Effects of laboratory versus field exercise on leukocyte subsets and cell adhesion molecule expression in children

Acceptance: 27 June 2001 / Published online: 6 September 2001
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Abstract In adults, exercise is a powerful and natural stimulator of immune cells and adhesion molecules. Far less is known about these exercise responses during childhood and whether or not exercise in real-life activities of healthy children might influence immune responses. We compared laboratory exercise (10×2 min periods of heavy, constant intensity, cycle ergometer exercise with 1 min rests between exercise in nine subjects, aged 9–15 years) with field exercise (90 min soccer practice in nine different subjects, aged 9–11 years). Blood was sampled before both protocols, 5 min after the 30 min laboratory protocol, and 10–15 min after the 90 min field protocol. Both field and laboratory exercise protocols led to significant (P < 0.05) increases in granulocytes, monocytes, and all lymphocyte subpopulations. The mean (SEM) increases were similar for the two protocols except for the significantly greater increase in laboratory compared with field protocols for natural killer cells [142 (39)% vs 12 (16)%, P < 0.001] and monocytes [64 (22)% vs 32 (19)%, P < 0.001]. Both protocols significantly influenced adhesion molecules (such as CD54) which have not been previously studied in children. However, the adhesion molecule CD8⁺CD62L⁻ increased to a significantly (P < 0.001) greater extent in the laboratory [101 (25)%] versus field [34 (25)%] protocol. Finally, the density of CD62L on lymphocytes significantly decreased with laboratory exercise but showed no change in the field protocol [−20 (3)% vs −3 (3)%, P < 0.001]. The rapid and substantial immune response in both laboratory and field protocols suggests that exercise stimulation of the immune system occurs commonly in the real lives of children and may play a role in their overall immune status.

Keywords Exercise · Children · White blood cells · Lymphocytes · Adhesion molecules

Introduction

It is increasingly recognized that brief episodes of physical exercise can substantially influence the type and numbers of circulating white blood cells (Nieman and Pedersen 1999; Pedersen and Hoffman-Goetz 2000). Naturally, most investigators have focused on the importance of this effect with regard to immune function and the body’s ability to respond to infection. During childhood adaptive consequences of white blood cell responses to exercise may prove to be important for the overall development of the hematopoietic and immune systems as well as for the fundamental processes of growth. Few studies of exercise immune effects have been made in children compared with adults (Boas et al. 1996, 2000; Eliakim et al. 1997; Wolach et al. 1998), and, to our knowledge, no studies have been made in children under field conditions, i.e. those mimicking the kind of exercise performed by children in daily life activities.

In addition to the general phenomenon of leukocyte redistribution in response to exercise (Boas et al. 1996; Eliakim et al. 1997; Pedersen and Hoffman-Goetz 2000) recent studies in adults show that exercise also affects the expression of certain leukocyte adhesion molecules. The cell adhesion molecule L-selectin (CD62L), for example, is important for the trafficking of leukocytes in and out of the circulation, mediating the rolling and adhesion to the vessel wall (Adams and Shaw 1994; Springer 1994). Findings in adults show that exercise leads to a preferential increase of CD62L⁺ versus CD62L⁻ lymphocytes, as well as a decrease in the cell surface density of CD62L (Kurokawa et al. 1995; Miles et al. 1998; Mills et al. 1999). The CD54, found on the surface of lymphocytes
and endothelial cells, is responsible for firm adhesion and transendothelial migration. Few studies have examined the effects of exercise on adhesion molecules in children.

The goal of the present study was to compare acute exercise-induced alterations in certain aspects of immune function (white blood cell subpopulations and their associated adhesion molecules) in two groups of children: one performing laboratory exercise protocols and the other engaging in soccer practice such as is now frequently encountered in the lives of millions of healthy children throughout the world. We hypothesized that field studies would lead to substantially greater alterations in these immune related factors for the following reasons: first, it is our experience that children are unwilling (or unable) to exercise as vigorously and/or for as long a duration in the laboratory as they are in real life settings which tend to be more fun and varied compared to what is possible in a laboratory. Secondly, real life exercise, such as a soccer practice, more likely involves those forms of exercise (resistive, eccentric) that are known to cause local inflammation if not frank microfiber injury.

