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Effect of Intense Exercise on Inflammatory Cytokines and Growth Mediators in Adolescent Boys

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ABSTRACT. *Objective.* **Exercise can enhance growth and development in children, but recent investigations have revealed an intriguing paradox. Namely, the early (4–5 weeks) response to training programs in children lead to a catabolic, growth hormone (GH)–resistant state rather than the expected anabolic activation of the GH→insulin-like growth factor-I (IGF-I) axis. This paradox led us to hypothesize that single bouts of exercise in children could stimulate proinflammatory cytokines known to inhibit directly anabolic activity of the GH→IGF-1 axis (interleukin [IL]-6, IL-1β, and tumor necrosis factor-α [TNF-α]).**

Methods. **Eleven healthy high school–age boys, age 14 to 18.5 years, performed a single, typical, 1.5-hour wrestling practice session. Blood was sampled before and after the session.**

Results. **We found significant decreases in anabolic mediators: total IGF-I (−11.2 ± 2.3%), bound IGF-I (−11.2 ± 2.4%), and insulin (−42 ± 10%. However, there was no change in unbound IGF-I. Remarkable increases were found in proinflammatory cytokines IL-6 (795 ± 156%), TNF-α (30 ± 12%), and IL-1β (286 ± 129%) and in IGF-binding protein-1 (835 ± 234%), which itself is stimulated by inflammatory cytokines and is known to inhibit IGF-I. Evidence for compensatory mechanisms to counter the antianabolic inflammatory response to acute exercise were also noted: IL-1ra increased (80 ± 20%) and IGF-binding protein-3 proteolysis (which can maintain unbound, biologically active IGF-I despite losses in total IGF-I) increased significantly (101 ± 39%) as well.**

Conclusions. **These data demonstrate that an intense exercise bout in male adolescents leads to reductions in anabolic mediators and profound increases in inflammatory cytokines. This might explain the development of what seems to be a paradoxical catabolic state in the initial phases of exercise training programs.** *Pediatrics* **2002;110:681–689;** ***adolescence, wrestling, sports, fitness, BMI, IGFBP-3 proteolysis.***

ABBREVIATIONS. GH, growth hormone; IGF, insulin-like growth factor; IL, interleukin; TNF, tumor necrosis factor; IGFBP, IGF-binding protein; ra, receptor antagonist; BMI, body mass index; $\dot{V}o_2$, volume of oxygen uptake; HR, heart rate; bpm, beats per minute; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; TBST, Tris-buffered saline with 0.1% Tween-20.

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Levels of physical activity during childhood can influence growth and development of muscle, fat, and bone. Recent data suggest that exercise alteration of the growth hormone (GH)→insulin-like growth factor-I (IGF-I) axis (GH→IGF-I), a system of hormones and mediators that modulate growth in many tissues, may be involved. Basal levels of IGF-I are correlated with muscle mass and fitness in prepubertal children, adolescents, and adults.[1–3]

Paradoxically, there is increasing evidence in both children and adults that even relatively brief periods of aerobic exercise training (5 weeks) can lead to reductions, rather than expected increases, in basal, resting levels of IGF-I.[2,4,5] Thus, training in children initially leads to a state of GH resistance (ie, reduced GH binding protein and IGF-I), more often associated with catabolic rather than anabolic hormonal activity.[6]

This paradox led us to the idea that single bouts of exercise in children could, as in adults,[7,8] stimulate proinflammatory cytokines known to inhibit directly anabolic activity of the GH→IGF-1 axis (namely, interleukin [IL]-6, IL-1β, and tumor necrosis factor-α [TNF-α], each of which has been shown previously in adults to be increased with exercise). The cumulative effect of these individual exercise perturbations would be to lower basal levels of IGF-I. As an extreme example of this paradigm, in children with systemic inflammatory diseases,[9–11] chronically elevated IL-6 leads to reduced basal IGF-I and impaired somatic growth.

With the possible exception of GH, little is known about the effects of single bouts of exercise on growth mediators, proinflammatory cytokines, and potential compensatory mechanisms in childhood or adolescence, a period of rapid fluctuation in circulating levels of anabolic hormones. This is important for a number of reasons that, ironically, seem to involve either too much or too little physical activity during childhood. An increasing number of common adult-onset diseases and conditions (eg, obesity, osteoporosis, cardiovascular disease) are now known to be associated with physical inactivity during childhood and adolescence,[12,13] but hormonal markers of adequate levels of physical activity in this age group have yet to be determined. In contrast, the American Academy of Pediatrics recently highlighted the potential risks of high-intensity training and sports specialization in young athletes[14] and noted, "Although many concerns surround intense sports competition

in children, little scientific information is available to support or refute these risks."

We hypothesized that acute alterations in the circulating levels of the GH→IGF-I axis and in the proinflammatory cytokines would be substantially catabolic in response to single bouts of intense exercise in adolescents. We further hypothesized that factors such as increasing fitness would attenuate the catabolic response, suggesting an ability of growing children to adapt hormonally to repeated exercise bouts (ie, training). To test these hypotheses, we studied the effect of a single, typical, high school wrestling practice session. Wrestling was chosen specifically because individual practice sessions are intense and because high school wrestlers are known to develop GH resistance during the course of a standard 3- to 4-month season.[15]

The complexity of anabolic and catabolic responses to exercise has become increasingly apparent in recent years. Clearly, measurement of individual mediators can no longer suffice to gain insight into mechanisms of growth regulation. Thus, in the present investigation, we focused on both well-studied and novel mediators that are influenced by exercise and act in concert to regulate growth. Likely indicators of an "anabolic" response included free and bound IGF, IGF-binding protein-3 (IGFBP-3), and insulin. Indicators that represent a "catabolic" response included IGFBP-1, IL-6, IL-1β, and TNF-α. Finally, we also tested for evidence of compensatory mechanisms that might mitigate the catabolic response, such as IGFBP-3 proteolysis and IL-1 receptor antagonist (IL-1ra).

METHODS

Sample Population

The study was approved by the Institutional Review Board, University of California, Irvine, and informed consent as well as assent were obtained. Eleven healthy adolescent boys participated in the study (Table 1). No participants were on any medications at the time of the study.

Height, Weight, and Body Mass Index Measurements

Standard, calibrated scales and stadiometers were used to determine height, weight, and body mass index (BMI; weight/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 and Prevention, National Center for Health Statistics.[16] Weight was measured before and at the end of the practice.

Measurement of Cardiorespiratory Fitness

On a separate day, within 1 week of the wrestling practice, each participant performed standard measurements of cardiorespiratory fitness. Each participant performed a ramp-type progressive exercise test on a cycle ergometer in which the participant exercised to the limit of his tolerance. Participants were vigorously encouraged during the high-intensity phases of the exercise protocol. Gas exchange was measured breath by breath,[17] and the volume of oxygen uptake ($\dot{V}o_2$) peak was determined as previously described for children and adolescents.[18] Mean heart rate (HR) peak was 184 ± 4 bpm, and mean respiratory exchange ratio peak was 1.14 ± 0.01, suggesting that close-to-maximal values likely were achieved.

TABLE 1. Participant Characteristics (n = 11)

Age (y)	16.5 ± 0.5 (range: 14–18.5)
Weight (kg)	75.4 ± 2.9 (range: 59.5–92.3)
Height (cm)	171.8 ± 1.8 (range: 161–178)
BMI (kg/m^2)	25.5 ± 0.8 (range: 22.2–30.8)
BMI percentile	83.6 ± 4 (range: 49–98)
Peak $\dot{V}o_2$ (ml/min/kg)	44.5 ± 2 (range: 37–54)

Data presented as mean ± standard error of the mean.

Field Study

The wrestling practice was held approximately 6 weeks after the end of the wrestling season. The field study was designed to mimic a real-life exercise paradigm, such as encountered in the daily activities of these adolescents. To accomplish this, we arranged a 1.5-hour wrestling practice modeled after typical sessions of this sport. The practice was coached by 1 of the wrestling team coaches. None of the participants trained during the day preceding the blood sampling. The wrestlers were instructed to have a light breakfast on the morning of the test, and the exercise session began at approximately noon. Participants were admitted to the General Clinical Research Center at the University of California, Irvine. The study took place at the wrestling facility of the University of California, Irvine, faculty/student recreation center.

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

Warm-up (20 minutes): Jogging and "stretch" exercise with sports-specific calisthenics such as push-ups and sit-ups.

Technique drills (20 minutes): The participants 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 participants were paired or placed in groups of 3. Specific wrestling positions were assigned, and participants wrestled from the given situation practicing a specific move and its counters. This involved exercise at maximal effort in bursts of 15 to 20 seconds.

"Iron man" (15 minutes): Wrestlers were placed in groups of 5 or 6 with 1 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 approximately 3 to 4 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 6-minute match.

Immediately before and after exercise, blood samples were obtained by standard phlebotomy. The time interval between the end of the training session and phlebotomy was 41 ± 4 seconds (27–70 seconds). Samples were placed in ice bath and were immediately taken to centrifugation. Aliquots of the resulting plasma were stored at −80°C until analyzed. Urine samples were collected immediately before and after exercise into sterile specimen cups and were placed on ice until the aliquot was frozen at −80°C. 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 3 time points (20, 50, and 80 minutes) during the practice. As is typically the case in high school wrestling practices, participants 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 mg/dL.

Albumin

Albumin levels were determined by colorimetric determination by the use of the Sigma BCP albumin procedure #256 (Sigma Diagnostic, St Louis, MO).

GH

GH serum concentrations were determined by enzyme-linked immunosorbent assay (ELISA) with the use of the DSL-10-1900 Active kit (Diagnostic System Laboratories, Webster, TX). Intra-

assay CV was 3.3% to 4.3%, interassay CV was 6.3% to 6.5%, and the sensitivity was 0.03 ng/mL.

IGF-I

IGF-I was extracted from IGFBPs using the acid-ethanol extraction method.[19] Serum IGF-I concentrations were determined by a 2-site immunoradiometric assay using the DSL-5600 Active kit (Diagnostic System Laboratories). IGF-I interassay CV was 3.7% to 8.2%, and intra-assay CV was 1.5% to 3.4%. Assay sensitivity was 0.8 ng/mL. 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% to 4.8%, interassay CV was 6.2% to 11.1%, and the sensitivity was 0.015 ng/mL.

