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# Acute Effect of Brief Low- and High-Intensity Exercise on Circulating Insulin-Like Growth Factor (IGF) I, II, and IGF-Binding Protein-3 and Its Proteolysis in Young Healthy Men\*

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## ABSTRACT

We measured circulating levels of the GH insulin-like growth factor (IGF) system in response to brief exercise of different intensities. Ten males (mean age  $28 \pm 5$  yr) were studied on three separate occasions: once under resting conditions (control) and once each performing 10 min of low- or high-intensity exercise. Blood samples were assayed by RIA for GH, IGF-I and -II, IGF-binding protein-3 (IGFBP-3), and IGFBP-3 proteolytic activity. After 10 min of low-intensity exercise, IGF-I and IGFBP-3 had increased over preexercise baseline by  $7.7 \pm 2.7\%$  ( $P < 0.05$ ) and  $12.5 \pm 3.3\%$  ( $P < 0.004$ ), respectively. After 10 min of high-intensity exercise, all measured components of the IGF system were increased: IGF-I by  $13.3 \pm 3.2\%$  ( $P < 0.002$ ),

IGF-II by  $15.7 \pm 3.1\%$  ( $P < 0.01$ ), and IGFBP-3 by  $23 \pm 6\%$  ( $P < 0.001$ ). IGFBP-3 proteolytic activity also was increased ( $44 \pm 14\%$  above baseline,  $P < 0.05$ ). GH reached its peak 10 min after the cessation of high-intensity exercise, unlike the earlier peaks of IGF-I and II. In summary: 1) brief exercise leads to small but significant increases in circulating IGF-I, IGF-II, IGFBP-3, and IGFBP-3 proteolysis; and 2) these responses may be influenced by exercise intensity. The IGF responses seem to be unrelated to GH. Acute exercise-induced proteolysis of IGFBP-3 may contribute to anabolic effects of physical activity by increasing the bioavailability of IGF-I. (*J Clin Endocrinol Metab* 81: 3492-3497, 1996)

EXERCISE IS a potent stimulus for a wide variety of anabolic processes (1), but the molecular signals that mediate this are not well understood. Insulin-like growth factors (IGFs) seem to be involved (2-4), and both IGF-I and -II are present in relatively high concentrations in the circulation of humans. Despite these high circulating levels, most IGFs circulate almost entirely complexed with specific binding proteins (IGFBPs) (5), allowing very little free IGF to circulate. Thus, to understand the effect of a stimulus like exercise on circulating IGF, one must consider the regulation of IGFBPs as well as the IGFs themselves.

We hypothesized: 1) that brief bouts of exercise would influence circulating levels of IGF-I, -II, and their binding proteins; and 2) that these effects would be determined, in part, by the relative intensity of the exercise input. We focused on brief exercise because spontaneous, naturally occurring physical activity tends to be of short duration. On three separate occasions, subjects either rested or performed

10 min of high- or low-intensity cycle ergometer exercise. We measured serum levels of GH, IGF-I and -II, and IGFBP-3 (the predominate serum IGF-binding protein).

Finally, we determined whether brief exercise affected serum IGFBP-3 proteolytic activity. IGFBP-3 proteolysis is increased in a number of conditions such as pregnancy (6), CNS tumors (7), diabetes (8), surgery (9), and severe illness (10). In these clinical conditions, increased bioavailability of IGF might be advantageous, and proteolysis of the IGFBP-3 could lead to increased free circulating and/or locally available IGF-I and IGF-II. Whether brief exercise leads to increased IGFBP-3 proteolysis has not been examined previously.

## Subjects and Methods

### Subjects (Table 1)

Ten healthy adult male volunteers participated in the study ranging in age from 20- to 34-yr-old (mean  $27.5 \pm 1.7$ ). None of these individuals trained as competitive athletes, but all engaged in some form of personal exercise. The study was approved by the institutional Human Subjects' Committee, and all subjects granted informed consent.

### Protocol

Each volunteer performed progressive ramp-type cycle ergometry to determine that individual's work rate at his maximal oxygen uptake ( $O_2$ max) and lactate or anaerobic threshold (LT). The LT indicates the work rate above which lactic acidosis accompanies physical exercise and is increasingly used to delineate low- and high-intensity exercise (11, 12). These values were then used to determine a high-intensity work rate

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**TABLE 1.** Subject age, weight,  $\dot{V}O_2$ max, LT, and relative work intensity

Subject	Age (yr)	Wt (kg)	$\dot{V}O_2$ max (mL/min kg)	LT (% $\dot{V}O_2$ max)	Low-intensity exercise (% $\dot{V}O_2$ max)	High-intensity exercise (% $\dot{V}O_2$ max)
1	30	80	52	59	44	83
2	33	85	45	53	47	79
3	30	72	50	50	39	75
4	20	80	38	47	42	66
5	20	90	37	48	42	79
6	26	75	39	57	48	79
7	21	97	40	59	51	77
8	34	83	40	58	51	85
9	32	75	47	66	55	83
10	29	66	39	58	46	85
Mean	28	80	42	56	46	79
SD	5.4	9.0	5.4	5.9	4.9	5.7

equal to 50% of the difference between the subjects LT and  $O_2$ max and a low-intensity work rate equal to 80% of the individual's LT. A similar strategy has been used successfully in a variety of studies designed to examine metabolic consequences of different exercise intensities (13).

The next three sessions occurred on separate mornings (between 0800–0900 h) at least one week apart, after an overnight fast. An antecubital venous catheter was placed for intermittent blood sampling. During two of the three sessions, the subjects performed 10 min of constant-work-rate exercise on the cycle ergometer, one session at high-intensity and one session at low-intensity. During the control session, the subject performed no exercise but rested comfortably. The order of the sessions was determined randomly.

Blood samples were drawn 5 min before and at the onset of exercise, 5 min into the exercise burst, at the end of exercise, and every 10 min after exercise during the 1-h recovery period. Blood samples were spun at 3000 rpm at 4 C for 20 min and the serum split, separated, and stored at -20 C until assayed for GH, IGF-I, IGF-II, and IGFBP-3 and at -70 C until assayed for proteolysis of IGFBP-3.

#### Gas exchange measurements

Breath-to-breath measurements of gas exchange during exercise are routinely made in our laboratory and are well described (14). Similarly, the LT commonly is determined by gas exchange criteria in many laboratories (15).

#### Serum lactate

Serum lactate was measured at rest (preexercise), at endexercise, and at the end of 1 h of recovery. Lactate was measured using the YSI 2300 Stat Lactate Analyzer (Yellow Springs, OH). The lactate intraassay variability is 2.8%, the interassay variability is 3.5%, and the sensitivity is 0.55 mmol/L.

#### Hematocrit

Hematocrit was measured at rest (preexercise), at endexercise, and at the end of 1 h of recovery. A capillary tube sample of blood was spun at 3000 rpm for 3 min, and the hematocrit was determined in conventional fashion.

#### GH

GH concentrations were determined using the fluoroimmunoassay technique (16). Monoclonal antibody pair was obtained from Medix Biotex Inc. (San Carlos, CA). Europium-labeled streptavidin was obtained from Delfia (by Wallace, Inc., Gaithersburg, MD). Interassay coefficient of variation was 5.7–10.1%, and intraassay coefficient of variation was 4.9–8.3%. Assay sensitivity was 0.1 ng/mL.

#### IGF-I and IGF-II

IGFs were extracted from IGFBPs using the acid-ethanol extraction method (17). Double-antibody RIA was performed to measure IGF-I and IGF-II serum concentrations. Polyclonal recombinant IGF-I antiserum was

obtained from the NIH (Bethesda, MD). Radioactively labeled  $^{125}$ I IGF-I tracer was purchased from Amersham (Arlington Heights, IL). IGF-I was obtained from Bachem Chemicals (Torrance, CA). IGF-I assay interassay coefficient of variation was 5.4–7.5% and intraassay coefficient of variation was 4.5–6.2%. Assay sensitivity was 0.1 ng/mL. IGF-II monoclonal antibody was purchased from Amano International (Troy, VA). IGF-II was obtained from Bachem Chemicals (Torrance, CA).  $^{125}$ I IGF-II tracer was iodinated using the modified chloramine-T method. IGF-II interassay coefficient of variation was 5.1–7.6%, and the intraassay coefficient of variation was 4.4–6.8%. Assay sensitivity was 0.1 ng/mL.

#### IGFBP-3 Western ligand blot (WLB)

Six  $\mu$ L human serum were electrophoresed on 12.5% sds-acrylamide gel under nonreducing conditions. The size-fractionated proteins were then electroblotted onto nitrocellulose and incubated overnight with  $^{125}$ I IGF-I at 4 C, washed, and visualized by autoradiography according to the method described by Hossenlopp *et al.* (18).

#### IGFBP-3 RIA

IGFBP-3 was measured by RIA using the Nichols kit (San Juan Capistrano, CA). Because of the high cost of IGFBP-3 RIA, three points felt to best represent acutely measurable changes in IGFBP-3 during the exercise protocol were chosen for study. These time points were pre-exercise, endexercise, and 30 min into recovery. Interassay coefficient of variation was 5.3–6.3% and intraassay coefficient of variation was 3.8–7.3%. Assay sensitivity is 0.25 ng/mL.

#### IGFBP-3 proteolysis

Serum from six time points during each protocol (preexercise, midexercise, endexercise, and every 10 min into recovery for 30 min) were assayed for the presence of IGFBP-3 protease activity, as described by Lamson (19). Briefly, nonglycosylated *E. coli* derived recombinant hIGFBP-3 was iodinated by a modification of the chloramine T method to an SA of 150–300 mCi/mcg. Then 2.5 mL of serum were mixed with 0.05 mol/L Tris-HCl pH 7.4, 0.5 mmol/L  $CaCl_2$ , and  $^{125}$ I-IGFBP-3 to a total volume of 25 mL and incubated for 5 h. At the completion of incubation, the samples were subjected to 12.5% sds-PAGE overnight under nonreducing conditions. The gels were subsequently dried and exposed to x-ray film at -20 C for 18 h. The intensities of the autoradiographic bands were then determined by scanning photodensitometry. The amount of proteolysis was calculated as the percentage of the optical density of fragmented IGFBP-3 over the sum of all IGFBP-3-related optical densities.

#### Statistical analysis

Standard techniques of linear regression techniques were used to determine the slope of  $O_2$  over time during constant-work-rate high- and low-intensity exercise from 3–10 min of exercise (20). Repeated-measures ANOVA was used to determine the effect of different exercise intensities on GH, IGF-I and -II, IGFBP-3, IGFBP-3 proteolysis, lactate, and hematocrit. When ANOVA was found to be significant, intergroup

comparisons were made, using modified *t* tests by the method of Duncan. In addition, we calculated the maximal change in growth factor or binding protein for the low- and high-intensity protocol in each subject. These variables were then compared, using paired *t* tests, modified for multiple comparisons. Data are presented as mean  $\pm$  SE, unless otherwise stipulated.

## Results

### Gas exchange parameters

The individual subject age, weight, height, and exercise gas exchange characteristics are shown in Table 1. Mean  $O_2$  max was  $42 \pm 5$  mL/min·kg (range 37–52 mL/min·kg). These values are well within the normal range previously established in our laboratory (21). As expected, the  $O_2$  reached a steady state during the low-intensity constant-work-rate sessions, but during the high-intensity protocols,  $O_2$  continued to increase throughout the 10 min of exercise [*i.e.* the linear regression slope was significantly positive (20)].

### Lactate and hematocrit

No changes in serum lactate were observed during the control session. Mean serum lactate for the low-intensity protocol increased from  $0.6 \pm 0.1$  mmol/L (range 0.5–0.7 mmol/L) to a peak of  $1.3 \pm 0.2$  mmol/L (range 0.9–2.2 mmol/L) ( $P < 0.01$ ). Mean serum lactate for high-intensity exercise increased from  $0.6 \pm 0.1$  mmol/L (range 0.5–0.7 mmol/L) at rest to a peak of  $6.9 \pm 0.5$  mmol/L (range 5.0–10.1 mmol/L) ( $P < 0.001$ ). The increase in lactate during high-intensity exercise was significantly greater than during low-intensity exercise.

Serum hematocrit was not significantly changed during the control session or during 10-min of below-LT exercise. In contrast, the hematocrit increased significantly from a baseline of  $44 \pm 1.5\%$  preexercise to  $50 \pm 1.0\%$  at the end of above-LT exercise ( $P < 0.001$ ), returning to  $44 \pm 1.1\%$  at the end of 1 h of recovery.

### GH (Fig. 1)

GH levels were unchanged during the control session and did not increase significantly from preexercise baseline during low-intensity exercise. GH levels began to rise significantly with above-LT exercise by 5 min into exercise, peaked at 10 min of recovery, and remained significantly elevated for 50 min of recovery.

### IGF-I (Fig. 2)

The average of the initial two blood samples for each individual subject was taken to establish baseline preexercise IGF-I and IGF-II levels. During the resting control session, we observed that IGF-I concentration fell between the first blood sample (drawn shortly after the antecubital catheter was placed) and all subsequent blood samples drawn. The mean fall in IGF-I was  $6.8 \pm 2.4\%$  [from  $240 \pm 11$  ng/mL to  $224 \pm 11$  ng/mL ( $P < 0.02$ )].

In contrast, the mean IGF-I concentration increased in 9 of 10 subjects after 10 min of low-intensity exercise by  $7.7 \pm 2.7\%$  ( $P < 0.04$ ). IGF-I levels quickly fell back to baseline within 10 min after exercise and remained at baseline throughout recovery.

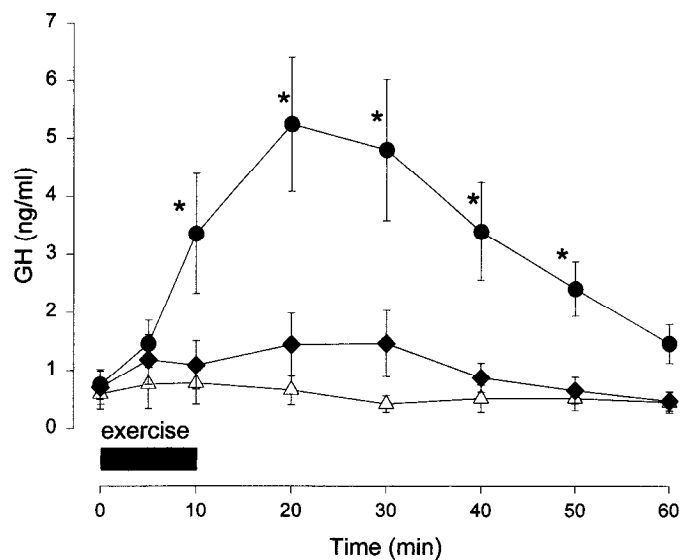


FIG. 1. Effect of brief exercise on circulating GH. Data represented are: control (open triangles); low-intensity exercise (closed diamonds); and high-intensity exercise (closed circles). GH was significantly increased only in the high-intensity exercise protocols and peaked at 10 min into recovery. (\* $P < 0.05$ ).

