Time course changes in IGFBP-1 after treadmill exercise and postexercise food intake in rats

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Received 12 July 2000; accepted in final form 4 December 2000

Anthony, T. G., J. C. Anthony, M. S. Lewitt, S. M. Donovan, and D. K. Layman. Time course changes in IGFBP-1 after treadmill exercise and postexercise food intake in rats. Am J Physiol Endocrinol Metab 280: E650–E656, 2001.—Prolonged exercise increases circulating insulin-like growth factor binding protein-1 (IGFBP-1) in humans and animals, but its physiological significance is unknown. This study examined 1) time-course changes in plasma IGFBP-1 and hepatic IGFBP-1 mRNA expression after exercise, 2) changes in IGFBP-1 in relation to plasma glucose, insulin, and IGF-I, and 3) the impact of feeding a postexercise meal on the IGFBP-1 response. Food-deprived male rats were vigorously run on a treadmill and compared with nonexercised controls at 15 min and 1, 4, 8, and 12 h after exercise. Circulating insulin concentrations in exercised rats were lower than in controls at 15 min and 1 h, whereas plasma glucose and IGF-I remained unaffected. Circulating and hepatic expression of IGFBP-1 was markedly increased above that of controls at 15 min, 1 h, and 12 h. In a separate experiment, one-half of the exercised animals received a nutritionally complete meal immediately after the experimental run. The meal elevated plasma insulin and glucose concentrations at 15 min and 1 h. Despite this change in nutritional status, serum IGFBP-1 concentrations and hepatic IGFBP-1 abundance remained elevated at 15 min and 1 h. These results demonstrate that the IGFBP-1 response to a single bout of treadmill exercise is short in duration and independent of insulin, glucose, and amino acid availability.

INSULIN-LIKE GROWTH FACTOR I (IGF-I) is a small polypeptide that is important for a wide range of cellular functions, including proliferation, differentiation, and cell survival. In the whole animal, IGF-I can act in an endocrine as well as an autocrine/paracrine fashion and has both metabolic (insulin-like) and anabolic (growth) functions. These functions are affected by a variety of nutritional, hormonal, and environmental stimuli (see review in Ref. 13).

Although most tissues in the body are capable of producing IGF-I, the liver is the primary source of circulating IGF-I (25). The majority of IGF-I in circulation is bound to the IGF binding proteins (IGFBP), a family of structurally homologous proteins that influence the half-life and bioavailability of IGF-I to the tissues (1). Most (>80%) IGF-I in plasma is bound to IGFBP-3 and an acid-labile subunit, forming a large 150-kDa ternary complex that is unable to cross the endothelial barrier. The remainder of IGF-I in plasma is bound to one of the lower molecular mass IGFBPs, whereas only a very small amount (<1%) circulates in the free form (1).

Of all the currently identified IGF binding proteins, IGFBP-1 is the only one to show rapid and marked fluctuation in human plasma (16, 17). The major physiological regulator of tissue IGFBP-1 expression is insulin (16, 17). However, the IGFBP-1 promoter contains a glucocorticoid response element, a hepatic nuclear factor-1 element, and a cAMP response element addition to an insulin regulatory sequence (17). Hence, the potential for regulation of this rapidly metabolized protein by other factors exists.

Several reports note that prolonged endurance exercise induces very high circulating concentrations of IGFBP-1 in humans and rats (12, 15, 21, 27). The mechanism for such high circulating concentrations and their physiological significance are unclear. Infusion of IGFBP-1 neutralizes insulin-like activity of IGF-I and raises fasting blood glucose concentrations in vivo (18). Therefore, it is proposed that IGFBP-1 may have an important role in glucoregulation during and after exercise (12, 15). However, consumption of a purely carbohydrate drink during cycling exercise to maintain plasma glucose and insulin levels does not prevent plasma IGFBP-1 levels from rising in humans (12). Hence, factors other than glucose and insulin appear to induce IGFBP-1 production during and after exercise.