IGFBPs

IGFBP-1 was measured by coated-tube immunoradiometric assays with the use of the DSL-10-7800 Active kit. For IGFBP-1, interassay CV was 1.7% to 6.7% and intra-assay CV was 2% to 4%. Assay sensitivity is 0.33 ng/mL (results for IGFBP-1 were not obtainable for 1 participant). 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% to 9.6%, interassay CV was 8.2% to 11.4%, and the sensitivity was 0.04 ng/mL.

IGFBP-3 Proteolysis

IGFBP-3 proteolysis was determined by Western immunoblot analysis. Serum (1 μL) or urine (60 μL) from the participants was fractionated by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis under nonreducing conditions. Fractionated proteins were electrotransferred onto Hybond-ECL nitrocellulose (Amersham Pharmacia, Arlington, VA). The membranes were blocked in 5% nonfat dry milk in Tris-buffered saline with 0.1% Tween-20 (TBST), and incubated with primary antibody diluted in TBST (1:3000) for 2 hours at room temperature or overnight at 4°C. Membranes were washed in TBST, then incubated with horseradish peroxidase–conjugated secondary antibody (Southern Biotechnology Associates, Birmingham, AL), diluted 1:7000, for 1 hour at room temperature. Immunoreactive proteins were detected using the Renaissance Western Blot Chemiluminescence reagents (NEN, Boston, MA). Densitometric measurement of immunoblots was performed using a Bio-Rad GS-670 Imaging densitometer (Bio-Rad, Melville, NY). All experiments were conducted at least 3 times. Monoclonal antibody against IGFBP-3 was provided by Diagnostic Systems Laboratories. In the serum, we expressed IGFBP-3 proteolysis first as a percentage of intact IGFBP-3 and second as the relative concentration of the IGFBP-3 fragments normalized to serum albumin. Urine IGFBP-3 fragment concentrations were normalized to creatinine.

TNF-α

TNF-α serum levels were determined by ELISA with the use of the R&D Systems Quantikine High Sensitivity kit (R&D Systems; Minneapolis, MN). Intra-assay CV was 8.7% to 14.8%, interassay CV was 16.1% to 22.6%, and the sensitivity was 0.18 pg/mL.

IL-6

IL-6 serum levels were determined by ELISA with the use of the R&D Systems Quantikine High Sensitivity kit. Intra-assay CV was 3.8% to 11.1%, interassay CV was 7.1% to 29.5%, and the sensitivity was 0.0094 pg/mL. IL-6 concentrations were also measured in the urine using these techniques. Data were normalized to urine creatinine measured by standard techniques as previously described.[20,21]

IL-1β

IL-1β serum levels were determined by ELISA with the use of the R&D Systems Quantikine High Sensitivity kit. Intra-assay CV was 1.6% to 4.0%, interassay CV was 5.3% to 9.0%, and the sensitivity was 0.059 pg/mL.

IL-1ra

IL-1ra serum levels were determined by ELISA with the use of the R&D Systems Quantikine High Sensitivity kit. Intra-assay CV was 3.1% to 6.2%, interassay CV was 4.4% to 6.7%, and the sensitivity was 22 pg/mL.

Glucose

Serum glucose levels were determined by quantitative enzymatic measurements with the use of Sigma diagnostic kit #510 (Sigma Diagnostics).

Insulin

Insulin serum levels were determined by ELISA with the use of the DSL-10-1600 Active kit. Intra-assay CV was 1.3% to 2.6%, interassay CV was 5.2% to 6.2%, and the sensitivity was 0.26 μIU/mL.

Statistical Analysis

Paired *t* tests were used to determine before versus after exercise differences; α was set at 0.05. Correlation and linear regression analyses were computed among growth factors, cytokines, BMI, and indexes of fitness. Data are presented as mean ± standard error of the mean. Statistical analysis of growth factors and cytokines was performed using before and after concentration values as shown in Tables 1 and 2. In Figs 1, 2, 5, and 6, we present the mean and standard error of the mean of the percentage changes found in each participant. The latter value is not identical with the percentage change that would be calculated using the mean before and after values of the whole group.

RESULTS

Height, Weight, BMI, and Fitness Level

Participant characteristics are presented in Table 1. Weight decreased significantly after the practice (75.4 ± 2.9 kg to 74.7 ± 2.9 kg; $P < .018$).

Cardiorespiratory Effects of the Wrestling Practice

All 11 participants completed the 1.5-hour practice. Mean baseline HR was 74 ± 2 bpm. Mean HR at 3 measurement points during the practice (20, 50, and 80 minutes) was 163 ± 3 bpm, 160 ± 4 bpm, and 163 ± 3 bpm, respectively. Lactate increased by 441 ± 67% ($P < .005$).

Albumin

There was a significant change in albumin levels after the exercise (from 4.47 ± 0.1 g/dL to 4.96 ± 0.1 g/dL; $P < .005$); consequently, concentrations of catabolic and anabolic mediators in the circulation are expressed as their ratio to albumin levels. Although it is clear that shifts in plasma water occur immediately with heavy exercise, a standardized approach toward adjusting solute concentrations has not been universally accepted.[22] Using hematocrit alone (as suggested by Dill and Costill in 1974[23]) is not sufficient because it is documented that exercise-associated splenic transfusion of highly concentrated blood into the central circulation may have a measurable effect on hematocrit.[24] A number of investigators have used albumin specifically to adjust for vascular water changes in IGF-I or BP-3 with exercise because these molecules approximate the size of albumin.[25] We used this approach in the present study.