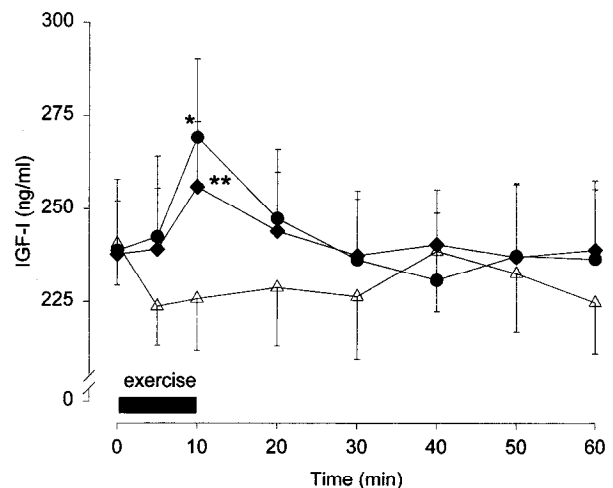


FIG. 2. Effect of brief exercise on circulating IGF-I. Data represented are: control (open triangles); low-intensity exercise (closed diamonds); and high-intensity exercise (closed circles). By the end of exercise, there were significant increases in IGF-I for both low- and high-intensity exercise. There was no significant difference between the magnitude of these increases. During the control session, there was a significant, unexplained, reduction in IGF-I between the first and second samples (see Text).

During high-intensity exercise, mean IGF-I concentration also increased by  $13.3 \pm 3.2\%$  ( $P < 0.002$ ) at the end of exercise. Nine out of 10 subjects demonstrated an increase. There was no significant difference between the increase in IGF-I for the low- and high-intensity protocols.

### IGF-II (Fig. 3)

IGF-II concentrations throughout the control session remained at baseline levels. During low-intensity exercise, IGF-II tended to increase but not significantly so. During high-intensity exercise, IGF-II increased significantly within

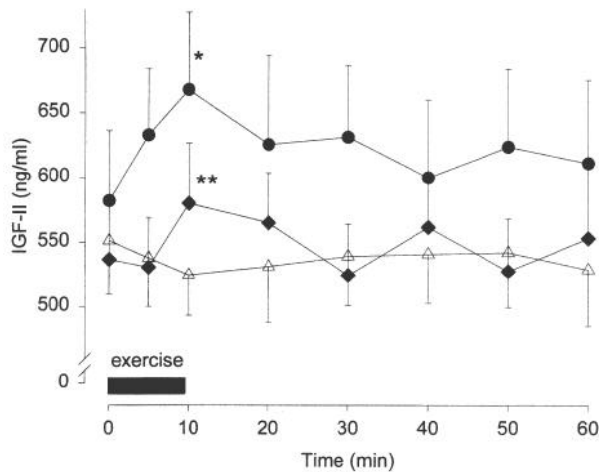


FIG. 3. Effect of brief exercise on circulating IGF-II. Data represented are: control (open triangles); low-intensity exercise (closed diamonds); and high-intensity exercise (closed circles). By the end of exercise, there were significant increases in IGF-II only during high-intensity exercise.

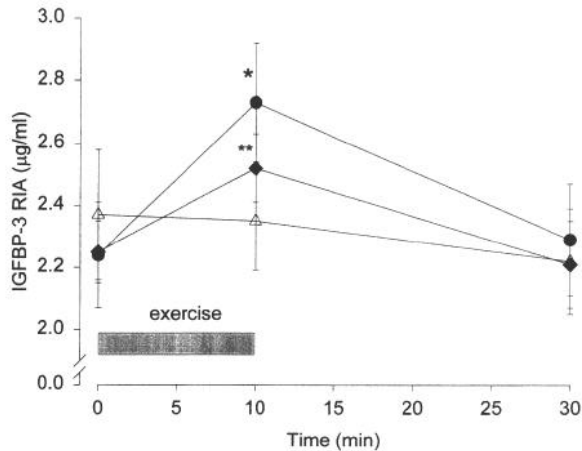


FIG. 4. Effect of brief exercise on circulating IGFBP-3 determined by RIA. Data represented are: control (open triangles); low-intensity exercise (closed diamonds); and high-intensity exercise (closed circles). IGFBP-3 did not change during the control session. Both low- and high-intensity exercise were associated with increased IGFBP-3. The increase associated with high-intensity exercise was significantly greater than during low-intensity exercise ( $P < 0.05$ ). In contrast, IGFBP-3 measured by Western ligand blot did not change during control or exercise sessions (see text).

5 min and peaked at the end of the exercise period (10 min). The mean peak percentage increase in IGF-II by endexercise was  $15.7 \pm 3.1\%$  ( $P < 0.01$ ). Serum concentrations fell to within baseline values by 10 min of recovery.

#### IGFBP-3 RIA (Fig. 4) and WLB

There was no significant difference in IGFBP-3 levels drawn during the control session. IGFBP-3 levels increased significantly during low-intensity exercise by  $12 \pm 3\%$  ( $P < 0.004$ ) at endexercise. IGFBP-3 levels measured by RIA also increased significantly during high-intensity exercise. The mean percentage increase was  $23 \pm 6\%$  at endexercise ( $P < 0.001$ ). Moreover, the increase in IGFBP-3 during high-in-

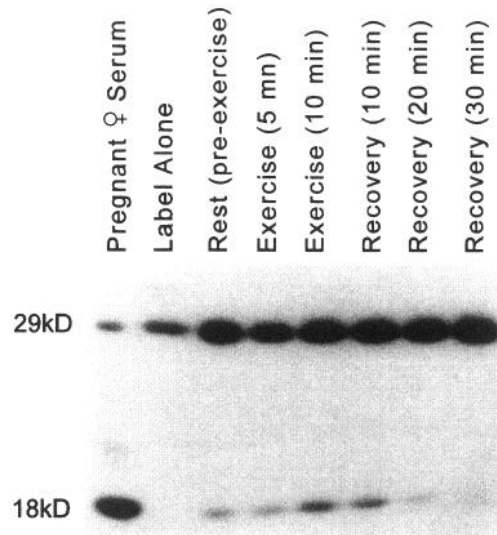


FIG. 5. An example of the gel-assay for IGFBP-3 proteolysis during exercise. Shown here are the intact IGFBP-3 and the 18-kD cleavage band.

tensity exercise was significantly greater than during low-intensity exercise.

In contrast to the RIA results, WLB analysis of above LT serum IGFBP-3 did not reveal a statistically significant change from baseline at any period during the exercise protocols. For example, during high-intensity exercise, IGFBP-3 by WLB at endexercise had not significantly changed ( $91 \pm 7\%$  of preexercise values), nor did it differ by the end of the recovery session ( $107 \pm 15\%$  of preexercise values).

#### IGFBP-3 proteolysis (Figs. 5 and 6)

There was no detectable significant difference in IGFBP-3 proteolysis at any time during the control session. There was a trend (but not statistically significant,  $P < 0.07$ ) toward increased proteolysis of IGFBP-3 by the end of below-LT exercise. Proteolysis of IGFBP-3 rose  $44 \pm 14\%$  ( $P < 0.01$ ) above baseline by the end of 10 min of above-LT exercise. This increase was seen in 9 of 10 subjects.

## Discussion

This study demonstrates that acute brief exercise increases circulating levels of IGFBP-3 and its proteolysis and confirms the previously reported increases in serum IGF-I and IGF-II levels (22, 23) (Fig. 4–6). The magnitude of these effects of exercise on IGF-I and -II, IGFBP-3, and IGFBP-3 proteolysis was determined, in part, by the intensity of the exercise performed. In addition, these increases in IGFs and IGFBP-3 and its proteolysis seem temporally unrelated to the rise in GH. Thus, physical activity might influence circulating IGF not only through changes in levels of the IGFs in the circulation but through regulation of the binding of the IGFs in the circulation, as well.

