement points during the practice (20, 50, and 80 minutes) was 163 (3), 160 (4), and 163 (3) beats/min.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject characteristics (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>16.5 (0.5) (14–18.5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.4 (2.9) (59.5–92.3)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.8 (1.8) (161–178)</td>
</tr>
<tr>
<td>BMi (kg/m²)</td>
<td>25.5 (0.8) (22.2–30.8)</td>
</tr>
<tr>
<td>BMI centile</td>
<td>83.6 (4) (49–98)</td>
</tr>
<tr>
<td>Peak V_o_2 (ml/min/kg)</td>
<td>44.5 (2) (37–54)</td>
</tr>
</tbody>
</table>

Values are mean (SEM) (range). BMi, body mass index.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Subject characteristics (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak V_o_2 (ml/min/kg)</td>
<td>44.5 (2) (37–54)</td>
</tr>
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expressing CD62L (CD4:CD62L T helper cells, the number of T helper cells not (CD8:CD62L) increased significantly, as did the number of natural killer cells. and adhesion molecules. than the large changes in peripheral blood mononuclear cells, the change in packed cell volume was substantially smaller was equivalent to only a 4.4% change in concentration. Thus, from 0.47 to 0.49 (p
CD11a (table 3). of CD54 on mixed lymphocyte increased, as did the density of mixed lymphocytes increased in the circulation. The density cytoytic cells, including granulocytes, monocytes, mixed lymphocytes, CD19 B cells, CD3 T cells, CD3CD8 T cytotoxic cells, CD3CD4 T helper cells, and CD3*CD16CD56 natural killer cells. The CD4:CD8 ratio had decreased after exercise (table 2). The number of CD3CD8 T cytotoxic cells expressing CD62L (CD8CD62L) increased significantly, as did the number of CD4CD62L T helper cells and the number of T helper cells not expressing CD62L (CD4CD62L). Both CD54 and CD54* mixed lymphocytes increased in the circulation. The density of CD54 on mixed lymphocyte increased, as did the density of CD11a (table 3). Packed cell volume had increased by the end of exercise from 0.47 to 0.49 (p<0.005). However, the overall change was equivalent to only a 4.4% change in concentration. Thus, the change in packed cell volume was substantially smaller than the large changes in peripheral blood mononuclear cells and adhesion molecules. respectively. Lactate concentration increased by 441 (67)\% (p<0.005).

**Response to the wrestling exercise**

As shown in tables 2 and 3 and figs 1 and 2, the wrestling exercise led to a significant increase in the number of all circulating white blood cells, including granulocytes, monocytes, mixed lymphocytes, CD19 B cells, CD3 T cells, CD3CD8 T cytotoxic cells, CD3CD4 T helper cells, and CD3*CD16CD56 natural killer cells.

The CD4:CD8 ratio had decreased after exercise (table 2). The number of CD3CD8 T cytotoxic cells expressing CD62L (CD8CD62L) increased significantly, as did the number of CD4CD62L T helper cells and the number of T helper cells not expressing CD62L (CD4CD62L). Both CD54 and CD54* mixed lymphocytes increased in the circulation. The density of CD54 on mixed lymphocyte increased, as did the density of CD11a (table 3).

Packed cell volume had increased by the end of exercise from 0.47 to 0.49 (p<0.005). However, the overall change was equivalent to only a 4.4% change in concentration. Thus, the change in packed cell volume was substantially smaller than the large changes in peripheral blood mononuclear cells and adhesion molecules.

**Correlation between fitness, BMI, and increase in lactate concentrations with leucocyte responses to the wrestling practice**

We found no correlation between fitness (peak VO2/kg) or BMI and the magnitude of change in response of any of the leucocytes or adhesion molecules to exercise. Exercise associated increases in lactate, however, correlated significantly with the increases in leucocytes (r = 0.644, p<0.009, fig 3), granulocytes (r = 0.675, p<0.023), and CD54 lymphocytes (ΔCD54 lymphocytes, r = 0.75, p<0.004).

