Alteration of IGFBP-1 in Soccer Players Due to Intensive Training

Dragana Lagundžin, Vesna Vučić, Marija Glibetić, and Olgica Nedić

Physical activity is accompanied by the changes in Insulin-like Growth Factor I (IGF-I)/IGF-Binding Protein 1 (IGFBP-1) axis. Inconsistent results concerning IGF-I and IGFBP-1 levels were reported. In this study we have raised some questions on the events that occur at the molecular level of the exercise-related IGFBP-1 changes. We have examined the fragmentation pattern of IGFBP-1, IGFBP-1 protease activity, interaction between IGFBP-1 and alpha2-macroglobulin (α2M), and possible existence of minor structural changes of IGFBP-1 in professional soccer players. Athletes had significantly greater amounts of fragmented IGFBP-1, whereas no difference was found in the amount of intact IGFBP-1 compared with controls. An increased activity of matrix metalloprotease-9 (MMP-9) was detected in athletes, causing IGFBP-1 degradation down to the fragment of 9 kDa as the major one. The amount of α2M, which protects IGFBP-1 from proteolysis, or the amount of IGFBP-1/α2M complexes was unaltered. Finally, we have examined whether IGFBP-1 isolated from soccer players exhibited altered reactivity with several chemical surfaces used in surface-enhanced laser desorption/ionization-time of flight mass spectrometry (SELDI-TOF MS). Different reactivity was detected with anion and cation exchangers, suggesting existence of at least one sequence within IGFBP-1, whose ionization pattern was not equal in athletes and controls. Differences in spectra obtained with ion exchanges may reflect differences in IGFBP-1 phosphorylation. Physiological implications of the events described in this study on the IGF-1 availability are, at this time, unknown. It can be hypothesized that IGFBP-1 proteolysis leads to altered distribution of IGF-I among IGFBPs, which may affect the final IGF-associated response.

Keywords: IGFBP-1, soccer players, proteolysis, SELDI-TOF MS

Insulin-like Growth Factor I (IGF-I) is an anabolic hormone associated with muscle glucose and amino acid uptake promoting anabolism (Fryburg et al., 1995). It is primarily synthetized in the liver under stimulation of growth hormone (GH), but during exercise the interaction between GH and IGF system is unclear (He and Zeng, 2006; Frystyk, 2010). IGF-I levels were reported to increase (Manetta et al., 2002; Rosendal et al., 2002; De Palo et al., 2008), decrease (Koistinen et al., 1996; Rarick et al., 2007; Nishida et al., 2010), or remain unaltered (Nindl et al., 2001; Kanaley et al., 2005; Arikawa et al., 2010) after the exercise.

IGF-I availability is controlled via interaction with IGF binding proteins (IGFBPs). Levels of IGFBP-1 were found most often to increase due to training (Hopkins et al., 1994; Koistinen et al., 1996; Berg et al., 2006), but some authors have reported no change in IGFBP-1 levels (Rosendal et al., 2002; Arikawa et al., 2010) or even its reduction (Gregory et al., 2013).

Carbohydrate feeding during exercise does not prevent elevation of IGFBP-1 (Hopkins et al., 1994), and IGFBP-1 is significantly increased only in the case of energy deficit (Rarick et al., 2007). Hoene and Weigert (2010) suggested upregulation of the IGFBP-1 gene in the liver in response to activation of MAPK, p53, and IL-6 signaling pathways and increased IGFBP-1 seems to be linked to a decreased amount of liver glycogen (Lavoie et al., 2002). IGFBP-1 responds differently to different types of exercise (for example, resistance versus endurance exercise; Gregory et al., 2013).

The aim of this study was to examine molecular aspects of the IGFBP-1 in athletes, namely the fragmentation pattern, IGFBP-1 protease activity, interaction between IGFBP-1 and alpha2-macroglobulin (α2M), and possible existence of minor structural changes of IGFBP-1.

Proteolysis of IGFBP-1 is a mechanism for increasing IGF-I bioavailability, as IGFBP-1 fragments have significantly reduced affinity for IGF-I (Gibson et al., 2001). Immunoassays do not offer information on the relative presence of intact IGFBP-1 and its immunoreactive fragments. In this study, the fragmentation pattern of IGFBP-1 and the presence of different fragments in the circulation of soccer players were investigated. Matrix metalloproteases (MMPs) modulate the activity of growth factors through degradation of their binding proteins, including IGFBP-1 (Lee et al., 2008). As gelatinases (MMP-2 and MMP-9) play a critical role in cleaving
muscle-specific proteins (Amirsasan et al., 2011), we examined the activity of MMP-9.

A protease inhibitor α2M, also known to interact directly with IGFBP-1, is another possible participant in IGFBP-1 proteolysis (Westwood et al., 2001). It is not known whether IGFBP-1 in a complex with α2M may be detected by immunoassays. We have determined the relative presence of α2M and IGFBP-1/α2M complexes in sera.

IGFBP-1 is secreted in a highly phosphorylated form (Lewitt, 1994), which may be partly or completely dephosphorylated, giving rise to several phosphoforms. Phosphorylated IGFBP-1 has six- to eightfold greater affinity for IGF-1 compared with non- and less phosphorylated IGFBP-1. Exercise was reported to induce significant elevation only of the heavily phosphorylated IGFBP-1 (Berg et al., 2006). Using surface-enhanced laser desorption/ionization-time of flight mass spectrometry (SELDI-TOF MS), we aimed to detect structural alterations, if they existed, by analyzing reactivity of the isolated IGFBP-1 with three different arrays: immobilized metal ion or anion or cation exchanger.

Thus, we have raised questions on the events that occur at the molecular level of the exercise-related IGFBP-1 changes, which may be part of the adaptive response to physical activity.

Materials and Methods

Samples
Blood samples were obtained from soccer players (n = 30, age 22 ± 1.5 years, BMI 22.3 ± 1.35 kg/m²) from the Serbian professional soccer club, having a combination of aerobic and anaerobic daily trainings. Samples were collected in September, at the beginning of playing season. The regular training regimen consisted of the morning outdoor running and exercise, followed by training with the ball for 3 hr, and the afternoon indoor training composed of mixed exercise with and without gym equipment and/or ball for 2 hr. Blood was taken in the morning—after overnight fasting and 16 hr after the last training. The concentration of hemoglobin was measured in samples collected into anticoagulant solution, whereas concentrations of other parameters were measured in sera. Control samples were taken from the age- and BMI-matched healthy male volunteers (n = 25, age 23 ± 1.4 years, BMI 22.8 ± 2.54 kg/m²) that were not subjected to regular exercise. A body fat percentage was measured by a skin-fold test.

Determination of Biochemical Parameters
Concentrations of biochemical parameters were determined using commercial diagnostic tests (Human, Wiesbaden, Germany). The concentration of IGF-1 was measured using IGF-1 RIA kit (INEP, