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Effect of Water Polo Practice on Cytokines, Growth Mediators, and Leukocytes in Girls

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ABSTRACT

NEMET, D., C. M. ROSE-GOTTRON, P. J. MILLS, and D. M. COOPER. Effect of Water Polo Practice on Cytokines, Growth Mediators, and Leukocytes in Girls. *Med. Sci. Sports Exerc.*, Vol. 35, No. 2, pp. 356–363, 2003. **Purpose:** The effects of exercise on growth and development are mediated through a complex interaction between the endocrine, immune, and nervous systems. Very little is known about how these systems respond to exercise in children or adolescents. Moreover, there are few studies that have examined growth factors, inflammatory cytokines, and leukocyte responses to “real-life” or field exercise solely in girls. Thus, the goal of the present study was to determine the acute exercise-induced alterations in the growth hormone → insulin-like growth factor-I axis, inflammatory cytokines, and certain aspects of immune function in a group of adolescent girls after a typical water polo practice. **Methods:** Ten, healthy, high-school female subjects, 14–16 yr old, performed a single, typical, 1.5-h water polo practice session. Blood was sampled before and after the session. **Results:** The exercise resulted in an increase in HR (from 82 ± 2 to 161 ± 5 beats·min⁻¹ at 30 min, $P < 1.4 \cdot 10^{-6}$), as well as in circulating lactate levels ($375 \pm 66\%$, $P < 0.0005$). Significant increases were noted in circulating IL-6 ($396 \pm 162\%$, $P < 0.005$) and IL-1ra ($71 \pm 20\%$, $P < 0.015$). A substantial increase in the level of IGFBP-1 ($1344 \pm 344\%$, $P < 0.001$) was also observed. Interestingly, TNF- α levels decreased after the exercise ($-10.4 \pm 3.8\%$, $P < 0.04$) as did insulin ($55 \pm 12\%$, $P < 0.005$). The exercise led to significant increases in granulocytes, monocytes, and lymphocytes. The exercise significantly influenced adhesion molecules (such as CD62L and CD54), which has not been previously studied in adolescent girls. **Conclusions:** These data demonstrate that an intense “real-life” exercise bout in adolescent females leads to profound increases in inflammatory cytokines and reductions in anabolic mediators with substantial alterations in white blood cell subpopulations and adhesion molecules. The role of these frequent, almost daily immune and cytokine changes on growth and development have yet to be determined. **Key Words:** PHYSICAL ACTIVITY, WHITE BLOOD CELLS, ADHESION MOLECULES, IL-6, TNF- α

Although exercise can influence the growth of muscle, bones, vascular, and adipose tissues, the specific mediators that cause these changes have not been elucidated. Recent data indicate, somewhat surprisingly, that exercise can simultaneously stimulate seemingly antagonistic mediators arising from very different organ systems: on the one hand, pituitary growth hormone (GH) and hepatic insulin-like growth factor-I (IGF-I) (7), both anabolic in nature, and, on the other hand, proinflammatory catabolic cytokines like tumor necrosis factor- α (TNF- α) or interleukin-1 (IL-1)—typically produced by immunologically active peripheral blood mononuclear cells (PBMC) (1). To date, very little is known about how these antagonistic responses are balanced after exercise in children or adolescents.

Moreover, there are few studies that have examined growth factors, proinflammatory cytokines, and PBMC response to “real-life” or field exercise solely in girls. Gender effects on the growth factor and immune response to exercise have been reported in adults. For example, Stupka et al. (36) noted that the muscle inflammatory response to exercise is attenuated in women compared with men, and Pritzlaff-Roy et al. (24) found that the exercise-induced GH response was greater in women than in men.

Thus, the goal of the present study was to determine the acute exercise-induced alterations in GH→IGF-I axis, inflammatory cytokines, and certain aspects of immune function in a group of adolescent girls after a typical water polo practice. We focused on white blood cell (WBC) subpopulations and their associated adhesion molecules, which regulate the mobilization and indicate immunological activity of PBMC. We hypothesized that water polo exercise would lead to acute alterations in the circulating levels of GH→IGF-I axis mediators (GH would be increased, IGF-I decreased), proinflammatory cytokines [increase in interleukin-6 (IL-6) and TNF- α], and WBC subpopulations and adhesion molecules (increase in WBC subpopulations and intercellular adhesion molecule-1).

Water polo was chosen specifically because individual practice sessions involve high metabolic demand. Moreover, water polo is composed of intense bursts of activity of

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TABLE 1. Subject characteristics ($N = 10$).

Age (yr)	15.1 ± 0.3 (range,14–16)
Weight (kg)	55.5 ± 1.8 (range,46–62.2)
Height (cm)	162.7 ± 1.6 (range,152–171)
BMI ($\text{kg}\cdot\text{m}^{-2}$)	20.9 ± 0.7 (range,18.2–23.8)
BMI percentile	57.7 ± 6.9 (range,25–83)
Peak VO_2 ($\text{mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$)	30.5 ± 1.9 (range,22.6–38.9)

Data presented as mean ± SEM.

