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Peak oxygen uptake, muscle volume, and the growth hormone-insulin-like growth factor-I axis in adolescent males

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ABSTRACT

ELIAKIM, A., J. A. BRASEL, T. J. BARSTOW, S. MOHAN, and D. M. COOPER. Peak oxygen uptake, muscle volume, and the growth hormone-insulin-like growth factor-I axis in adolescent males. *Med. Sci. Sports Exerc.*, Vol. 30, No. 4, pp. 512–517, 1998. **Purpose:** The growth effects of exercise appear to be mediated in part by central neuroendocrine control reflected in circulating levels of growth hormone (GH), insulin-like growth factor-I (IGF-I), and their binding proteins (BP). In previous studies positive correlations between peak $\dot{V}O_2$ and circulating IGF-I have been demonstrated. The relationship between peak oxygen uptake and these potential regulating factors has not been examined in adolescent males where patterns of GH pulsatility and levels of IGF-I are rapidly changing. **Methods:** Forty-three healthy adolescent males (age 16 ± 0.7 yr, 70% at Tanner V) performed cycle ergometry to determine p oxygen uptake (peak $\dot{V}O_2$), and magnetic resonance images to determine the thigh muscle volume. Baseline blood samples were collected for GHBP, the extracellular portion of the GH tissue receptor (by ligand mediated immunofunctional assay), IGF-I (by RIA), and IGFBPs 1–5 (by RIA). Mean GH was determined from samples obtained every 20 min overnight. **Results:** Peak $\dot{V}O_2$/kg was positively correlated with mean overnight GH levels ($r = 0.41$, $P < 0.005$). Both peak $\dot{V}O_2$/kg and thigh muscle volume/kg were negatively correlated with GHBP ($r = -0.33$, $P < 0.02$) and IGFBP-4 ($r = -0.52$, $P < 0.005$). There were no correlations between peak $\dot{V}O_2$/kg and IGF-I or IGFBPs 1–3, and 5. **Conclusions:** GH pulsatility is increased adolescent males who have higher peak $\dot{V}O_2$, but this did not translate into increases in IGF-I. We speculate that in the fitter males, lower GHBP levels may reduce hepatic sensitivity to GH. Thus, circulating IGF-I was unchanged despite higher mean GH in subjects with higher peak $\dot{V}O_2$. IGFBP-4 which is known to inhibit IGF-I was negatively correlated with peak $\dot{V}O_2$ leading, possibly, to increased IGF-I bioactivity. Fitness (as assessed by muscle mass and peak $\dot{V}O_2$) does modulate the GH-IGF-I axis, but not solely through circulating IGF-I; both GHBP and IGFBPs play important roles. **Key Words:** EXERCISE, BINDING PROTEINS, PEAK OXYGEN UPTAKE, MUSCLE MASS

It is now well established that many anabolic effects of growth hormone (GH) are mediated by insulin-like growth factor-I (IGF-I) (18). Exercise training involves, to some extent, anabolic adjustments of certain tissues like muscle and bone. Thus, it is not unexpected that a number of investigators have found that peak $\dot{V}O_2$ is correlated with circulating levels of GH and IGF-I (11,16,29,39).

There is mounting evidence that the bioactivity of GH and IGF-I is modified by a group of functionally important binding proteins that can alter the physiological function of the two growth factors themselves. However, the relationship between peak $\dot{V}O_2$ and the GH and IGF binding proteins has been inadequately examined. Moreover, in any study of the relationship between peak $\dot{V}O_2$ and circulating growth factors, attention must be paid to maturational status. Adolescence is of particular interest because growth spurts commonly occur, and, like exercise, these anabolic events are controlled through the GH-IGF-I axis.

GH, GHBP, IGF-I, and IGFBP-3 all increase during normal puberty in humans (21,27). These relationships are unique since both GH *and* its receptor (i.e., GHBP) seem to be increasing simultaneously, defying the well-described phenomenon of ligand induced receptor down-regulation. Moreover, even though IGF-I inhibits pituitary secretion of GH (30), this negative feedback loop does not appear to predominate during pubertal growth when both GH and IGF-I are increasing.

More is known about the acute effect of energy-deficient, catabolic states on the GH-IGF-I axis than about the effect of anabolic states. In experimentally induced states of malnutrition, mean GH is elevated, but GHBP (the extracellular portion of the tissue GH receptor, which may reflect tissue GH capacity (21)) is low. The functional result of these alterations is one of tissue GH "resistance," and circulating levels of IGF-I and IGFBP-3, both of which are produced in the liver and stimulated by GH (19), are low (25,35,37).