It has been reported that feeding rats a low-protein diet induces hepatic and kidney IGFBP-1 mRNA expression (28) and that withdrawal of amino acids from culture media results in increased expression of
IGFBP-1 mRNA in HepG2 cells and isolated hepatocytes (14). These data suggest that amino acid availability may influence IGFBP-1 gene expression. Therefore, we were interested in exploring the effects of feeding a protein-containing meal on IGFBP-1 production after prolonged exercise. The objectives of this investigation were threefold: 1) to characterize the plasma and hepatic IGFBP-1 profile during a 12-h recovery period after treadmill exercise, 2) to define the changes in IGFBP-1 in relation to plasma glucose, insulin, and IGF-I, and 3) to determine the impact of postexercise meal feeding on the IGFBP-1 response. This study demonstrates that the IGFBP-1 response to exercise is short in duration and is not related to nutrient consumption after exercise.

EXPERIMENTAL PROCEDURES

Materials

(3-[125I]iodotyrosyl)insulin-like growth factor I and [32P]dCTP were purchased from Amersham (Arlington Heights, IL).

Animals and Experimental Design

The animal facilities and protocol were reviewed and approved by the Institutional Animal Care Review Board of the University of Illinois. In both experiments, male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), initially weighing 140–150 g, were individually housed in wire-bottom cages in a room maintained at 23–25°C with a 12:12-h light-dark cycle. All animals were freely provided tap water and a commercial diet (Harlan-Teklad Rodent Chow, Madison, WI) until the experimental run. The day after arrival, all animals began an 8-day treadmill acclimation schedule that gradually increased in either speed or duration up to 26 m/min for 15 min. Animals that refused to run during the acclimation period were eliminated from the study. All exercise sessions commenced at the beginning of the light cycle after recording of body weight and were performed on a motor-driven treadmill. The experimental run consisted of 2 h of treadmill running at 26 m/min (1.5% grade). Previous reports on energy expenditure in this strain of rat indicate the intensity of the experimental run to be ~75% maximal oxygen consumption (24). In both experiments, food was removed from all animals 4 h before the experimental run.

**Experiment 1.** On the day of the experiment, rats were randomly assigned to one of two treatment groups: C, nonexercised control rats, or E, exercised rats. Animals were allowed free access to water after the treadmill bout, but no food was available throughout the 12-h recovery period. Four rats per treatment group were killed at 15 min and 1, 4, 8, and 12 h postexercise.

**Experiment 2.** On the day of the experiment, animals were randomly assigned to one of three treatment groups: C, nonexercised control rats; E, exercised and food deprived after the experimental run; M, exercised and administered a nutritionally complete meal immediately after exercise. The nutritionally complete meal was prepared and administered exactly as previously described (10). The caloric density was 8.8 kJ/ml (54.5% energy from carbohydrate, 31.5% energy from fat, and 14% energy from protein). The experimental dose was administered by gavage immediately after the experimental run and provided ~15% of daily energy intake for this age and strain of rat (10). All animals were allowed free access to water after the treadmill bout, but no food was available after exercise beyond the defined postexercise meal. Four rats per treatment group were killed at 15 min and four at 1 h, four at 3 h, and four at 12 h postexercise.

**Biological Samples**

At each designated time point, animals were anesthetized by carbon dioxide overdose and killed by decapitation. Trunk blood was collected in heparinized tubes and centrifuged at 1,800 g for 10 min to obtain plasma. The liver was quickly excised, rinsed in ice-cold saline, blotted, and frozen in liquid nitrogen before storage at −80°C.