Methods

Sample population

The study was approved by the Institutional Review Board, University of California, Irvine (UCI). The experiment complied with the current laws of the State of California, USA. Informed consent as well as assent were obtained from the total of 18 healthy children who participated in the study (9 field study, 9 laboratory; Table 1). We did not think it feasible that sufficient numbers of children would agree to participate in both types of studies since this would involve both taking multiple blood samples and insertions of intravenous catheters. Therefore, we excluded a paired sample study design. Two separate groups were recruited and, despite our efforts, the groups did not match precisely for age, body mass, and height (see Table 1). No subjects were receiving any medications at the time of the study. The field studies were performed in December of 1999 and the laboratory studies between April and September of 2000.

Field study

The field study was designed to mimic real-life exercise, such as might be encountered in the daily activities of children. To accomplish this, we arranged a 1.5 h soccer practice modeled on typical sessions of this sport. The soccer practice was coached by one of the UCI team coaches who also had particular experience in working with children. The experiment goal was for the children to engage in approximately 40–50 min of vigorous aerobic-type exercise over the 1.5 h period. During the soccer practice, there were many water breaks and brief rest periods, first, to ensure adequate hydration and second, to reflect the intensity and tempo of this type of activity typically found in the community.

The children were instructed to have a light breakfast on the morning of the test, and after this the participants reported to the laboratory at 0900 hours. Blood samples were obtained and the children proceeded to the soccer practice on a site adjacent to the General Clinical Research Center (GCRC). After the 1.5 h practice, the children jogged back to the GCRC where further blood samples were obtained. There was a 5–15 min interval between the end of the jog back to the GCRC and the time that the blood samples were obtained. The effect of this protocol on cytokines and growth factors in these children has been published (Scheett et al. 1999).

Laboratory study

The exercise tests were performed at the Human Performance Core Laboratory of the UCI University of California San Diego Satellite Center. Each subject underwent two separate exercise test sessions performed on different days. First, to measure cardiorespiratory responses to exercise and assess fitness, we used a ramp-type progressive exercise test on an electronically braked cycle ergometer (SensorMedics Vmax 229, Yorba Linda, Calif.). The exercise intensity increased by 10 W-min⁻¹, and each subject exercised to the limit of his or her tolerance. Gas exchange was measured breath-by-breath (Beavere et al. 1981; Cooper et al. 1984). This approach has been used extensively in children and adolescents.

The second session consisted of a series of 10×2 min periods of constant intensity cycle ergometry with 1 min rests between each period of exercise. The exercise intensity was individualized for each subject and calculated to be equivalent to the intensity corresponding to 50% of the difference between the anaerobic or lactate threshold (determined noninvasively from the ramp test) and peak oxygen uptake. We have used this approach in previous investigations to ensure that the exercise was standardized to physiological indicators of each individual subject’s exercise capability (Cooper et al. 1989). In addition, our experience with heavy intensity exercise (i.e. exercise intensities above the subject’s lactate threshold) is that children often do not maintain constant exercise for more than several minutes at a time. The total duration of the second exercise protocol was 30 min (20 min of cycle ergometer exercise interspersed with 10 min of rest).

Flow cytometry

Whole blood was preserved with EDTA and maintained at room temperature (23°C). As previously described (Mills et al. 1999), flow cytometry using CellQuest software (FACSCalibur, Becton Dickinson, San Jose, Calif.) was used to quantify leukocytes and lymphocyte subsets and CD62L and CD54 expression. A complete blood count (CBC) analysis was performed by using a Coulter STKS CBC Counter. Blood was processed within 12 h of collection and whole blood was stained with monoclonal antibodies conjugated to various fluorochromes (Becton-Dickinson and PharMin- gen). The lysing reagent was FACS Brand Lysing Solution (Becton-Dickinson) which resulted in a simultaneous lysis of red blood cells and partial fixation of leukocytes. Fluorescence compensation was performed using CalibRITE beads and FACScComp software (Becton-Dickinson). Optimal amounts of antibodies were used and 8,000–15,000 events were analyzed per tube. Isotypic controls were used for each assay to determine non-specific staining. In addition to determining CD62L and CD54L expression, we determined CD62L density on mixed lymphocytes. For CD62L density, flow cytometric estimation of antibodies bound/cell (ABC) was performed using Quantibrite PE beads (Becton-Dickinson). The ABC, being the number of antibodies that bind to the specific cell or microbead population, provides a good approximation of antigen density expressed on the cell. The Quantibrite PE beads

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*P < 0.02