<15-s duration with intervening, lower-intensity intervals averaging <20-s duration. This is qualitatively similar to the characteristic exercise pattern of children involving many brief pulses of activity of varying intensities. Finally, water polo and other swimming sports are increasingly popular among female high school students.

METHODS

Approach to the Problem and Experimental Design

The study was designed to examine growth mediators, cytokines, and PBMC responses to intense exercise in a field setting in which each participant served as her own control. There was no independent, nonexercising control group. Because frequent blood sampling would have been unfeasible in the context of a vigorous swimming exercise, we sampled blood twice, before and immediately after the exercise training session.

Sample Population

The study was approved by the Institutional Review Board, University of California, Irvine (UCI), and informed written consent as well as a written assent were obtained. Ten healthy adolescent girls participated in the study (Table 1). No subjects were on any medications at the time of the study. Due to the known abnormalities in menstrual status with this population, we did not attempt to study the subjects at the same point in their menstrual cycle.

Height, Weight, and Body Mass Index (BMI) Measurements

Standard, calibrated scales and stadiometers were used to determine height, weight, and BMI ($\text{weight}/\text{height}^2$). Because BMI changes with age, we calculated BMI percentile for each child using the recently published standards from the Centers for Disease Control, National Center for Health Statistics (15). Weight was measured before and at the end of the practice.

Measurement of Cardiorespiratory Fitness

On a separate day, within 1 wk of the water polo practice, each volunteer performed standard measurements of cardiorespiratory fitness. These studies were done at the UCI General Clinical Research Center (GCRC). Each subject performed a ramp-type progressive exercise test on a cycle ergometer in which the subject exercised to the limit of her tolerance. Subjects were vigorously encouraged during the

TABLE 2. Water polo practice description.

	Duration (min)	Description
Dry-land warm-up	10	Sit-ups, push-ups, sport-specific stretch drills.
Swimming warm-up	10	300–600 yards swimming at a moderate pace.
Swimming conditioning	20	Swimming drills with increased intensity. At least 10 min of high intensity.
Leg work	15	5 min of treading water. 10 min of moving up and down the pool, both hands out of the water, passing a ball from hand to hand.
Passing	15	Treading water in stationary position, both arms out of the water while passing a ball with a partner at increasing distances.
Shooting	10	Swimming toward the goal, changing position in the water (horizontal to vertical), and shooting the ball, a high-intensity short-burst drill.
Game drills	10	Game-like situations are practiced.

high-intensity phases of the exercise protocol. Gas exchange was measured breath-by-breath and the $\text{VO}_{2\text{peak}}$ was determined using standard techniques.

Field Study

The water polo practice was held approximately 2 wk before the start of the water polo season. The field study was designed to mimic a real-life exercise paradigm, such as encountered in the daily activities of these adolescent girls. To accomplish this, we arranged a 1.5-h water polo practice modeled after typical sessions of this sport. The practice was coached by one of the water polo team coaches. None of the subjects trained during the day preceding the blood sampling. Participants were instructed to have a light breakfast on the morning of the test, and the exercise session began at approximately 12 p.m. The study took place at the swimming pool facility of the UCI faculty/student recreation center.

The 90-min water polo practice components are presented in Table 2. It is important to note that once the subjects completed the swimming-conditioning portion of the workout, they treaded water or swam for the entire remainder of the practice.

Pre- and postexercise blood samples were obtained by standard phlebotomy. The time interval between the end of the training session and phlebotomy was 104 ± 19 s (55–240 s). Samples used for flow cytometry were preserved with EDTA and maintained at room temperature (23°C). All other samples were placed in an ice bath and were immediately centrifuged. Aliquots of the resulting plasma were stored at -80°C until analyzed. All pre- and postintervention specimens were analyzed in the same batch by technicians who were blinded to the order of the samples. HR was measured by individual palpation at baseline and at two time points (30 and 60 min) during the practice. As is typically the case in high school water polo 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 YSI lactate analyzer (YSI 1500, Yellow Springs, OH). The intra-

assay coefficient of variation (CV) was 2.8%, The interassay CV was 3.5%, and the sensitivity was $0.2 \text{ mg}\cdot\text{dL}^{-1}$ ($0.022 \text{ mmol}\cdot\text{L}^{-1}$).

Albumin. Albumin levels were determined by colorimetric determination by the use of the Sigma BCP albumin procedure no. 256 (Sigma Diagnostic, St. Louis, MO). Albumin has been measured by other investigators in studies of the effects of exercise on growth factors like IGF-I to estimate possible effects of exercise on plasma volume because albumin is similar in molecular weight to IGF-I or IGFBP-3 (6). We recognize, however, that multiple approaches exist to this complex problem.