**Plasma Measurements**

Plasma glucose was analyzed by a glucose oxidase automated method (YSI model 2300 analyzer, Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was analyzed by radioimmunoassay (Linco Research, St. Louis, MO). Plasma IGF-I was measured by a specific RIA following acid chromatography to remove IGFBP, as previously described (29). All samples were analyzed within a single assay with an intra-assay coefficient of variation of 6%. Plasma IGFBP profiles were characterized by SDS-PAGE and Western ligand blotting, as previously described (29). Plasma samples (3 μl) were separated on a 12% SDS-polyacrylamide gel, the proteins were transferred to a nitrocellulose membrane, and the membrane was incubated overnight with [125I]-labeled IGF-I. IGFBP-1 was visualized by autoradiography for 7 days at −80°C. Plasma IGFBP-1 was measured by a specific RIA,

![Fig. 1. Plasma insulin (A) and insulin-like growth factor I (IGF-I, B) in food-deprived male rats after 2 h of treadmill exercise. Values are means ± SE; n = 4 animals in each group at each time point. Means at each time point not sharing the same letter are significantly different, P < 0.05.](http://ajpendo.physiology.org/Downloadedfrom/10.22033.5)
as previously described (19), with an intra-assay coefficient of variation of 5.4%.

**RNA Preparation and Tissue mRNA Analysis**

Frozen hepatic tissue was powdered under liquid nitrogen with a mortar and pestle. Total cellular RNA was isolated from 100-mg samples of liver by the TRI-Reagent method (7), and the quantity and purity of the RNA were determined by the absorbance ratio at optical densities of 260 and 280 nm. Hepatic IGFBP-1 and -2 mRNA expression was determined by Northern analysis. For Northern blot, 20-μg samples were size-fractionated on a 1.2% agarose-formaldehyde gel and capillary transferred to a 0.45-μm nitrocellulose membrane in 10× SSC (1.5 mol/l NaCl and 0.15 mol/l trisodium citrate). The membrane was baked for 1 h at 80°C before hybridization. Also, before hybridization, membranes were incubated in 225-mm bottles in a hybridization oven (Bellco, Vineland, NJ) for 2 h at 65°C in a buffer solution containing 10 g/l BSA, 70 g/l SDS, 0.45 mol/l NaHP₄ (pH 7.2), 0.25 mol/l NaCl, and 0.001 mol/l EDTA. Hybridizations were performed in this same buffer at 65°C for 12–24 h, and membranes were probed with random-prime [³²P]dCTP-labeled rat cDNAs for IGFBP-1 (20) and IGFBP-2 (4). Membranes were also probed with a cDNA for chicken β-actin (generously provided by Dr. Lawrence Schook, University of Minnesota) to correct for loading variation. After hybridization, membranes were rinsed three times with 1 g/l SDS in 0.2× SSC buffer (0.03 mol/l NaCl and 0.003 mol/l trisodium citrate) for 10 min at 65°C. Membranes were wrapped in plastic wrap and visualized by autoradiography at −80°C for 2–3 days.

**Results**

**Experiment 1**

Plasma insulin after exercise was significantly lower than that for controls at 15 min and 1 h but not different at 4, 8, or 12 h postexercise (Fig. 1). Plasma IGF-I concentrations in exercised animals were not

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**Fig. 2.** Plasma IGF binding protein (IGFBP) profiles of food-deprived control (C) and exercised (E) male rats after 2 h of treadmill running. Plasma samples were resolved on a 12% SDS-polyacrylamide gel, Western ligand blotted with [¹²⁵I]-labeled IGF-I, and exposed to X-ray film for 7 days at −80°C. Three bands with an apparent molecular retention (Mr) of 39–46 kDa, 29–31 kDa, and 24 kDa were visualized.
different from controls at 15 min and 1, 4, and 8 h postexercise and did not change over time (Fig. 1). Plasma IGF-I in the nonexercised rats was significantly lower than in exercised rats at 12 h.