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Less is known about IGFBPs 1,2, 4, and 5 during catabolic states, but circulating levels of IGFBP-2 (which are sensitive to insulin rather than to GH (32)) have been shown to increase during malnutrition (25,35,37). Reproducible assays for IGFBP-4 and -5 have only become available recently, and the limited data that are available suggest that IGFBP-4 levels change in an inverse manner with circulating IGF-I while IGFBP-5 parallels IGF-I (13,22–24). Thus, one can infer that IGFBP-2 and -4 would decrease while IGFBP-5 increases in anabolic states, although definitive studies have not yet been done.

We hypothesized that exercise training in adolescence enhances the naturally occurring anabolic changes in GH, IGF-I, and their binding proteins. To test this hypothesis we conducted a cross-sectional study of the relationships between fitness (reflecting the trained state) and components of the GH-IGF-I axis in healthy adolescent boys. The term "fitness" is used to describe a number of physiological adaptations to physical activity including cardiorespiratory, anatomic, biochemical, and molecular. Thus, to assess fitness, we used both functional and anatomic measurements. The functional measurements were obtained from progressive exercise tests with gas exchange (e.g., peak $\dot{V}O_2$). The anatomic assessment was derived from magnetic resonance imaging (MRI) of the right thigh muscles, a technique that is increasingly used to determine muscle volume *in vivo* (28,33).

METHODS

Sample population. Forty-three healthy adolescent boys participated in this cross-sectional study. The participants were all students at Torrance High School (Torrance, CA) and enrolled in an anatomy and physiology class during the summer of 1996 (July-August) with class hours from 8 a.m. to 12:30 p.m. The ethnic configuration of the group was 71% Asian, 20% Caucasian, and 9% Hispanic. No attempt was made to recruit subjects who participated in competitive extramural athletic programs. The study was designed to examine late pubertal subjects with an age range of 15–17. Measurements of height and weight were made using standard techniques. Assessment of pubertal status (testicular volume, penile length, and pubic hair) was performed by examination in all of the subjects. Seventy percent of the subjects were at Tanner level V, 26% at Tanner level IV, and 4% at Tanner level III. A standardized test developed by Killen et al. (17) was used to screen the subjects for eating disorders, and none were found. The study was approved by the Institutional Human Subject Review Board and informed consent was obtained from the subjects and their parents or guardians. Many of the subjects in the present cross-sectional study also participated in a prospective endurance training intervention study, some of the results of which have been published elsewhere (12).

Measurements of peak $\dot{V}O_2$. Each subject performed a ramp-type progressive exercise test on a cycle ergometer in which the subject exercised to the limit of his tolerance. Gas exchange was measured breath-by-breath (2) and the peak $\dot{V}O_2$ was determined as previously described in children and adolescents (6). To minimize the confounding effect of size alone, the peak $\dot{V}O_2$ was normalized to body weight. This is a common normalization technique and is especially effective when dealing with a homogeneous sample of same-gender subjects in a narrow age range (5,41).

MRI of thigh musculature. We chose to examine the musculature of the right thigh since these muscles are likely to be involved in the kinds of activities (both resistance and endurance) that children and adolescents naturally perform. The MRI procedure was previously described (10). Briefly, the thigh muscle cross-sectional areas (CSA) of 13 consecutive 2-cm slices (beginning at the knee to a level of 2–3 cm below the femoral neck) were measured using computerized planimetry. The volume (cm^3) of each slice was estimated as CSA (cm^2) × 2 cm. These 13 measurements were then summed to estimate the thigh muscle volume. To minimize confounding effects of size alone, muscle volume was normalized to body weight.

Blood sampling protocols. Subjects were admitted to the Clinical Research Center (CRC) at Harbor-UCLA Medical Center at about 4 p.m. and a dinner was served shortly thereafter. An indwelling venous catheter was inserted in a forearm vein at 6:00 p.m. Baseline blood samples were collected for circulating GHBP, IGF-I, IGFBP 1–5, and testosterone levels at 8:00 p.m. Serial blood sampling for GH was initiated at 8:00 p.m. and continued for 12 h. Samples were collected at 20-min intervals. Subjects were restricted to limited physical activity (e.g., walking in the confines of the CRC). Th lights were turned off in the subjects' rooms at 10:00 p.m..

Growth Hormone (GH). GH serum concentrations were determined using the fluoroimmunoassay technique (36). The monoclonal antibody pair was obtained from Medix Biotex Inc. (San Carlos, CA). Europium labeled streptavidin was obtained from Delfia (Wallac, Inc., Gaithersburg, MD). Inter-assay coefficient of variation (CV) was 5.7–10.1%, and intra-assay CV was 4.9–8.3%. Assay sensitivity was 0.1 $ng \cdot mL^{-1}$.

GHBP. GHBP was measured using the Ligand-Mediated Immunofunctional Assay (4). Inter-assay CV was 9.7–12.9%, and intra-assay CV was 6.3–8.9%. Assay sensitivity was 7.8 $pmol \cdot L^{-1}$.