Effect of Wrestling Practice on Anabolic Mediators

A mean increase of 821 ± 452% in circulating GH levels was found, but this was not statistically significant. There was a small but significant decrease in the level of total IGF-I and bound IGF-I. The decrease

TABLE 2. Effect of Exercise on Cytokines and Growth Factors

	Before Exercise	After Exercise
Lactate*	13.6 ± 0.8 mg/dL (308 ± 21 mg/g albumin)	81.5 ± 11.6 mg/dL (1660 ± 252 mg/g albumin)
Serum IL-6*	1.35 ± 0.3 pg/mL (31 ± 6 pg/g albumin)	10.9 ± 1.4 pg/ml (219 ± 28 pg/g albumin)
Urine IL-6*‡	0.623 ± 0.138 pg/mg creatinine	1.515 ± 0.335 pg/mg creatinine
IL-1ra*	221 ± 14 pg/mL (4965 ± 310 pg/g albumin)	425 ± 40 pg/mL (8506 ± 726 pg/g albumin)
TNF-α*	3.1 ± 0.25 pg/mL (67 ± 5 pg/g albumin)	4.2 ± 0.4 pg/mL (86 ± 9 pg/g albumin)
GH	3.83 ± 1.65 ng/mL (87 ± 37 ng/g albumin)	5.62 ± 1.62 ng/mL (116 ± 35 ng/g albumin)
Total IGF-I†	480 ± 36 ng/mL (10855 ± 936 ng/g albumin)	474 ± 39 ng/mL (9648 ± 854 ng/g albumin)
Bound IGF-I†	476 ± 36 ng/mL (10769 ± 927 ng/g albumin)	471 ± 39 ng/mL (9579 ± 841 ng/g albumin)
Free IGF-I	3.7 ± 0.7 ng/mL (85.5 ± 18.2 ng/g albumin)	3.4 ± 0.7 ng/mL (69.3 ± 13.6 ng/g albumin)
IL1-β*	0.061 ± 0.024 pg/mL (1.33 ± 0.5 pg/g albumin)	0.136 ± 0.031 pg/mL (2.76 ± 0.6 pg/g albumin)
IGFBP-1*	24 ± 14 ng/mL (516 ± 291 ng/g albumin)	96 ± 19 ng/mL (1919 ± 351 ng/g albumin)
IGFBP-3	3694 ± 290 ng/mL (83581 ± 7472 ng/g albumin)	3821 ± 283 ng/mL (77366 ± 6018 ng/g albumin)
Glucose	81 ± 6 mg/dL (1833 ± 165 mg/g albumin)	87 ± 11 mg/dL (1770 ± 250 mg/g albumin)
Insulin†	15.2 ± 4.2 μIU/mL (349 ± 99 μIU/g albumin)	7.6 ± 2.2 μIU/mL (149 ± 46 μIU /g albumin)

Data presented as mean ± standard error of the mean.
Numbers in parentheses are concentrations divided by albumin.
* Values corrected for albumin: increase with exercise, $P < 0.05$.
† Values corrected for albumin: decrease with exercise, $P < 0.05$.
‡ Corrected to urine creatinine levels.

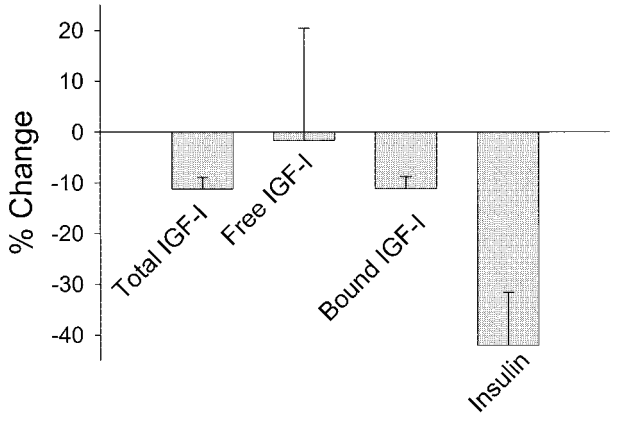

Fig 1. Effect of wrestling practice on the likely anabolic mediators: total IGF-I, free and bound IGF-I, and insulin. A significant decrease was noted for total ($P < .002$) and bound IGF-I ($P < .002$) and for insulin ($P < .037$). No significant change was found for free IGF- I.

in unbound IGF-I was not statistically significant (Fig 1, Table 2).

Serum IGFBP-3 did not significantly change. However, IGFBP-3 proteolysis in the serum increased from 11.3 ± 2.5% of intact IGFBP-3 at baseline to 16.6 ± 2.2% of intact IGFBP-3 at the end of exercise ($P < .044$). When analyzed as concentration of IGFBP-3 fragments in the serum (normalized to serum albumin), there was an overall increase of 101 ± 39% ($P < .03$; Fig 4). Similarly, in the urine there was a significant increase (120 ± 33%; $P < .002$) in IGFBP-3 proteolysis when analyzed as concentration of IGFBP-3 fragments normalized to urine creatinine (Fig 4). The wrestling practice was followed by a 42 ± 10% decrease in insulin ($P < .037$), but no change in glucose levels was noted.