GH. GH serum concentrations were determined by ELISA with the use of the DSL-10-1900 Active kit (Diagnostic System Laboratories, Webster, TX). Intra-assay CV was 3.3–4.3%, interassay CV was 6.3–6.5%, and the sensitivity was $0.03 \text{ ng}\cdot\text{mL}^{-1}$

IGF-I. IGF-I was extracted from IGFBP by using the acid-ethanol extraction method. Serum IGF-I concentrations were determined by a two-site immunoradiometric assay (IRMA) by using the DSL-5600 Active kit (Diagnostic System Laboratories). IGF-I interassay CV was 3.7–8.2% and intra-assay CV was 1.5–3.4%. Assay sensitivity was $0.8 \text{ ng}\cdot\text{mL}^{-1}$. Free IGF-I was determined by ELISA with the use of the DSL-10-9400 Active kit (Diagnostic System Laboratories). Intra-assay CV was 3.74–4.8%, interassay CV was 6.2–11.1%, and the sensitivity was $0.015 \text{ ng}\cdot\text{mL}^{-1}$.

IGFBP. IGFBP-1 was measured by ELISA with the use of the DSL-10-7800 Active kit (Diagnostic System Laboratories). For IGFBP-1, interassay CV was 6.2–7.6% and intra-assay CV was 1.7–4.6%. Assay sensitivity was $0.25 \text{ ng}\cdot\text{mL}^{-1}$ (results for IGFBP-1 were not obtainable for one subject). IGFBP-3 serum concentrations were determined by ELISA with the use of the DSL, 10–6600 Active kit (Diagnostic System Laboratories). Intra-assay CV was 7.3–9.6%, interassay CV was 8.2–11.4%, and the sensitivity was $0.04 \text{ ng}\cdot\text{mL}^{-1}$.

TNF- α . TNF- α serum levels were determined by ELISA with the use of the R&D system Quantikine High Sensitivity kit (R&D system; Minneapolis, MN). Intra-assay CV was 8.7–14.8%, interassay CV was 16.1–22.6%, and the sensitivity was $0.18 \text{ pg}\cdot\text{mL}^{-1}$.

IL-6. IL-6 serum levels were determined by ELISA with the use of the R&D system Quantikine High Sensitivity kit (R&D system). Intra-assay CV was 3.8–11.1%, interassay CV was 7.1–29.5%, and the sensitivity was $0.0094 \text{ pg}\cdot\text{mL}^{-1}$.

IL-1 β . IL-1 β serum levels were determined by ELISA with the use of the R&D system Quantikine High Sensitivity kit (R&D system). Intra-assay CV was 1.6–4.0%, interassay CV was 5.3–9.0%, and the sensitivity was $0.059 \text{ pg}\cdot\text{mL}^{-1}$.

IL-1ra. IL-1ra serum levels were determined by ELISA with the use of the R&D system Quantikine High Sensitivity kit (R&D system). Intra-assay CV was 3.1–6.2%, interassay CV was 4.4–6.7%, and the sensitivity was $22 \text{ pg}\cdot\text{mL}^{-1}$.

Glucose. Serum glucose levels were determined by quantitative enzymatic measurements with the use of Sigma

diagnostic kit no. 510 (Sigma Diagnostics). Intra-assay assay CV was 3.5%, interassay CV was 2–5%, and the dynamic range of the kit was $25\text{--}300 \text{ mg}\cdot\text{dL}^{-1}$ ($1.4\text{--}16.6 \text{ mmol}\cdot\text{L}^{-1}$).

Insulin. Insulin serum levels were determined by ELISA with the use of the DSL-10-1600 Active kit (Diagnostic System Laboratories). Intra-assay CV was 1.3–2.6%, interassay CV was 5.2–6.2%, and the sensitivity was $0.26 \mu\text{IU}\cdot\text{mL}^{-1}$.

Flow cytometry. Flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA) using CellQuest software 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 (Beckman Coulter Counter, Fullerton, CA). Whole blood was stained with monoclonal antibodies conjugated to various fluorochromes (Becton Dickinson and PharMingen, San Diego, CA). The lysing reagent was FACS Brand Lysing Solution (Becton Dickinson), which results in a simultaneous lysis of red blood cells and partial fixation of leukocytes. Fluorescence compensation was performed using CaliBRITE beads (Becton Dickinson) and FACSComp software. 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 nonspecific staining. In addition to determining CD62L and CD54 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). 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 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 pre versus post exercise differences. α was set at 0.05. Data are presented as mean \pm SEM. Statistical analysis of growth factors and cytokines were performed using pre- and postconcentration values as shown in the tables. In Figures 1 and 2, we present the mean and SEM of the percent changes found in each subject. The latter value is not identical with the percent change that would be calculated using the mean pre- and postvalues of the whole group. Standard linear regression analysis was used to determine any correlations among fitness, body composition, lactate, circulating catabolic and anabolic mediators, and the PBMC response.

RESULTS

Height, weight, BMI, and fitness level. Subject characteristics are presented in Table 1. No change in weight was noted after exercise.