Representative Western ligand blots for the IGFBP are shown in Fig. 2. The 29- to 31-kDa IGFBP band (containing IGFBP-1 and -2) in the exercised animals was elevated threefold above controls at 15 min [E vs. C, 85.4 ± 13.3 vs. 27.3 ± 3.9 arbitrary units (AU)] and twofold above controls at 1 h (E vs. C, 62.1 ± 3.5 vs. 26.2 ± 13.1 AU). There were no differences in IGFBP-1 and -2 protein expression between treatment groups at any other time point after exercise, but protein expression in both groups at 12 h was higher than in controls at 1 h (C and E at 12 h vs. C at 1 h, 75.7 ± 3.8 and 62.5 ± 4.3 vs. 23.4 ± 2.7 AU, respectively). There were no differences in the 39- to 45-kDa band (corresponding to IGFBP-3) or the 24-kDa band (corresponding to IGFBP-4) between groups at any time point or across time.

Because both IGFBP-1 and IGFBP-2 migrate in the 29- to 31-kDa region on SDS-polyacrylamide gels, Northern analysis was utilized to determine whether one or both IGFBPs were expressed in the liver after exercise (Fig. 3). At 15 min and 1 h after exercise, hepatic IGFBP-1 mRNA expression in the exercised animals was increased sixfold and threefold, respectively, compared with nonexercised controls (Table 1). Hepatic IGFBP-2 mRNA expression was low or not detectable in both exercised and nonexercised rats at 15 min and 1 h, respectively (Fig. 4). Additionally, IGFBP-1 concentrations in exercised meal-fed animals were 13- and 16-fold higher than in controls at 15 min and 1 h, respectively, and was not different one from the other (Fig. 5). There were no differences among groups in IGFBP-1 mRNA expression after a prolonged bout of treadmill exercise, and it provides additional insight into the physiological role of IGFBP-1. After a single bout of exercise, we found dramatic increases in circulating IGFBP-1, reduced levels of insulin, and stable levels of IGF-I and glucose. Stimulation of circulating IGFBP-1 after exercise was short in duration and correlated with increased hepatic mRNA expression. The high levels of expression induced by exercise were unrelated to circulating levels of insulin, IGF-I, or glucose. Feeding a complete meal after exercise did not alter the acute-phase IGFBP-1 response, suggesting that neither amino acid nor glu-

<table>
<thead>
<tr>
<th>Time</th>
<th>IGFBP-1</th>
<th>IGFBP-2</th>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>E</td>
</tr>
<tr>
<td>15 min</td>
<td>10 ± 1</td>
<td>64 ± 25*</td>
</tr>
<tr>
<td>1 h</td>
<td>17 ± 5</td>
<td>50 ± 5*</td>
</tr>
<tr>
<td>4 h</td>
<td>32 ± 5</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>8 h</td>
<td>12 ± 2</td>
<td>21 ± 8</td>
</tr>
<tr>
<td>12 h</td>
<td>37 ± 4</td>
<td>49 ± 9*</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as arbitrary densitometric units normalized by β-actin; n = 3–4/group. C, nonexercised controls; E, exercised animals run on a treadmill for 1.5 h at 26 m/min; NDE, no detectable expression. Means in a row not sharing the same superscript letter are significantly different, P < 0.05. *Different from 15-min controls, P < 0.055.

**DISCUSSION**

This investigation characterizes for the first time the time-course changes in liver IGFBP-1 mRNA expression after a prolonged bout of treadmill exercise, and it provides additional insight into the physiological role of IGFBP-1. After a single bout of exercise, we found dramatic increases in circulating IGFBP-1, reduced levels of insulin, and stable levels of IGF-I and glucose. Stimulation of circulating IGFBP-1 after exercise was short in duration and correlated with increased hepatic mRNA expression. The high levels of expression induced by exercise were unrelated to circulating levels of insulin, IGF-I, or glucose. Feeding a complete meal after exercise did not alter the acute-phase IGFBP-1 response, suggesting that neither amino acid nor glu-