IGF-I. IGFs were extracted from IGFBPs using the acid-ethanol extraction method (7). Double antibody radioimmunoassay (RIA) was performed to measure IGF-I serum concentrations. Polyclonal recombinant IGF-I antiserum was obtained from the NIH (Baltimore, MD). Radio labeled $^{I\text{-}125}$ IGF-I tracer was purchased from Amersham (Arlington Heights, IL). IGF-I was obtained from Bachem Chemicals (Torrance, CA). IGF-I inter-assay CV was 5.4–7.5%, and intra-assay CV was 4.5–6.2%. Assay sensitivity was 0.1 $ng \cdot mL^{-1}$.

IGFBPs 1–5. IGFBP-1 and 3 were measured by coated-tube Immunoradiometric Assays (IRMA). IGF BP-2, -4 and -5 were measured by RIA. IGFBP 1–3 were measured using commercially available kits; (Diagnostic System Laboratories Inc. kits, Webster, TX). IGFBP-4 and -5 were measured

TABLE 1. Anthropometric, fitness, and hormonal characteristics of the participating subjects (cross-sectional study, $N = 43$).

	Mean ± SE
Height (cm)	170.5 ± 1.1
Weight (kg)	63.9 ± 1.9
Peak $\dot{V}O_2$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	39.3 ± 0.8
Thigh muscle volume ($cm^3 \cdot kg^{-1}$)	22.1 ± 0.4
GH ($ng \cdot mL^{-1}$)—mean value	2.53 ± 0.28
GH peaks (number/12 h)	2.1 ± 0.2
GH peak width (min)	116 ± 9
GH peak height ($ng \cdot mL^{-1}$)	9.9 ± 0.9
GHBP ($pmol \cdot L^{-1}$)	207.3 ± 15
IGF-I ($ng \cdot mL^{-1}$)	223.9 ± 8
IGF BP-1 ($ng \cdot mL^{-1}$)	8.4 ± 1.8
IGF BP-2 ($ng \cdot mL^{-1}$)	213.3 ± 16
IGF BP-3 ($ng \cdot mL^{-1}$)	3494 ± 78
IGF BP-4 ($ng \cdot mL^{-1}$)	171.6 ± 8
IGF BP-5 ($ng \cdot mL^{-1}$)	264.5 ± 8
IGF-I/IGFBP-4 ratio	1.38 ± 0.06
Testosterone ($ng \cdot mL^{-1}$)	3.4 ± 0.3

in our coauthor's laboratory (SM) as recently described (23,24). For IGFBP-1, inter-assay CV was 1.7–6.7% and intra-assay CV was 2–4%. Assay sensitivity is 0.11 $ng \cdot mL^{-1}$. For IGFBP-2, inter-assay CV was 6.4% and intra-assay CV was 6.5%. Assay sensitivity is <0.6 $ng \cdot mL^{-1}$. For IGFBP-3, inter-assay CV was 0.6–1.9%, and intra-assay CV was 1.8–3.9%. Assay sensitivity is 0.5 $ng \cdot mL^{-1}$. For IGFBP-4 inter-assay CV was <8.1% and intra-assay CV was <5%. Assay sensitivity is <0.5 $ng \cdot mL^{-1}$. For IGFBP-5 inter-assay CV was <8% and intra-assay CV was <4%. Assay sensitivity is <5 $ng \cdot mL^{-1}$.

Statistical analysis. GH peaks (number, width, amplitude) were determined using statistical algorithms developed previously (38). Standard techniques of regression and correlation were used for the cross-sectional studies relating GH-IGF-I axis components and the functional and structural fitness variables. Calculations were performed using the statistical package JMP (SAS Institute, Cary, NC). The alpha level was set a $P = 0.05$. Data are presented as mean ± SE.

RESULTS

Mean baseline values for height, weight, peak $\dot{V}O_2$, thigh muscle volume, mean overnight GH level, number of GH peaks, GH peak width and amplitude, GHBP, IGF-I, IGFBP 1–5, IGF-I/IGFBP-4 ratio, and testosterone levels are shown in Table 1. Exercise testing revealed that the subjects had made a substantial effort since the mean peak HR was 192 ± 2 $beats \cdot min^{-1}$ and the respiratory exchange ratio was 1.24 ± 0.02.