**DISCUSSION**

This is the first study to show acute, substantial responses of leucocytes and adhesion molecules to field exercise in a population of healthy adolescent boys. Although representing an intense level of physical activity, the wrestling practice protocol is encountered in the lives of many adolescents and is not atypical of the intensity found in other high school level individual and/or team sports. The data also show that exercise intensity (represented by the change in lactate concentration) can influence the immune response to brief periods of intense exercise in adolescent boys.

It has been shown that sustained exercise leads to an abrupt (first 10–20 minutes) increase in leucocytes in adults.13 This initial increase in the number of circulating leucocytes is believed to result from recruitment of cells from the marginal pool.14 The pulmonary and splanchnic vascularature are considered to be important reservoirs of this population of healthy adolescent boys. Although representing an intense level of physical activity, the wrestling practice is not atypical of the intensity found in other high school level individual and/or team sports. The data also show that exercise intensity (represented by the change in lactate concentration) can influence the immune response to brief periods of intense exercise in adolescent boys.

**Table 2** Circulating leucocytes and lymphocyte subsets before and after exercise

<table>
<thead>
<tr>
<th>Subset</th>
<th>Before</th>
<th>After</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cells</td>
<td>6993 (423)</td>
<td>13436 (1339)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>3848 (296)</td>
<td>7719 (885)</td>
<td>0.001</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2548 (179)</td>
<td>4651 (408)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Monocytes</td>
<td>597 (50)</td>
<td>1069 (128)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD8*CD16CD56 NK cells</td>
<td>434 (47)</td>
<td>1466 (207)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD19 B cells</td>
<td>458 (54)</td>
<td>540 (46)</td>
<td>0.029</td>
</tr>
<tr>
<td>CD3 T cells</td>
<td>1616 (127)</td>
<td>2571 (221)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD3CD4 T helper cells</td>
<td>859 (69)</td>
<td>1215 (91)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD3CD8 T cytotoxic cells</td>
<td>271 (38)</td>
<td>1214 (158)</td>
<td>0.002</td>
</tr>
<tr>
<td>CD4:CD8 ratio</td>
<td>1.31 (0.07)</td>
<td>1.06 (0.07)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values are cells/μl and are expressed as mean (SEM).

**Table 3** Expression of lymphocyte adhesion molecules before and after exercise

<table>
<thead>
<tr>
<th>Lymphocyte adhesion molecules</th>
<th>Before</th>
<th>After</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8CD62L</td>
<td>416 (38)</td>
<td>557 (42)</td>
<td>0.002</td>
</tr>
<tr>
<td>CD8CD62L*</td>
<td>255 (20)</td>
<td>657 (116)</td>
<td>0.003</td>
</tr>
<tr>
<td>CD62L density on CD8*</td>
<td>10103 (633)</td>
<td>7677 (621)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD62L density on lymphocytes</td>
<td>10806 (453)</td>
<td>8089 (432)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD4*CD62L</td>
<td>748 (63)</td>
<td>1030 (76)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD4CD62L*</td>
<td>111 (4)</td>
<td>185 (13)</td>
<td>0.009</td>
</tr>
<tr>
<td>CD62L density on CD4*</td>
<td>11309 (446)</td>
<td>9053 (426)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD54 lymphocytes</td>
<td>899 (128)</td>
<td>1428 (224)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD54* lymphocytes</td>
<td>1649 (51)</td>
<td>3223 (184)</td>
<td>0.0005</td>
</tr>
<tr>
<td>CD54 density on lymphocytes</td>
<td>980 (46)</td>
<td>1154 (59)</td>
<td>0.007</td>
</tr>
<tr>
<td>CD11a density on lymphocytes</td>
<td>19130 (1075)</td>
<td>23993 (1328)</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Values are antibodies bound/cell. *Antibodies bound/cell.
In contrast, fitter elderly subjects tend to have higher immune compromise, particularly in the recovery period. This decrease may contribute to the exercise induced first line of the host defence system to foreign pathogens, but the change in natural killer cell number to be the most prominent change (fig 2). Natural killer cells play a pivotal role in the increase of T cytotoxic cells, T helper cells, and B cells. T cells, and natural killer cells (table 2, fig 1) after the wrestling exercise.