Fig. 3. Representative Northern blot visualizing hepatic IGFBP-1 and IGFBP-2 mRNA levels in food-deprived control and exercised male rats after 2 h of treadmill running. Nonexercised controls (C) and exercised (E) animals were killed at 15 min or 1, 4, 8, or 12 h postexercise. Subsequent densitometric measurements were corrected for loading variation by β-actin.
cose availability is a key factor in the short-term up-regulation of IGFBP-1 gene expression after exercise. After exercise, sensitivity of skeletal muscle glucose transport activity in response to insulin and IGF-I is increased (11). Enhanced insulin sensitivity in muscle is counterbalanced by a reduction in plasma insulin at the onset of exercise (3), presumably to prevent exercise-induced hypoglycemia. Conversely, circulating concentrations of IGF-I show little to no change after exercise (5, 12, 15). When the potential for IGF-I to bind both the type I receptor and insulin/type I hybrid receptors (8) is considered, an increase in the sensitivity of muscle to IGF-I could lead to increased glucose uptake by muscle. This could potentially result in a disruption of glucose homeostasis, leading to hypoglycemia after exercise.

These observations led to the hypothesis that increases in circulating IGFBP-1 after exercise may prevent IGF-I-induced hypoglycemia by binding free IGF-I in plasma (18). Indeed, infusion of IGFBP-1 into rats prevents the hypoglycemic effect of an infused equimolar solution of IGF-I, and infusion of IGFBP-1 alone causes a small but significant increase in fasting blood glucose levels (18). However, maintenance of euglycemia during cycling exercise did not prevent circulating IGFBP-1 from rising in humans (12). Similarly in the current study, oral administration of a complete meal immediately after exercise did not prevent an acute rise in circulating IGFBP-1. These data collectively suggest that low plasma glucose concentrations are not required to trigger the IGFBP-1 response to exercise and do not support a direct role for glucose in modulating IGFBP-1 production. However, other factors involved in maintaining glucose homeostasis may play a role.

Table 2. Plasma glucose, insulin, and IGF-I concentrations in male rats during exercise recovery

<table>
<thead>
<tr>
<th>Time</th>
<th>Glucose, mM</th>
<th>Insulin, µg/l</th>
<th>IGF-I, µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>E</td>
<td>M</td>
</tr>
<tr>
<td>15 min</td>
<td>5.66 ± 0.43b</td>
<td>5.67 ± 0.35b</td>
<td>10.34 ± 0.65*</td>
</tr>
<tr>
<td>1 h</td>
<td>5.63 ± 0.36b</td>
<td>6.57 ± 0.44b</td>
<td>11.37 ± 2.14a</td>
</tr>
<tr>
<td>3 h</td>
<td>6.72 ± 0.6</td>
<td>6.73 ± 0.26</td>
<td>6.59 ± 0.29</td>
</tr>
<tr>
<td>12 h</td>
<td>5.78 ± 0.54</td>
<td>5.32 ± 0.12</td>
<td>5.83 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 3–4 in each group at each time point. C, nonexercised controls; E, exercised animals not fed after exercise; M, exercised animals fed a nutritionally complete meal immediately after the 2-h experimental run. For each plasma measurement, means in a row not sharing the same letter(s) are different, P < 0.05.
Insulin is proposed to be a primary regulator of circulating IGFBP-1 under most physiological conditions (17). However, this does not appear to be the case after prolonged exercise in humans, for plasma levels of IGFBP-1 are significantly elevated immediately after a marathon run despite similar pre- and postmarathon plasma insulin values (15). Additionally, maintenance of plasma insulin levels by consumption of a purely carbohydrate drink during exercise did not prevent IGFBP-1 levels from rising (12). In the current study, plasma insulin did not correlate with plasma IGFBP-1 or hepatic IGFBP-1 mRNA abundance, further demonstrating that plasma concentrations of insulin do not predict circulating levels of IGFBP-1 after exercise.