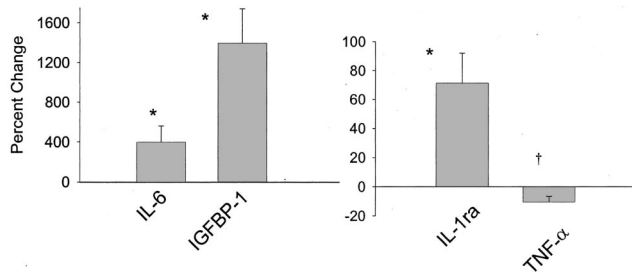


FIGURE 1—Effect of a water polo practice on likely catabolic mediators. Substantial elevations in IGFBP-1 ($P < 0.001$) and IL-6 ($P < 0.005$) followed exercise. Significant increases in IL-1ra ($P < 0.015$) were also observed but at about an order of magnitude less. TNF- α decreased after exercise ($P < 0.04$).

Cardiorespiratory effects of the water polo practice. All 10 subjects completed the 1.5-h practice. Mean baseline HR was 82 ± 2 beats·min⁻¹. Mean HR during the practice measured at 30 and 60 min was 161 ± 5 ($P < 1.4 \cdot 10^{-6}$ increase from baseline) and 154 ± 6 beats·min⁻¹ ($P < 2.6 \cdot 10^{-5}$ increase from baseline), respectively. Lactate increased by $375 \pm 66\%$ ($P < 0.0005$).

Albumin. There was a significant change in albumin levels after the exercise (from 4.8 ± 0.1 to 5.1 ± 0.1 g·dL⁻¹, $P < 0.018$); consequently, concentrations of catabolic and anabolic mediators in the circulation are expressed as their ratio to albumin levels.

Effect of water polo practice on anabolic mediators. The water polo practice was followed by a $55 \pm 12\%$ decrease in insulin ($P < 0.005$). No change in glucose levels was noted. No change was noted in circulating GH, total, bound, and free IGF-I levels (Fig. 1, Table 3). The exercise associated decrease in insulin was correlated with the increase in lactate ($r = 0.64$, $P < 0.05$), but no other correlations were found among the changes in the anabolic mediators, body composition, or fitness.

Effect on catabolic mediators. Significant increases were noted in circulating IL-6 ($P < 0.005$) and IL-1ra ($P < 0.015$, Table 3). A substantial increase in the level of

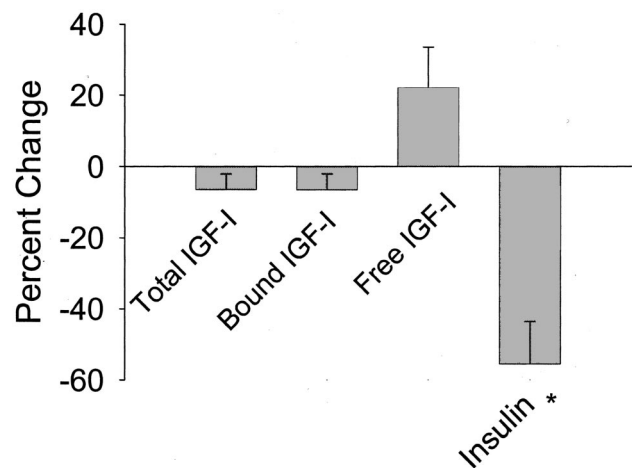


FIGURE 2—Effect of water polo practice on the likely anabolic mediators. A significant decrease was noted for insulin ($P < 0.005$). Total, free, and bound IGF-I did not significantly change after the exercise.

IGFBP-1 was also observed ($1396 \pm 344\%$, $P < 0.001$). Interestingly, TNF- α levels decreased after the exercise ($P < 0.04$, Table 3, Fig. 2). No correlations were found among the changes in the catabolic mediators, body composition, lactate, or fitness.

Effect on PBMC and adhesion molecules. There were no significant changes in hematocrit or hemoglobin levels after the water polo practice (43.35 ± 0.55 to $43.1 \pm 0.79\%$ and 13.94 ± 0.19 to 13.99 ± 0.24 g·dL⁻¹ respectively, NS). As shown in Tables 4 and 5 and Figures 3 and 4, the water polo practice led to a significant increase in the number of circulating WBC, including granulocytes, monocytes, mixed lymphocytes, CD3⁺ T cells, CD3⁺CD8⁺ T-cytotoxic cells, CD3⁺CD4⁺ T-helper cells, CD3⁻CD16⁺56⁺, natural killer cells, and platelets. No change was noted in CD19⁺ B cell response. Both CD3⁺CD8⁺ T-cytotoxic cells expressing CD62L (CD8⁺CD62L⁺) and T-cytotoxic cells not expressing CD62L (CD8⁺CD62L⁻) increased after exercise; however, only the increase in T-cytotoxic cells not expressing CD62L was statistically significant. CD62L density on CD8⁺ lymphocytes decreased after the exercise (Fig. 5). The number of CD3⁺CD4⁺ T-helper cells expressing CD62L (CD4⁺CD62L⁺), increased significantly; however, the number of T-helper cells not expressing CD62L did not increase significantly (CD4⁺CD62L⁻). Both CD54⁺ and CD54⁻ mixed lymphocytes increased in circulation (Table 5). No correlations were found among the changes in the PBMC, body composition, lactate, or fitness.