The determinants of the transient increase in circulating IGF-I in the present study are not readily apparent. One possibility would be the classic mechanism of increased hepatic IGF release because of exercise-induced secretion of GH. However, our data suggest that the increase in IGFs

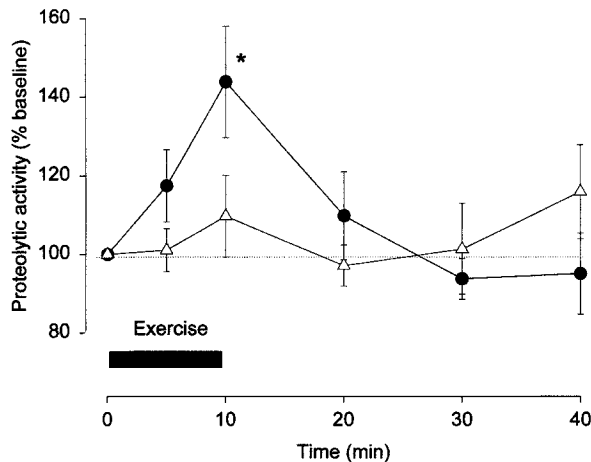


FIG. 6. Effect of brief exercise on IGFBP-3 proteolysis. Data represented are: control (*open triangles*); and high-intensity exercise (*closed circles*). There were no significant changes in proteolysis during the control session. Because of technical difficulties, we obtained IGFBP-3 proteolysis assays in only four subjects during low-intensity exercise, but no changes in proteolysis were observed in these samples. There was a significant increase in IGFBP-3 proteolysis at end-exercise during the high-intensity protocol.

accompanying exercise is, in fact, not related to GH. GH increased significantly only in response to high-intensity exercise [consistent with our previous studies (24)], yet IGF-I increased for both low- and high-intensity exercise. Moreover, in the present study, circulating IGF-I and II reached their peaks before the GH peak, whereas IGF-I is known to increase in the circulation several hours after the administration of endogenous GH (25).

Our results are consistent with observations of Bang *et al.* (23), who showed that exercise led to increases in IGF even in subjects with pituitary insufficiency, and it is now understood that many aspects of IGF control are independent of GH function (2). In addition, the effect of exercise on IGF-I seems to depend upon the type of the exercise performed. For example, increases in circulating IGF-I are not observed in training programs that consist primarily of resistance exercise (*i.e.* weight lifting) (26). The mechanism of these different IGF responses to the type of exercise is not known.

The observed changes in circulating IGFs must reflect rapid changes in the balance among one or all of: 1) IGF input into the circulation from the liver and/or other sources; 2) distribution within the circulating blood; and 3) removal from the circulation. The transient nature of the increases suggests that hemodynamic or metabolic effects of exercise *per se* might play a role. Exercise in humans is accompanied by the rapid autotransfusion of hemoconcentrated blood from the spleen into the cellular circulation (27), by increased blood flow to the exercising muscle, and by loss of plasma water (28). Each of these phenomenon might explain, in part, an increased IGF concentration by changes in IGF flux and/or volume of distribution, but measurements of circulating IGF concentration alone are not sufficient to isolate which of these mechanisms is most important.

We noted an initial small, but significant, fall in IGF-I, during the control sessions, between the first and second serum samples (Fig. 2). There is little support for systematic

circadian patterns of circulating IGF-I or -II in healthy young adults (29); thus, the mechanism for this unexpected fall is probably related to other, as yet unidentified, factors. Whatever the mechanism might have been for the early reduction in IGF-I, it was not apparent during the separate sessions of both low- and high-intensity exercise.

There have been far fewer investigations in humans of the physiological responses of IGF-II compared with IGF-I. In part, this lack of attention to IGF-II may have resulted because, in the rat (the most commonly used animal model for IGF-I molecular biology and physiology), IGF-II seems to exert its most important effects prenatally, and circulating levels are quite low during all of postnatal life (30). In contrast, IGF-II levels remain high throughout life in humans (31) and may play a particularly important role in bone growth and development (32). Our studies confirm those of Bang *et al.* (23), who also showed an acute, endurance exercise-associated rise in IGF-II, but the biological importance of this has yet to be determined.