There were significant positive correlations between peak $\dot{V}O_2$/kg and 1) mean overnight GH level ($r = 0.41$, $P < 0.005$, Fig. 1, *top panel*); 2) GH peak width ($r = 0.36$, $P < 0.02$) and 3) GH peak amplitude ($r = 0.39$, $P < 0.01$). There were no statistically significant correlations between thigh muscle volume and mean GH levels or GH pulsatility patterns. GHBP was negatively correlated with both peak $\dot{V}O_2$/kg ($r = -0.33$, $P < 0.02$, Fig. 1, *bottom panel*) and thigh muscle volume ($r = -0.45$, $P < 0.005$). GHBP was inversely correlated with mean GH levels ($r = -0.37$, $P < 0.02$).

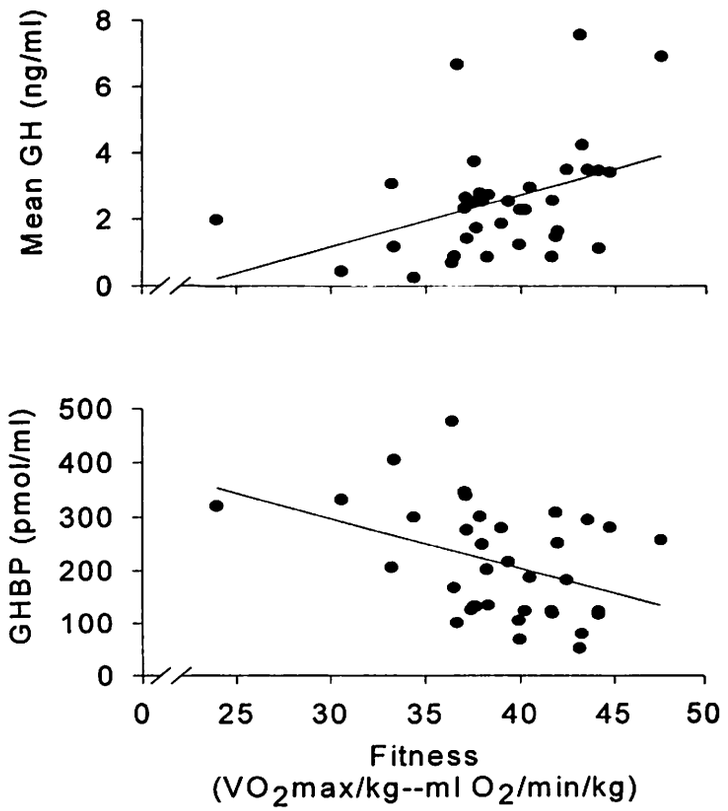

Figure 1—*Top Panel*: Relationship between peak $\dot{V}O_2$/kg and mean overnight GH concentrations ($y = 0.15 \times x - 3.5$, $r = 0.41$, $P < 0.005$). *Bottom Panel*: Relationship between peak $\dot{V}O_2$/kg and GHBP ($y = -9.25 \times x + 575$, $r = -0.33$, $P < 0.02$).

There was no significant correlation between IGF-I and either peak $\dot{V}O_2$ or thigh muscle volume. There were significant negative correlations between IGFBP-4 and peak $\dot{V}O_2$/kg ($r = -0.52$, $P < 0.005$, Fig. 2, *top panel*) and thigh muscle volume ($r = -0.38$, $P < 0.02$, Fig. 2, *bottom panel*). There were significant positive correlations between IGF-I/IGFBP-4 ratio and peak $\dot{V}O_2$/kg ($r = 0.33$, $P < 0.02$) and thigh muscle volume ($r = 0.31$, $P < 0.02$). Both peak $\dot{V}O_2$ and thigh muscle volume were not correlated with the other IGFBP's (1, 2, 3, or 5) or with testosterone.

DISCUSSION

These data add to the growing evidence supporting a possible role of the GH-IGF-I axis in the long-term adaptation to increased levels of physical activity in adolescent males. As expected, mean GH was positively correlated with peak $\dot{V}O_2$; but in contrast to our hypothesis, GHBP was negatively correlated with both peak $\dot{V}O_2$ and thigh muscle volume (Table 2). IGF-I was not correlated with either peak $\dot{V}O_2$ or thigh muscle volume in adolescent males even though IGF-I was previously noted to be related to peak $\dot{V}O_2$ in older men (29), women (16), and recently by us in adolescent females (11). Interestingly, the strongest relationship was the inverse correlation between IGFBP-4 and both peak $\dot{V}O_2$ and thigh muscle volume. This finding is particularly intriguing because IGFBP-4 seems to inhibit mitogenic effects of IGF-I at least in some tissues (22).