DISCUSSION

This study presents the first data demonstrating acute, substantial, and simultaneous catabolic and anabolic responses as well as immune system responses to field exercise in healthy adolescent females. Although the water polo practice protocol constitutes an intense level of physical activity (HR increase of about 100%, and lactate increase of about 375%), it 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 uniquely the PBMC and adhesion molecules response to a relatively brief intense exercise.

The regulation of growth in adolescents is not only of profound biological interest but also has great implications for health throughout life. Hormonal factors, including the cytokines and components of immune system function studied in the present paper, likely play a role in growth and development. Thus, this study of how “real life” physical activity influences these key regulating substances may, we believe, lead to novel ways in which we gauge levels of physical activity in children and adolescents and determine their optimal benefits.

The water polo practice was accompanied by a profound proinflammatory, catabolic response. IL-6, a cytokine usually associated with catabolic states, increased significantly after the practice (Fig. 1). IL-6 is known to increase with exercise in adults, and more recently, this has been shown in children as well (20).

TABLE 3. The effect of exercise on cytokines and growth factors.

	Preexercise	Postexercise
Lactate*	8.4 ± 0.7 mg·dL ⁻¹ (174 ± 16 mg·g ⁻¹ albumin)	41.5 ± 6.4 mg·dL ⁻¹ (814 ± 124 mg·g ⁻¹ albumin)
IL-6*	1.95 ± 0.45 pg·mL ⁻¹ (41 ± 10 pg·g ⁻¹ albumin)	6.72 ± 1.23 pg·mL ⁻¹ (132 ± 24 pg·g ⁻¹ albumin)
IL-1ra*	290 ± 58 pg·mL ⁻¹ (6084 ± 1237 pg·g ⁻¹ albumin)	464 ± 60 pg·mL ⁻¹ (9176 ± 1189 pg·g ⁻¹ albumin)
TNF-α†	2.58 ± 0.28 pg·mL ⁻¹ (53 ± 18 pg·g ⁻¹ albumin)	2.4 ± 0.25 pg·mL ⁻¹ (47 ± 15 pg·g ⁻¹ albumin)
Growth hormone	3.06 ± 0.44 ng·mL ⁻¹ (63 ± 29 ng·g ⁻¹ albumin)	3.07 ± 0.81 ng·mL ⁻¹ (62 ± 53 ng·g ⁻¹ albumin)
Total IGF-I	522 ± 24 ng·mL ⁻¹ (10826 ± 1531 ng·g ⁻¹ albumin)	510 ± 27 ng·mL ⁻¹ (10070 ± 559 ng·g ⁻¹ albumin)
Bound IGF-I	520 ± 24 ng·mL ⁻¹ (10766 ± 482 ng·g ⁻¹ albumin)	506 ± 27 ng·mL ⁻¹ (10011 ± 557 ng·g ⁻¹ albumin)
Free IGF-I	2.9 ± 0.7 ng·mL ⁻¹ (60.2 ± 14.7 ng·g ⁻¹ albumin)	3.03 ± 0.5 ng·mL ⁻¹ (59.6 ± 9.8 ng·g ⁻¹ albumin)
IL-1β	0.273 ± 0.061 pg·mL ⁻¹ (5.6 ± 1.2 pg·g ⁻¹ albumin)	0.259 ± 0.063 pg·mL ⁻¹ (5.1 ± 1.2 pg·g ⁻¹ albumin)
IGFBP-1*	3.5 ± 0.7 ng·mL ⁻¹ (72 ± 12 ng·g ⁻¹ albumin)	43.0 ± 7.8 ng·mL ⁻¹ (851 ± 154 ng·g ⁻¹ albumin)
IGFBP-3	3704 ± 168 ng·mL ⁻¹ (76860 ± 3821 ng·g ⁻¹ albumin)	3662 ± 122 ng·mL ⁻¹ (72275 ± 2632 ng·g ⁻¹ albumin)
Glucose	98 ± 3 mg·dL ⁻¹ (2058 ± 102 mg·g ⁻¹ albumin)	98 ± 3 mg·dL ⁻¹ (1947 ± 63 mg·g ⁻¹ albumin)
Insulin†	30.3 ± 6.8 μIU·mL ⁻¹ (621 ± 121 μIU·g ⁻¹ albumin)	11.85 ± 3.8 μIU·mL ⁻¹ (229 ± 72 μIU·g ⁻¹ albumin)

Data presented as mean ± SEM.

Number in parentheses are concentrations divided by albumin.

* Values corrected for albumin increase with exercise, *P* < 0.05.

† Values corrected for albumin decrease with exercise, *P* < 0.05.

Elevations in IL-6 in the same range as we observed in the adolescents after exercise have been noted in a wide variety of disease states including congestive heart failure, chronic idiopathic neutropenia, cardiomyopathy, and acute influenza (14). However, the present study, along with our recent studies in prepubertal children and adolescent boys, demonstrates a robust inflammatory response to exercise in healthy children (20), and this challenges the notion that during childhood proinflammatory cytokines are involved solely in pathologic conditions.

Although IL-6 traditionally has been considered a catabolic mediator, newer studies suggest that it may also have positive effects on growth in certain situations including exercise. Other members of the IL-6 family of cytokines (e.g., leukemic inhibitory factor) have been shown to regulate muscle growth (27), and IL-6 is known to stimulate angiogenesis mediated by vascular growth factors (fibroblast growth factor-2 (3) and vascular endothelial growth factor (8)). Angiogenesis and muscle hypertrophy are among the most important short-term adaptations to repeated exercise. Thus, the proinflammatory cytokine response to exercise may ultimately prove to play a mechanistic role in beneficial adaptive responses to exercise in healthy children. IL-1ra, which antagonizes the inflammatory effects of IL-6, primarily produced by mononuclear cells, typically increases in response to elevations in IL-6 itself and was, not surprisingly, elevated after exercise in the present study. This finding is in agreement with the IL-1ra response after exercise seen in adults (21) demonstrating that although IL-1ra does indeed peak after IL-6, significant

elevations in IL-1ra are typically observed in the same time that elevations in IL-6 occur after exercise.

We, in children (33), and several researchers in adults have reported significant, albeit modest, increases in the TNF-α response to strenuous exercise (22), whereas others have reported no change (38). In the present study, unexpectedly, we found a small but significant decrease in TNF-α of about 20% (Fig. 1, even after we corrected for the change in serum albumin). Possible explanations for this variability include the type of exercise, as well as its intensity and duration. Most relevant is the recent study of Rhind et al. (29), who demonstrated that prolonged cold exposure (despite the maintenance of core body temperature) down-regulates intracellular expression of TNF-α and leads to a decrease in TNF-α serum levels after exercise, similar to what we observed.

IGFBP-1 is found predominately in tissues, not in circulating blood, and acts primarily to inhibit anabolic effects of IGF-I. Circulating IGFBP-1 is elevated in pathologic, catabolic states like sepsis and burns, suggesting, most likely, a rapid secretion of IGFBP-1 into the central circulation from the liver (26). The robust IGFBP-1 response to exercise in adults was noted as early as 1989 (37), an observation that was corroborated recently in prepubertal children and adolescent boys as well (33). Thus, the IGFBP-1 response to acute exercise is substantial (an increase of about 14-fold in the present study (Fig. 1)) and reproducible in children and adolescents.

IGFBP-1 is known to be highly regulated by insulin, and increased insulin levels are usually associated with reduced circulating IGFBP-1 (26). Consistent with this inverse relationship was our own observation that the reduction in

TABLE 4. The effect of exercise on WBC population.

(Cells·μL ⁻¹)	Pre	Post
WBC*	7636 ± 601	13968 ± 1107
Granulocytes*	4687 ± 471	9554 ± 971
Lymphocytes*	2401 ± 227	3471 ± 290
Monocytes*	548 ± 71	932 ± 115
Platelets*	235 ± 8	298 ± 18
CD3 ⁻ CD16 ⁺ 56 ⁺ NK cells*	314 ± 37	796 ± 113
CD19 ⁺ B cells	328 ± 45	339 ± 43
CD3 ⁺ T cells*	1647 ± 81	2111 ± 189
CD8 ⁺ T-cytotoxic cells*	592 ± 66	826 ± 102
CD4 ⁺ T-helper cells*	921 ± 118	1050 ± 94

Mean ± SEM.

* Increase with exercise, *P* < 0.05.

TABLE 5. The effect of exercise on lymphocyte adhesion molecule expression.

(Cells·μL ⁻¹)	Pre	Post
CD8 ⁺ CD62L ⁺	383 ± 42	456 ± 42
CD8 ⁺ CD62L ⁻ *	209 ± 28	370 ± 65
CD8 ⁺ 62L density lymphocytes†	10427 ± 1021	7745 ± 712
CD4 ⁺ CD62L ⁺⁺ *	802 ± 105	913 ± 87
CD4 ⁺ CD62L ⁻	119 ± 18	136 ± 18
CD54 ⁺ lymphocytes*	1392 ± 142	2132 ± 219
CD54 ⁻ lymphocytes	1008 ± 128	1338 ± 137

* Increase with exercise, *P* < 0.05.

† Decrease with exercise, *P* < 0.05 (molecules per cell).

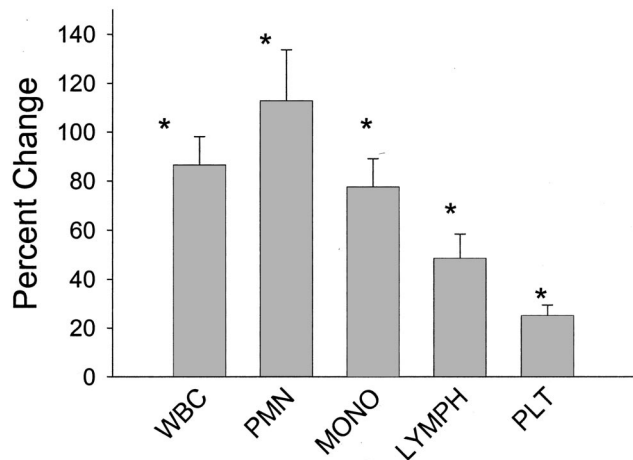


FIGURE 3—Effect of water polo practice on white blood cells (WBC), polymorphonuclear leukocytes (PMN), total lymphocytes (LYMPH), monocytes (Mono), natural killer cells (NK), and platelets (PLT). Data are presented as percent change. There were substantial and significant increases in all cell types shown ($P < 0.05$).

insulin accompanied the rise in IGFBP-1. But a number of researchers have reached the conclusion that IGFBP-1 is elevated with exercise even when insulin concentrations are constant (13). Finally, there is evidence that IGFBP-1 may actually be stimulated by proinflammatory cytokines (32). Thus, it is likely that the increase in IGFBP-1 with exercise is caused by a variety of mechanisms related to: 1) the release of proinflammatory cytokines, and 2) glucoregulatory factors that lead to the reduction in insulin; both may be related to the exercise associated IL-6 increase.

GH typically increases substantially with aerobic exercise, in intense swimming exercise (16), and is correlated with lactate levels (24). However, in the present study, the postexercise GH was not elevated, despite the increase in lactate. It is most likely that we may have “missed” the typical GH response to exercise because in an interval of 1.5 h (the time elapsed between our pre- and postblood

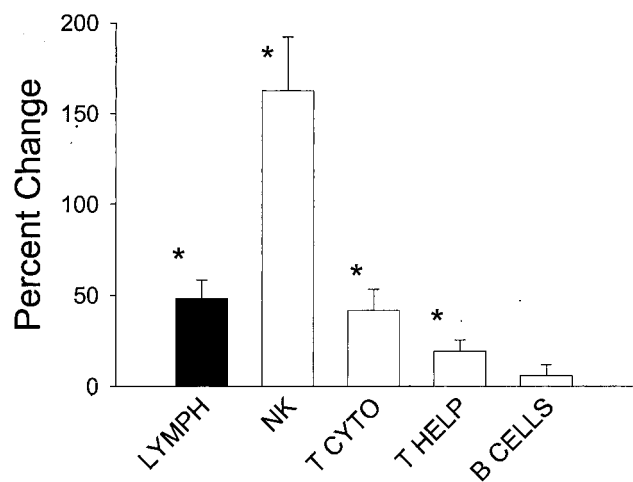


FIGURE 4—Effect of water polo practice on white total lymphocytes (Lymph), NK cells, T-cytotoxic cells (T cyto), T-helper cells (T help), and B-lymphocytes (B cells). Data are presented as percent change. Significant increases in total lymphocytes NK cells and all T cell subtypes were noted ($P > 0.05$).

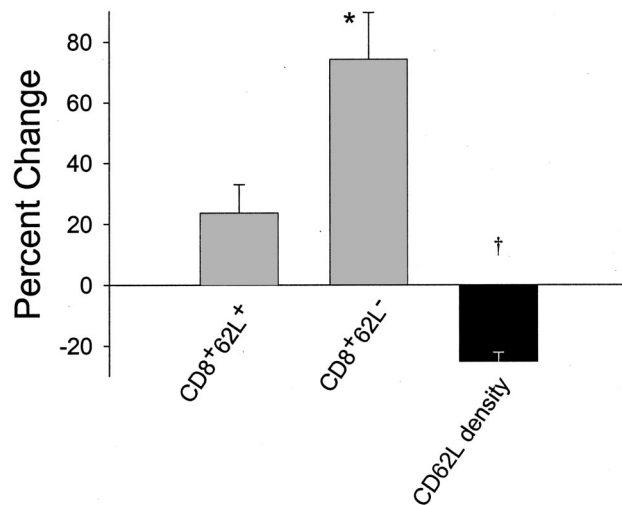


FIGURE 5—Effect of water polo exercise on CD62L (L-selectin). CD8⁺62L⁻ and CD8⁺62L⁺ T cell subsets both increased after exercise; however, only the increase of CD62L⁻ was statistically significant ($P < 0.005$ and $P < 0.056$, respectively). The number of CD62L molecules on circulating lymphocytes decreased significantly ($P < 0.0005$).

sampling), GH may have reached its peak and returned to baseline (25). However, it is also possible that other factors like heterogeneity in menstrual cycle (not controlled in the present study), or the pattern of exercise in the water polo practice, i.e., repeated bursts of high-intensity activity, may also have played a role in the attenuation of the GH response as we noted that a prior GH pulse may inhibit subsequent exercise associated GH release (4).

IGF-I levels appear to acutely increase with exercise (4), but with exercise of sufficient intensity and duration, IGF-I levels may fall (20). The bulk of circulating IGF-I is bound in a ternary complex (IGF-I, IGFBP-3, and an acid-labile subunit that is too large to cross the capillary membranes). Thus, an increasing number of investigators have chosen to examine the effect of physiological perturbations like exercise on the bound and free IGF-I pools. We recently demonstrated (20) in adolescent boys before and after a wrestling practice (also, very intense exercise) that there was a significant decrease in total IGF-I, no change in bound IGF-I, and a significant increase in free IGF-I. Although the pattern of IGF-I changes in the girls was qualitatively similar to the boys, it did not achieve statistical significance.

Insulin decreased substantially with exercise in the face of unchanging glucose concentration (Table 3, Fig. 2) consistent with previous studies in adults (9), adolescents (20), and children (30). Moreover, the decrease in insulin was correlated with the metabolic stress imposed (represented by a 375% increase in lactate). The acute reduction of insulin with heavy exercise is known to reflect increases in anti-insulin, counterregulatory hormones including catecholamines, glucagon, and GH (10), and as recently suggested IL-6 (35), all of which optimally regulate substrate flux during exercise when skeletal muscle substrate utilization increases dramatically, acting to decrease the levels of insulin to prevent hypoglycemia. Far less is known about the

long-term consequences of exercise-associated fluctuations in insulin metabolism on the process of growth and development in children. Insulin regulates metabolism and growth in a variety of tissues including muscle—prolonged hypoinsulinemia is associated with impaired growth whereas hyperinsulinemia leads to excessive growth (18)

Strenuous exercise is known to elicit a pronounced WBC response in adults, and more recent studies have corroborated that this occurs, at least qualitatively, in children (23). Consistent with this, we found marked changes in PBMC and adhesion molecules (Table 4 and 5 Figs. 3–5) with the water polo exercise. The exercise led to significant elevations in the number of circulating WBC, including granulocytes, monocytes, mixed lymphocytes, T cells, natural killer cells, and platelets (known to be activated after exercise). The predominant changes causing this leukocytosis were increases in granulocytes and lymphocytes (Fig. 3).

Lymphocyte subsets were also affected by exercise. The mechanism of the acute lymphocytosis associated with exercise is not yet fully understood but is clearly related to the generalized “stress” response in which key elements of the hypothalamic-pituitary-adrenal and sympathetic nervous system are globally activated by physical exercise. Consequently, it is not surprising that membrane density of β_2 receptor may determine the degree of exercise-induced lymphocyte mobilization into the circulating blood. NK cells have the greatest density of β_2 receptors (17), and, indeed, we and others have found the largest increase in NK cells after exercise (23), whereas B cells, which have the lowest density of β_2 receptors, did not increase at all with exercise (Fig. 4).

CD62L (L-selectin) mediates leukocyte rolling and adhesion to endothelium at the site of inflammation (34). We found an increase in both CD62L⁻ and CD62L⁺ with a preferential significant increase in CD62L⁻ T cell subsets (Fig. 5). The relative increase in circulating CD62L⁻ lymphocytes is believed to result from a preferential release into the circulation of CD62L⁻ cells from the marginal pool rather than an actual down-regulation of L-selectin in response to exercise (19). The decreased density of CD62L on circulating lymphocytes is consistent with prior exercise studies (11).

Because L-selectin is typically shed from T-lymphocytes as the cell transitions from a naïve to a postantigen presented

memory T cell (2), these findings suggest that the water polo exercise led to recruitment of memory T cells into the peripheral circulation. These memory T cells (marked also as CD45RO) are known to be associated with increased production of inflammatory cytokines such as IL-6 (12), which was, in fact, elevated in this study. Lymphocytes expressing CD54 [known as intercellular adhesion molecule-1 (ICAM-1)] also increased in response to exercise, as has been noted by other workers (28). CD54 is constitutively expressed on the surface of some lymphocytes and is upregulated in response to a variety of inflammatory mediators, including proinflammatory cytokines (like IL-6), certain hormones, cellular stresses, and virus infection (31). Interestingly, CD54 is now known to be elevated in children with asthma exacerbations (5). Whether the exercise associated increase in CD54 that we observed in healthy children under field conditions plays a role in the common occurrence of childhood exercise-induced asthma or in the water sports protective effect remains an intriguing, as yet unexamined, possibility.

In summary, we demonstrated that a typical water polo practice in high school girls led to substantial changes in growth factors, inflammatory cytokines, and cellular components of the immune system. The reductions in insulin, with the large increase in circulating IL-6 and IGFBP-1, are consistent with the hypothesis that acute exercise in female adolescents leads to a predominately catabolic response. In healthy adolescent girls, vigorous physical activity induces a proinflammatory response, mediated in part by leukocytes and manifested by elevated concentrations of circulating proinflammatory cytokines. The effect of these substantial exercise-associated alterations in growth mediators, cytokines, and immune mediators on the overall process of growth and development in adolescents has yet to be determined.

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