In response to exercise in both adults and children, stress hormones, including adrenaline (epinephrine) and noradrenaline (norepinephrine), increase (not measured in this study). The influence of exercise induced changes in catecholamines on lymphocyte trafficking is determined, in part, by the density of β2 adrenergic receptor on the lymphocytes. After the wrestling practice, we found increases in all lymphocyte subsets, but the magnitude of change within the subsets differed. Natural killer cells have the highest density of β2 receptors on their surface and are known to be the cell type most responsive to exercise or catecholamine injection. Consistent with this, we found the change in natural killer cell number to be the most prominent change (fig 2). Natural killer cells play a pivotal role in the first line of the host defence system to foreign pathogens, but also increase in response to other stress—for example, psychosocial.

In adults, the initial increase in natural killer cell number after exercise usually persists for a brief time (hours), and is followed by a decrease (exercise associated leucopenia). Whether this biphasic pattern exists in children and adolescents after exercise and the precise biological role has not been determined.

After natural killer cells, β2 receptor density decreases progressively on T cytotoxic cells, T helper cells, and B cells. T cytotoxic cells and T helper cells increased with exercise in our study while B cells showed a small significant change, and the pattern of these changes in lymphocyte subpopulations paralleled the pattern of β2 receptor density (fig 2).

Both CD4 (T helper) and CD8 (T cytotoxic) lymphocytes increased but the increase in CD8 was greater. Consequently, there was a significant decrease in the CD4:CD8 ratio, which has been used to gauge the degree of immunodeficiency in pathological states. Similarly to these findings in adolescents, strenuous exercise is known to elicit an initial decrease in the CD4:CD8 ratio in adults. Shek et al suggested that this decrease may contribute to the exercise induced immune compromise, particularly in the recovery period. In contrast, fitter elderly subjects tend to have higher CD4:CD8 ratios. The physiological importance of exercise alterations in the CD4:CD8 ratio of adolescents and/or its influence on the development of the immune system is not yet known.

This study shows that in children, as in adults, strenuous exercise influences adhesion molecules. CD62L (L-selectin) mediates leukocyte rolling and adhesion to endothelium at the site of inflammation. We found an increase in both CD62L and CD62L with a preferential significant increase in CD62L T cell subsets (table 3). The relative increase in circulating CD62L lymphocytes is believed to result from preferential release into the circulation of CD62L cells from the marginal pool (primarily the spleen) rather than actual downregulation of L-selectin in response to exercise. The decreased density of CD62L on circulating lymphocytes, mainly CD8 and CD4 cells, is consistent with previous exercise studies. As L-selectin is typically shed and CD11a is upregulated on T lymphocytes as the cell changes from a naïve to a post-antigen presented memory T cell, these findings suggest that the wrestling exercise led to recruitment of memory T cells into the peripheral circulation from the marginal pool—that is, the marginal pool serves as a reservoir to memory cells. It is well documented that, compared with naïve cells, memory cells have increased interaction capacity with activated endothelium as well as the ability to selectively recruit into inflammatory sites. Finally, these memory T cells (marked also as CD45RO) are also known to be associated with increased production of inflammatory cytokines such as interleukin 6. The influence on the development of the immune system is not long term effects on subsequent growth. Whether or not exercise associated immune stimulation can alter the immune system is not known. 

In summary, we here provide the first data showing significant effects of field exercise on immune cell subsets in healthy adolescents. We also show the effect of exercise on adhesion molecules correlates with the metabolic stress imposed by the wrestling exercise (represented by lactate produced by the working muscles, fig 3). Boas et al obtained similar findings on leucocyte number in children performing laboratory exercise. We found no correlation between fitness level or BMI centile and the magnitude of the immune response. This may be because the subjects in this study were all relatively fit, and their high BMI centile may actually be due to increased muscle mass rather than increased fat. We did not carry out an independent assessment of body composition—for example, by dual x-ray absorptiometry.
Take home message

During childhood and adolescence, intense bouts of exercise serve as powerful stimuli to the immune system. The overall effect of these stimuli on immunity, however, is yet to be determined.

ACKNOWLEDGEMENTS

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REFERENCES

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