There have been few studies of IGFBPs in response to brief exercise. Most have concentrated on IGFBP-1. Suikarri *et al.* (33) noted an increase in IGFBP-1 after 3 h of cycle ergometer exercise at a work rate comparable to 45–50% of the subjects'  $O_2$ max. Similarly, Hopkins *et al.* (34) reported an increase in IGFBP-1 after prolonged exercise to exhaustion. IGFBP-1 exists in the circulation in much smaller quantities than IGFBP-3, and its role in circulating IGF-I bioavailability, or, alternatively, as a reflection of tissue phenomenon, is not known (35).

IGFBP-3 levels measured by RIA increased with both low- and high-intensity exercise and were greater during the above-LT protocols (Fig. 4). In contrast to our RIA measurements, IGFBP-3 measured by WLB did not change. One potential explanation for the discrepancy between RIA and WLB data is that the antibody used in the RIA recognizes both intact and fragment forms of IGFBP-3, whereas the WLB method measures only the intact form of IGFBP-3. Discrepancies between RIA and WLB measurements of serum IGFBP-3 have been shown to occur secondary to the presence of IGFBP-3 proteolytic activity (2192). We, therefore, measured IGFBP-3 proteolysis and were intrigued to find that proteolysis did occur consequent to high-intensity exercise (Fig. 5 and 6).

Although we cannot determine the mechanism of the increased IGFBP-3 proteolysis from our data, the findings lead to several speculations. First, IGFBP-3 protease activity has been shown to be calcium dependent (36), and brief periods of heavy exercise can lead to increases in total and free-ionized serum calcium concentrations with a time course similar to the observed changes in IGFBP-3 proteolysis (37). Interestingly, Belcastro (38) demonstrated in the rat that exercise increases non-lysosomal calcium-specific protease activity of calpain, a protease that *in vitro* causes morphological changes in striated muscle similar to exercise. Although all IGFBP-3 proteases that have been observed clinically are not identical (39), the original pregnancy-associated IGFBP-3 protease(s) was characterized as a calcium-dependent serine protease (36). The cleavage patterns of the exercise-induced IGFBP-3 protease seems similar to the pregnancy-associated patterns (see Fig. 5) supporting, indirectly, the possibility that calcium may play a role in exercise-induced IGFBP-3 proteolysis.

The modest proteolytic activity that we observed in blood

sampled from the antecubital vein might actually reflect more substantial proteolytic activity in the local milieu of the exercising muscle. Along these lines, Lalou and Binoux (40) suggested that IGFBP-3 protease activity is more marked in the tissues than in the circulation. Both the gas exchange data and the lactate levels obtained in our study showed that marked acid-base changes had occurred during the high-intensity protocols, and serum changes of this magnitude are known to be associated with even more profound changes in the exercising muscle (41). *In vitro* studies demonstrate that IGFBP-3 undergoes limited proteolysis at acid pH (42). Whether the magnitude of these local changes in pH somehow stimulates IGFBP-3 proteolysis and the local release of bioactive IGFs into the exercising muscle has yet to be determined.

In summary, brief exercise leads to increased proteolysis of IGFBP-3, as well as small, but significant, increases in the serum concentrations of IGF-I and IGF-II. The increase in IGF-I and IGF-II, as well as the increase in IGFBP-3 proteolysis, are simultaneous, acute, of short duration, and precede the exercise-induced GH peak. In addition, the data suggest that in some cases these responses are dependent on the magnitude of the exercise input. These alterations in IGF and IGF regulation in the circulating blood may play a role in mediating the well-described anabolic effects of physical activity; alternatively, the exercise associated changes in circulating IGF and IGFBP-3 may reflect processes that occur in the exercising tissue itself.

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