Effect on Catabolic Mediators

Significant increases where noted in circulating IL-6, TNF-α, IL-1β, and IL-1ra (Fig 2, Table 2). Individual cytokine data are presented in Fig 3. Urine IL-6 also increased significantly with exercise. A substantial increase in the level of IGFBP-1 was also observed.

Correlation Among Fitness, BMI, and Increase in Lactate Levels With Mediator Responses to the Wrestling Practice

We found no correlation among fitness (peak $\dot{V}o_2$/kg) and the magnitude of change in any of the catabolic or anabolic mediators except for IGFBP-1 (Fig 5). Fitness was inversely correlated with the magnitude of the IGFBP-1 response ($r = -0.77$, $P < .01$). BMI percentile was positively correlated with the increase in IGFBP-1 ($r = 0.67$, $P < .033$). Finally, exercise-associated increases in lactate were significantly correlated with the increases in IL-6 ($r = 0.74$, $P < .01$; Fig 6), TNF-α ($r = 0.73$, $P < .01$), and IL-1β ($r = 0.66$, $P < .03$).

DISCUSSION

This study presents the first data demonstrating immediate, substantial, and simultaneous catabolic and anabolic responses to exercise in a population of healthy male adolescents. Although representing an intense level of physical activity, the wrestling prac-

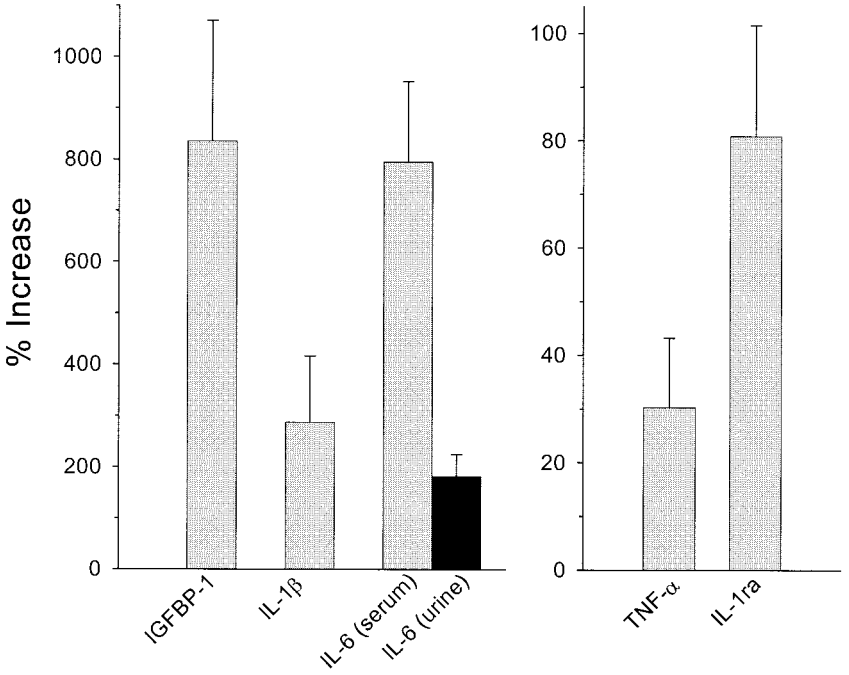

Fig 2. Effect of a wrestling practice on likely catabolic mediators. All values represent serum levels except for the additional measurement of IL-6 in the urine. Substantial elevations in IGFBP-1 ($P < .001$), IL-1β ($P < .034$), and IL-6 (blood, $P < .0005$; and urine, $P < .005$) followed exercise. Significant increases in TNF-α ($P < .05$) and IL-1ra ($P < .002$) were also observed, but at approximately 1 order of magnitude less.

tice 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 uniquely that factors such as BMI and relative fitness can influence the growth mediator response to brief periods of intense exercise.

GH typically increases substantially with exercise,[26] but in the present study, GH after exercise was elevated but not significantly. This is not surprising because in an interval of 1.5 hours (the time elapsed between our before and after blood sampling), the GH increase in response to exercise will typically have reached its peak and begun to return to baseline levels in most participants. Although GH stimulates IGF-I production at the tissue level, it seems that immediate changes in IGF-I are not influenced by the typical exercise-associated increase in GH.[27] Previous studies, although not entirely consistent, tend to indicate that circulating IGF-I has a biphasic response to an acute bout of exercise[27–29]: serum levels increase to a small but significant degree in the first 10 to 20 minutes, but as exercise progresses, IGF-I levels fall.

In the present study, we were unable to obtain blood samples early in exercise for obvious technical reasons, but by the end of the exercise period, we observed a small but significant fall in total IGF-I (Fig 1). Reductions in circulating IGF-I accompany many catabolic states such as sepsis and burns,[30] but the mechanisms for these immediate reductions either during exercise or in other catabolic states have yet to be elucidated.

The bulk of circulating IGF-I is bound in a ternary complex (IGF-I, IGFBP-3, and an acid-labile subunit[31]) that is too large to cross the capillary membranes. Consistent with this, the half-life of bound IGF-I is in the range of 12 to 15 hours, whereas the half-life of free IGF-I is on the order of 10 to 20 minutes.[32] Thus, an increasing number of investigators have chosen to examine the effect of physiologic perturbations such as exercise on the bound and free IGF-I pools. In the present study, no significant fall in free IGF-I occurred despite significant decreases in total and bound IGF-I. These data suggest a mechanism that preserves circulating concentrations of the free IGF-I during exercise.

One possibility is the proteolysis of IGFBP-3, which would lead to increased concentrations of free IGF-I in the circulation. Indeed, we did find increased IGFBP-3 proteolytic fragments in both the serum and the urine after the wrestling practice (Fig 4). IGFBP-3 proteolysis occurs in a number of conditions in which preservation of the circulating pool of IGF-I may be beneficial (eg, pregnancy,[33] diabetes,[34] severe illness,[35] and advanced malignancies[36,37]). A small nonsignificant decrease in normalized IGFBP-3 concentration was noted. However, we did not perform western ligand blot analysis specifically for intact IGFBP-3, and ELISA may not be able to distinguish intact from all proteolyzed fragments of IGFBP-3.

We speculate that the reduction in total and bound IGF-I accompanying heavy exercise in male adolescents may be compensated, in part, by IGFBP-3 proteolysis. Finally, factors such as relative fitness may play a role in the IGFBP-3 proteolysis response to exercise. For example, Dall et al[25] recently found no increased IGFBP-3 proteolysis with an acute, intense bout of exercise in highly fit, elite rowers whose peak

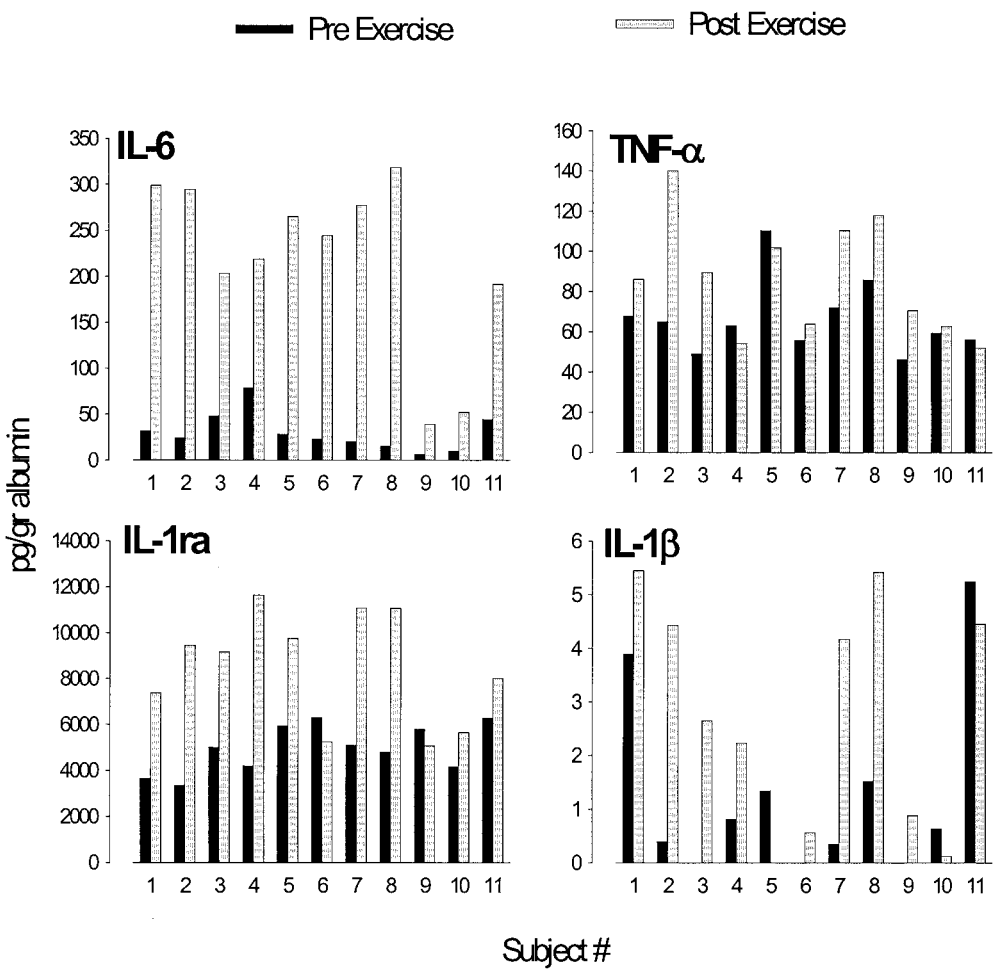

Fig 3. Effect of wrestling practice on serum cytokines (IL-6, TNF-α, IL-1β, and IL-1ra). Before and after exercise data are presented for each participant.

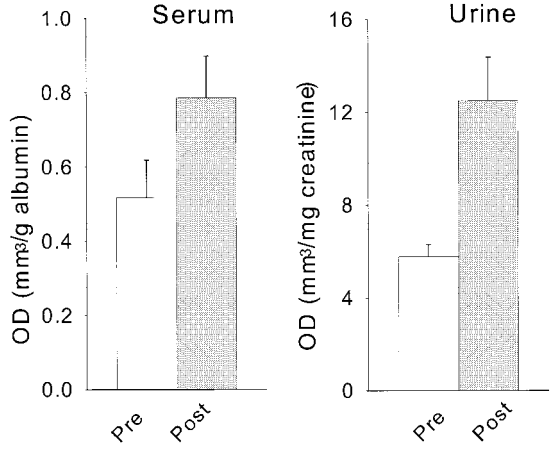

Fig 4. IGFBP-3 proteolysis in serum and urine. A statistically significant increase in IGFBP-3 fragments was noted in both serum ($P < .032$) and urine ($P < .002$).

$\dot{V}O_2$/kg was 70 mL/min/kg, substantially higher than the 44 mL/min/kg found in our group.

Insulin decreased substantially with exercise in the face of unchanging glucose concentration (Fig 1, Table 2), consistent with previous studies in both adults[38] and children.[39] The immediate reduction of insulin with heavy exercise in male adolescents likely reflects increases in anti-insulin, counterregulatory hormones, including catecholamines, glucagon, and GH, all of which optimally regulate substrate flux during exercise when skeletal muscle substrate utilization increases dramatically. 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.[40]

The attenuation of circulating anabolic factors was accompanied by increases in mediators and cytokines usually associated with catabolic states, namely, IL-6, TNF-α, and IL-1β (Figs 2 and 6). As noted, these proinflammatory cytokines are known to increase with exercise in adults, and this has been shown more recently in prepubertal children as well.[3,21] As in our previous study in younger children, the qualitatively similar changes of IL-6 in the blood and urine (Fig 2) support the potential use of urine IL-6 to indicate exercise effects on this particular inflammatory cytokine when blood sampling is not feasible. IL-1ra, which antagonizes the inflammatory effects of IL-6, typically increases in response to elevations in IL-6 itself and, not surprising, was ele-

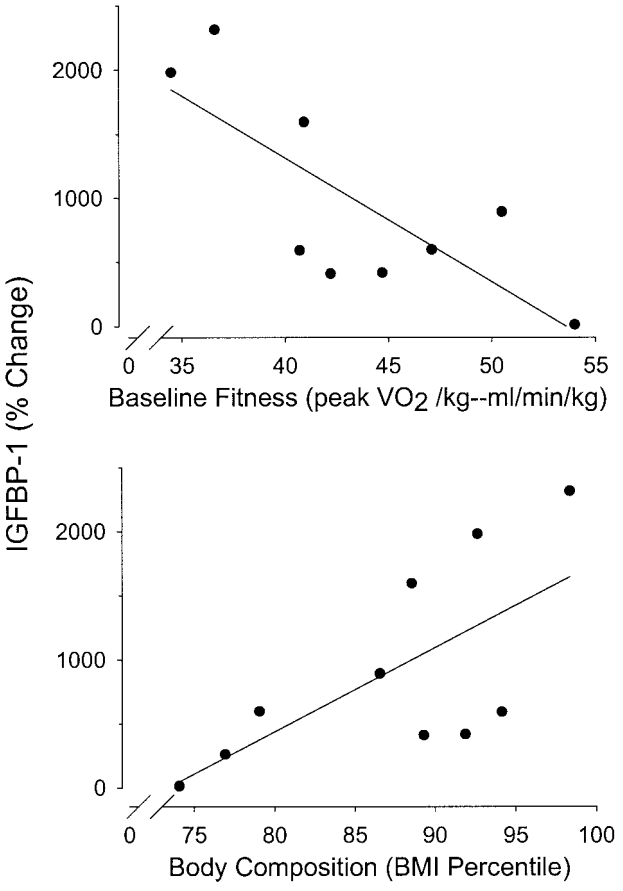

Fig 5. Top, correlation between exercise-associated increase in IGFBP-1 and fitness (expressed as peak $\dot{V}o_2$/kg). Bottom, correlation between exercise-associated increase in IGFBP-1 and BMI (expressed as BMI percentile). Fitness was inversely correlated with the magnitude of the IGFBP-1 response (the regression equation was $y = -97X + 5182$; $r = -0.77$, $P < .01$). BMI was positively correlated with the increase in IGFBP-1 (the regression equation was $y = 65.8X - 4830$; $r = 0.67$, $P < .033$).

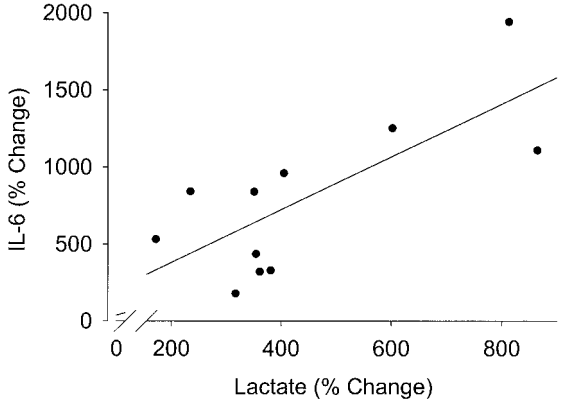

Fig 6. Significant correlation between the increases in lactate and IL-6 in blood associated with the wrestling practice ($r = 0.74$, $P < .009$). Data are expressed as the percentage change from preexercise, baseline measurements (the regression equation was $y = 1.71X + 37.6$).

vated after exercise in the present study. This finding is in agreement with the IL-1ra response after exercise seen in adults,[41,42] 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.