In previous studies, a purely carbohydrate beverage was the only dietary treatment utilized to examine the effect of feeding on plasma IGFBP-1 during and after exercise (12, 15). To date, no studies have investigated the impact of amino acids on IGFBP-1 production after exercise. Studies in human HepG2 cells and isolated rat hepatocytes demonstrate that amino acid limitation strongly induces IGFBP-1 expression without affecting IGF-I expression (14). Moreover, treatment of hepatic cells with amino acids at concentrations that mimic those observed in the blood of rats fed a low-protein diet also induces IGFBP-1 expression (14). With this in mind, we hypothesized that the provision of a nutritionally complete meal containing an exogenous source of amino acids would prevent increased hepatic expression of IGFBP-1 after exercise. However, consumption of the protein-containing meal immediately after exercise did not blunt circulating IGFBP-1 concentrations or hepatic mRNA expression of IGFBP-1 compared with exercised food-deprived animals. These data indicate that supplemental protein has no effect on IGFBP-1 regulation immediately after exercise.

An alternate role for increased production of IGFBP-1 is to modulate the anabolic, rather than the metabolic, effects of IGF-I. Studies in human skeletal muscle cells demonstrate the addition of IGFBP-1 to culture media to inhibit IGF-I-stimulated protein synthesis, suggesting that increased production of IGFBP-1 may prevent free IGF-I from interacting with the type I receptor in muscle after exercise (9). Indeed, prolonged exercise results in a significant reduction in the rate of protein synthesis in mixed skeletal muscle of rats (10). However, feeding a protein-containing meal immediately after exercise results in complete recovery of muscle protein synthesis by 1 h postexercise (10), whereas feeding this same protein-containing meal did not abate IGFBP-1 levels in the current study. Therefore, the two processes appear to be unrelated.

Although there were few or no differences in IGFBP-2 gene expression between control and exercised rats throughout the time course, mRNA expression of IGFBP-2 in both treatment groups increased steadily over time. Additionally, IGFBP-1 gene expression in control and exercised rats was elevated at 12 h compared with rats receiving a postexercise meal. These results are likely due to prolonged food deprivation, for rats killed at 12 h postexercise were food deprived for 18 h. Liver IGFBP-2 gene expression is sharply elevated by fasting and decreased by refeeding (6, 22). The hepatic mRNA abundance of IGFBP-1 is also increased in nutritionally restricted animals (22). These effects are caused primarily by an increase in transcription of the IGFBP-1 and IGFBP-2 genes (26). Therefore, nutritional status appears to regulate IGFBP-1 and IGFBP-2 transcription independently of exercise.

The hypothesis that IGFBP-1 is involved in glucose regulation cannot be discounted by the current findings, for many factors are involved in maintaining glucose homeostasis. One likely candidate that could maintain glucose homeostasis in part via increasing IGFBP-1 production is the steroid hormone cortisol. When we consider that the IGFBP-1 promoter contains a glucocorticoid response element (17) and that the glucocorticoids are intimately involved in glucose counterregulation, this is an attractive possibility. Prolonged exercise increases circulating concentrations of corticosterone in rats (10), and this hormone increases in relation to the degree of metabolic stress. The stress of exercise may be an important factor in the IGFBP-1 response. In support of this hypothesis, 30 min of cycle exercise did not alter plasma concentrations of IGFBP-1 (23), whereas exercise bouts of longer duration resulted in significant elevations in plasma IGFBP-1 (2, 15, 21, 27). Further investigation is needed to determine the factor(s) involved in regulating IGFBP-1 production after exercise.

In summary, this report describes the time course for changes in plasma concentrations and hepatic gene expression of IGFBP-1 after prolonged exercise. Plasma concentrations and hepatic mRNA expression of IGFBP-1 rise markedly immediately after exercise but return to control values within a few hours. Factors other than insulin, glucose, and amino acid availability directly regulate IGFBP-1 immediately after exercise, for circulating concentrations of insulin and glucose do not predict plasma IGFBP-1 concentrations or hepatic IGFBP-1 mRNA expression after exercise. Furthermore, ingestion of a nutritionally complete meal containing an exogenous source of amino acids does not influence the plasma or hepatic IGFBP-1 profile immediately after exercise. Investigation into other factors underlying the IGFBP-1 response to exercise is needed to elucidate the physiological role of IGFBP-1 during exercise recovery.

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