The higher levels of mean GH in subjects with higher peak $\dot{V}O_2$ result probably from an increase in peak width

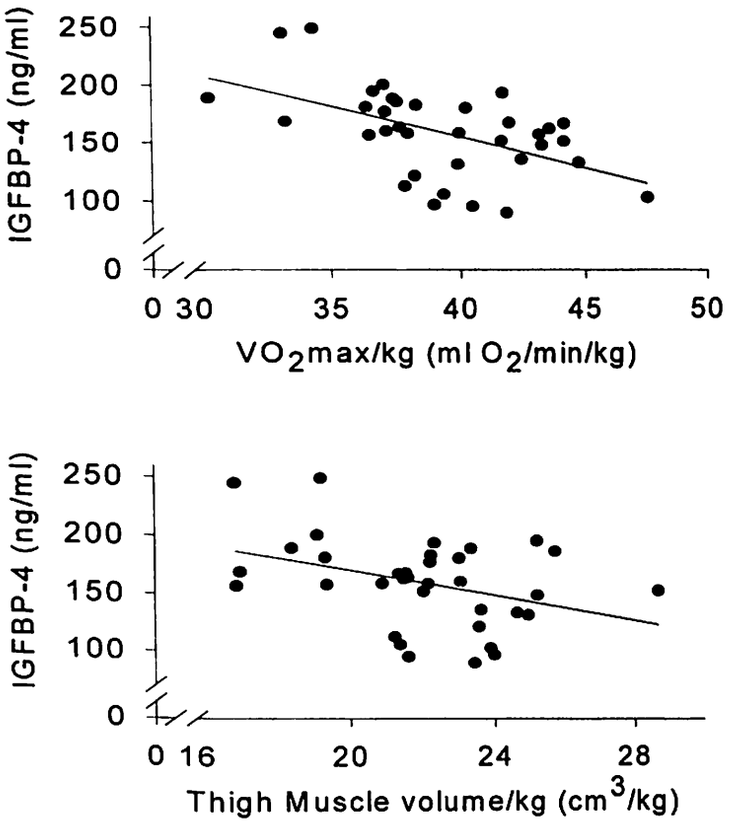

Figure 2—***Top Panel*: Cross sectional relationships between IGFBP-4 and peak $\dot{V}O_2$/kg (y= −5.4××x - 370, r = −0.52, $P < 0.005$). *Bottom Panel*: Cross-sectional relationships between IGFBP-4 and thigh muscle volume (y = −5.5××x + 280, r = −0.38, $P < 0.02$).

and height since both were significantly correlated with peak $\dot{V}O_2$, while GH peak frequency was not. This finding is consistent with studies of Weltman et al. (39) who noted correlations between 24 h integrated GH concentrations and peak $\dot{V}O_2$ in young healthy male adults. Moreover, Borer et al. (3) noted an increased GH pulse amplitude in physically active, rapidly growing hamsters.

The mechanisms of this neuroendocrinologic effect of chronic exercise are not understood. Our data is correlational and caution must be used in interpreting mechanisms from these results. Nonetheless, our observations on the inverse relationship between GHBP and both thigh muscle volume and peak $\dot{V}O_2$ and the inverse relationship between GHBP and mean GH levels suggest a possible mechanism to explain the increased GH in fit subjects. In this paradigm, fitness-associated down regulation of GH receptors is the primary adaptation. Circulating GH itself is known to inhibit pituitary GH secretion (30). The lower levels of circulating GHBP (as noted, reflecting lower tissue GH receptor number) in fit subjects may represent widespread tissue insensitivity to GH. Thus, in the fit, relatively GH-insensitive state, higher levels of GH would be required to limit pituitary secretion of GH. A similar mechanism has been demonstrated in the clinical syndrome of "thyroid resistance" in which reduced tissue sensitivity to thyroid hormone results in generally greater pituitary TSH secretion (20).

There are also other plausible explanations for the inverse relationship between GH and GHBP in fit subjects. Ligand mediated receptor down-regulation is a possibility in which higher circulating GH levels lead to lower tissue GH receptor. In this paradigm, GH secretion is enhanced in fit subjects and the increased secretion eventually down regulates the GH receptor.

IGF-I was not correlated with indices of fitness in this population of adolescent boys even though, as noted, IGF-I was correlated with peak $\dot{V}O_2$ in other studies of males (although not adolescents). The mechanism for this discrepancy is not readily apparent, but it is becoming clear that the level of circulating IGF-I does not invariably reflect IGF-I bioactivity.

Most circulating IGF-I is bound in a complex of IGFBP-3 and the "acid-labile subunit" which appears not to be bioavailable to most tissues (34). Moreover, increasing attention has been paid to the role of other IGFBPs (e.g., IGFBP-4) in regulating IGF bioactivity. We found a substantial inverse correlation between IGFBP-4 and both muscle mass and peak $\dot{V}O_2$. IGFBP-4 appears to inhibit mitogenic effects of IGF-I in bone culture studies (22). Interestingly, IGF-I itself leads to proteolysis of IGFBP-4, suggesting a possible mechanism whereby IGF-I acts to enhance its own bioactivity (9,26). These observations, along with the present findings relating IGFBP-4 to indices of fitness, may suggest a mechanism in which the fit state is anabolic not by acting on GH or IGF-I but by altering their binding proteins. We therefore calculated the ratio of circulating IGF-I/IGFBP-4 as an indicator of "effective" IGF-I activity and found that the ratio was positively correlated with indices of fitness in the adolescent males studied.