The proinflammatory cytokines IL-1β, IL-6, and TNF-α are known to inhibit the GH→IGF-I system through a variety of mechanisms, including depression of GH receptor gene expression, inhibition of IGF-I production, and stimulation of IGF binding proteins that act to inhibit IGF-I function.[43–48] Thus, the increase in catabolic mediators and decrease in anabolic mediators after wrestling may be related mechanistically. Finally, although there is still controversy surrounding the source of the proinflammatory response to exercise,[49,50] the strong correlation that we observed between the increase in IL-6, IL-1β, and TNF-α and the increase in lactate (Fig 6) supports the notion that whatever its source, the exercise inflammatory response is closely tied to the degree of metabolic stress imposed.

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.[51–54] However, the present study, along with our recent study in prepubertal children, demonstrates a robust inflammatory response to exercise in healthy children,[21] and this challenges the notion that during childhood proinflammatory cytokines are involved solely in pathologic conditions. Other members of the IL-6 family of cytokines (eg, leukemic inhibitory factor) have been shown to regulate muscle growth,[55] and IL-6 is known to stimulate angiogenesis mediated by vascular growth factors (fibroblast growth factor-2[56] and vascular endothelial growth factor[57]). Angiogenesis and muscle hypertrophy are among the most important short-term adaptations to repeated exercise. Thus, the proinflammatory cytokine response to exercise ultimately may prove to play a mechanistic role in beneficial adaptive responses to exercise in healthy children.

IGFBP-1 is found predominately in tissues, not in circulating blood, and acts primarily to inhibit anabolic effects of IGF-I.[31] Circulating IGFBP-1 is elevated in pathologic, catabolic states such as sepsis and burns, suggesting, most likely, a rapid secretion of IGFBP-1 into the central circulation from the liver.[58,59] The robust IGFBP-1 response to exercise in adults was noted as early as 1989,[60] an observation that was corroborated recently in prepubertal children as well.[3,21] Thus, the IGFBP-1 response to acute exercise seems to be substantial (an increase of approximately 8-fold in the present study [Fig 2]) 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.[31] Consistent with this inverse relationship was our own observation that the reduction in insulin accompanied the rise in IGFBP-1, but a number of researchers using both human[61] and animal[62] models have reached the conclusion that IGFBP-1 is elevated with exercise even when insulin concentrations are constant. Finally, there is evidence that IGFBP-1 may actually be stimulated by IL-1β, IL-6, and TNF-α.[47] 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.

We examined the relationship among growth factors, proinflammatory cytokines, BMI, and fitness to determine whether the response to an acute bout of exercise might be modified by these variables. We found strong correlations (Fig 5) between the IGFBP-1 response to exercise and fitness (assessed as peak $\dot{V}o_2$/kg body wt) and BMI (expressed as BMI percentile for age). The inverse correlation of IGFBP-1 change with relative fitness indicated that the better trained participants had an attenuated IGFBP-1 response. To the extent that the IGFBP-1 increase is an indicator of the metabolic and proinflammatory "stress" of the exercise, these data suggest the hypothesis that with repeated exercise (ie, training), participants adapt and circulating indicators of stress, in this case IGFBP-1, are attenuated.

Consistent with this notion was the observation that the IGFBP-1 response was positively correlated with BMI percentile. This suggests that leaner participants had a smaller IGFBP-1 response, and it is likely that the leaner participants were relatively fitter. However, in our participants, the interpretation of BMI can become problematic. The BMI calculates a relative relationship between body height and weight; one can only infer that an increased BMI means a greater fat contribution to body weight. Although this is often an accurate explanation for an increase in BMI, it may not be in the case of individuals who are involved in training, in whom the increase in body weight may actually be attributable to increase in muscle mass rather than fat. An independent assessment of body composition (eg, by dual radiograph absorptiometry) was not done in the present study. Whether the increase in IGFBP-1 response to exercise in adolescents with relatively higher BMI is a function of body composition or fitness has yet to be determined.

In summary, we demonstrated that a typical wrestling practice in high school boys led to substantial changes in growth factors and inflammatory cytokines. The reductions in insulin and in total and bound IGF-I along with the increases in IL-1β, IL-6, and TNF-α are consistent with the hypothesis that an acute bout of exercise in male adolescents leads to a predominately catabolic response. Compensatory mechanisms are also stimulated by exercise: 1) an increase in IGFBP-3 proteolysis and maintenance of the concentration of unbound IGF-I in the circulation and 2) an increase in IL-1ra.

These data might explain, in part, the cause of the IGF-I "paradox" that we have recently observed in children. We propose that the cumulative effect of multiple, intense exercise bouts (the essence of a training program) is initially to create a metabolic state similar in some respects to the GH-resistant, catabolic state often found in sepsis in which basal IGF-I is reduced. The time course of hormonal alterations after individual bouts of exercise has yet to be determined in children or adolescents.

As training proceeds, however, compensatory mechanisms and other as-yet-unknown adaptive mechanisms ensue and an anabolic "rebound" occurs. This would explain the positive correlation that we and others have observed among fitness, muscle mass, and circulating IGF-I in cross-sectional studies. At what point in training in children a rebound in IGF-I levels occurs is not yet known. We speculate that both the short- and long-term growth adaptations to exercise are regulated, in part, by alterations in the balance of specific catabolic and anabolic mediators. The effect of these substantial exercise-associated alterations in growth mediators on the overall process of growth and development in adolescents has yet to be determined.

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