There is increasing awareness that the effect of stimuli like exercise on growth factors may be different in local tissues than in the circulation (40). GH, for example, is produced only in the pituitary and is known to stimulate hepatic IGF-I. This process is responsible for most of the IGF-I found in the circulation (1). Moreover, factors like exercise training can stimulate local muscle production of IGF-I even in the absence of GH (8,40). Whether alternative tissue sources of IGF-I or IGFBPs contribute to the circulating levels is not known, but even if they do, locally produced growth factors will certainly be diluted and might undergo proteolysis or other degradation in the transit from tissue to the circulating blood. As a consequence, changes in growth factors like IGF-I detected in the circulation probably do not reflect the full response of a particular stimulus like exercise.

There were substantial differences in the relationships between the GH-IGF-I axis and both muscle mass and peak $\dot{V}O_2$ between adolescent males in the present study and adolescent females in our previous study (11). In males (Table 2), thigh muscle volume and peak $\dot{V}O_2$ were negatively correlated with GHBP, but in females we found positive correlations. Moreover, in males IGF-I was not correlated with indices of fitness, but in females IGF-I was positively correlated with muscle volume. This gender difference is at least internally consistent; both mean GH and GH receptor (as reflected by GHBP levels) were enhanced in the fitter subjects consistent with increased hepatic production of IGF-I and, consequently, to greater circulating

TABLE 2. Relationship between peak $\dot{V}O_2$ and the GH-IGF-I axis in adolescent boys and girls.

	Mean GH	GHBP	IGF-I	IGFBP-2	IGFBP-3	IGFBP-4	IGF-I/IGFBP-4	IGFBP-5
Observed ♂	+	−	NC	NC	NC	·	·	NC
Observed ♀	+	+	NC**	−	NC	−	·	NC

(+, positive correlation; −, negative correlation; NC, no correlation). Data for adolescent females was summarized from reference (11). ** The relationship between peak $\dot{V}O_2$ and IGF-I in adolescent females only approached significance ($P < 0.057$); however IGF-I was positively correlated with thigh muscle volume in adolescent females (11). Reference 11: Eliakim, A., J. A. Brasel, S. Mohan, T. J. Barstow, N. Berman, and D. M. Cooper. Physical fitness, endurance training, and the GH-IGF-I system in adolescent females. *J. Clin. Endocrinol. Metab.* 81:3986–3992, 1996.

IGF-I. In the fitter males mean GH was increased, but GHBP decreased; therefore IGF-I levels were unchanged.

The mechanism for the different gender-related relationships between both peak $\dot{V}O_2$ and thigh muscle volume and GHBP and IGF-I is not readily apparent. Recently discovered effects of estrogen and testosterone on GHBP could play a role: testosterone appears to decrease GHBP (14) while estrogen increases GHBP (15,31). In fact, the mean GHBP in the males (207 ± 15 pmol·L^{-1}) was significantly lower than in the females (394 ± 32 pmol·L^{-1}, $P < 0.05$). Perhaps, these large sex-steroid related effects may mask more subtle physical activity-associated effects on GHBP.

In summary, this study supports a growing body of evidence that functional and structural indices of fitness are correlated with components of the GH-IGF-I axis in adolescents. Whereas previous investigations have focused on mean GH and IGF-I, our data point toward involvement of GH binding proteins and IGF binding proteins. These relationships are different in males and females, indicating that the GH-IGF-I relationship to indices of fitness may be dynamically modified by sex steroids.

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REFERENCES

1. Adamo, M. L., M. A. Bach, C. T. Roberts, and D. Leroith. Regulation of insulin, IGF-1, and IGF-2 g, and IGF-2 gene expression. In: *Insulin-like Growth Factors: Molecular and Cellular Aspects* D. LeRoith (Ed.). Boca Raton: CRC Press, 1991, pp. 271–303.
2. Beaver, W. L., N. Lamarra, and K. Wasserman. Breath-by-breath measurement of true alveolar gas exchange. *J. Appl. Physiol.* 51:1662–1675, 1981.
3. Borer, K. T., D. R. Nicoski, and V. Owens. Alteration of pulsatile growth hormone secretion by growth- inducing exercise: involvement of endogenous opiates and somatostatin. *Endocrinology* 118: 844–850, 1986.
4. Carlsson, L. M., A. M. Rowland, R. G. Clark, N. Gesundheit, and W. L. Wong. Ligand-mediated immunofunctional assay for quantitation of growth hormone-binding protein in human blood. *J. Clin. Endocrinol. Metab.* 73:1216–1223, 1991.
5. Cooper, D. M. Development of the oxygen transport system in normal children. In: *Advances in Pediatric Sport Sciences: Volume 3–Biological Issues*, O. Bar-Or (Ed.). Champaign, IL: Human Kinetics Books, 1989, pp. 67–100.
6. Cooper, D. M., D. Weiler-Ravell, B. J. Whipp, and K. Wasserman. Aerobic parameters of exercise as a function of body size during growth in children. *J. Appl. Physiol.* 56:628–634, 1984.
7. Daughaday, W. H., M. Kapadia, and I. Mariz. Serum somatomedin binding proteins: physiologic significance and interference in radioligand assay. *J. Lab Clin. Med.* 109:355–363, 1987.
8. Devol, D. L., P. Rotwein, J. L. Sadow, J. Novakofski, and P. J. Bechtel. Activation of insulin-like growth factor gene expression during work-induced skeletal muscle growth. *Am. J. Physiol.* 259:E89–E951990.
9. Donnelly, M. J. and J. M. Holly. The role of IGFBP-3 in the regulation of IGFBP-4 proteolysis. *J. Endocrinol.* 149:R1–R7, 1996.
10. Eliakim, A., T. J. Barstow, J. A. Brasel, et al. The effect of exercise training on energy expenditure, muscle volume, and maximal oxygen uptake in adolescent females. *J. Pediatr.* 129:537–543, 1996.
11. Eliakim, A., J. A. Brasel, S. Mohan, T. J. Barstow, N. Berman, and D. M. Cooper. Physical fitness, endurance training, and the GH-IGF-I system in adolescent females. *J. Clin. Endocrinol. Metab.* 81:3986–3992, 1996.
12. Eliakim, A., L. G. Raisz, J. A. Brasel, and D. M. Cooper. Evidence for increased bone formation following a brief endurance-type training intervention in adolescent males. *J. Bone Mineral Res.* 12:1708–13, 1997.
13. Hayden, J. M., S. Mohan, and D. J. Baylink. The insulin-like growth factor system and the coupling of formation to resorption. *Bone* 17:93s–98s, 1995.
14. Ip, T.-P., D. M. Hoffman, A. J. O'Sullivan, K.-C. Leung, and K. K. Y. Ho. Do androgen regulate growth hormone binding protein in adult men? *J. Clin. Endocrinol. Metab.* 80:1278–1282, 1995.
15. Jospe, N., C. C. Orlowski, and R. W. Furlanetto. Comparison of transdermal and oral estrogen therapy in girls with Turner syndrome. *J. Pediatr. Endocrinol. Metab.* 8:111–116, 1995.
16. Kelley, P. J., J. A. Eisman, M. C. Stuart, N. A. Pocock, P. N. Sambrook, and T. H. Gwinn. Somatomedin-c, physical fitness, and bone density. *J. Clin. Endocrinol. Metab.* 70:718–723, 1990.
17. Killen, J. D., C. B. Taylor, L. D. Hammer, et al. An attempt to modify unhealthful eating attitudes and weight regulation practices of young adolescent girls. *Int. J. Eat. Disord.* 13:369–384, 1993.
18. Leroith, D., M. Adamo, H. Werner, and C. T. Roberts, Jr. Insulin-like growth factors and their receptors as growth regulators in normal physiology and pathologic states. *Trends Endocrinol. Metab.* 2:134–139, 1991.
19. Lowe, W. L., Jr. Biological actions of the insulin-like growth factors. In: *Insulin-Like Growth Factors: Molecular and Cellular Aspects*, D. LeRoith (Ed.). Boca Raton: CRC Press, 1991, pp. 49–86.
20. McDermott, M. T. and E. C. Ridgway. Thyroid hormone resistance syndromes. *Am. J. Med.* 94:424–432, 1993.
21. Merimee, T. J. and Z. Laron. Growth hormone binding proteins of serum. In: *Growth Hormone, IGF-I, and Growth: New Views of Old Concepts*, T. J. Merimee and Z. Laron (Eds.). London: Freund Publishing House Ltd., 1996, pp. 11–22.
22. Mohan, S., C. Baptista, J. E. Wergedal, and D. J. Baylink. Isolation of a novel inhibitory IGF binding protein, a potential local regulator of IGF action in bone cell conditioned medium. *Proc. Natl. Acad. Sci.* 86:8338–8342, 1989.
23. Mohan, S., C. Libanati, C. Dony, K. Lang, N. Srinivasan, and

D. J. Bayink. Development, validation, and application of a radioimmunoassay for insulin-like growth factor binding protein-5 in human serum and other biological fluids. *J. Clin. Endocrinol. Metab.* 80:2638–2645, 1995.
24. Mohan, S., Y. Nakao, Y. Honda, et al. Studies on the mechanisms by which insulin-like growth factor (IGF) binding protein-4 (IGFBP-4) and IGFBP-5 modulate IGF actions in bone cells. *J. Biol. Chem.* 270:20424–20431, 1995.
25. Mohnike, K., U. Kluba, W. F. Blum, V. Aumann, P. Vorwerk, and U. Mittler. Serum concentrations of insulin-like growth factors (IGF)-I and IGF-II and IGF binding proteins (IGFBP)-2 and IGFBP-3 in 49 children with ALL, NHL or solid tumors. *Klin. Padiatr.* 207:225–229, 1995.
26. Noll, K., B. R. Wegmann, K. Havemann, and G. Jaques. Insulin like growth factors stimulate the release of insulin like binding protein-3 and degradation of IGFBP-4 in non small cell lung cancer cell lines. *J. Clin. Endocrinol. Metab.* 81:2653–2662, 1996.
27. Olivie, M. A. A., R. V. Garcia-Mayor, D. G. Leston, et al. Serum insulin-like growth factor binding protein-3 and IGF-I levels during childhood and adolescence: a cross-sectional study. *Pediatr. Res.* 38:149–155, 1995.
28. Parkkola, R., U. Kujala, and U. Rytokoski. Response of the trunk muscles to training assessed by magnetic resonance imaging and muscle strength. *Eur. J. Appl. Physiol.* 65:383–387, 1992.
29. Poehlman, E. T. and K. C. Copeland. Influence of physical activity on insulin-like growth factor-I in healthy younger and older men. *J. Clin. Endocrinol. Metab.* 71:1468–1473, 1990.
30. Pontiroli, A. E., R. Lanzi, L. D. Monti, E. Sandoli, and G. Pozza. Growth hormone (GH) autofeedback on GH response to GH-releasing hormone: role of free fatty acids and somatostatin. *J. Clin. Endocrinol. Metab.* 72:492–495, 1991.
31. Rajkovic, I. A., E. Valiontis, and K. K. Ho. Direct quantitation of growth hormone binding protein in human serum by ligand-immunofunctional assay: comparison with immunoprecipitation and chromotographic methods. *J. Clin. Endocrinol. Metab.* 78:772–777, 1994.
32. Rechler, M. M. Insulin-like growth factor binding proteins. *Vitam. Horm.* 47:1–114, 1993.
33. Roman, W. J., J. Fleckenstein, J. Stray-Gundersen, S. E. Alway, R. Peshock, and W. J. Gonyea. Adaptations in the elbow flexors of elderly males after heavy-resistance training. *J. Appl. Physiol.* 74:750–754, 1993.
34. Rosenfeld, R. G., G. Lamson, H. Pham, et al. Insulin-like growth factor binding proteins. *Recent Prog. Horm. Res.* 46:99–163, 1990.
35. Smith, W. J., L. E. Underwood, and D. R. Clemmons. Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. *J. Clin. Endocrinol. Metab.* 80:443–449, 1995.
36. Strasburger, C., G. Barnard, L. Toldo, et al. Somatotropin as measured by a two-site time resolved immunofluorometric assay. *Clin. Chem.* 35:913–917, 1989.
37. Tonshoff, B., W. F. Blum, A. M. Wingen, and O. Mehls. Serum insulin-like growth factors (IGFs) and IGF binding proteins 1, 2, and 3 i, and 3 in children with chronic renal failure: relationship to height and glomerular filtration rate. The European Study Group for Nutritional Treatment of Chronic Renal Failure in Childhood. *J. Clin. Endocrinol. Metab.* 80:2684–2691, 1995.
38. Veldhuis, J. D., A. Iranmanesh, K. K. Ho, M. J. Waters, M. L. Johnson, and G. Lizarralde. Dual defects in pulsatile growth hormone secretion and clearance subserve the hyposomatotropism of obesity in man. *J. Clin. Endocrinol. Metab.* 72:51–59, 1991.
39. Weltman, A., J. Y. Weltman, M. L. Hartman, Relationship beween age, percentage body fat, fitness, and 24-hour growth hormone release in healthy young adults: effects of gender. *J. Clin. Endocrinol. Metab.* 78:543–548, 1994.
40. Zanconato, S., D. Y. Moromisato, M. Y. Moromisato, et al. Effect of training and growth hormone suppression on insulin-like growth factor-I mRNA in young rats. *J. Appl. Physiol.* 76:2204–2209, 1994.
41. Zanconato, S., G. Reidy, and D. M. Cooper. Calf muscle cross sectional area and maximal oxygen uptake in children and adults. *Am. J. Physiol.* 267:R720–R7251994.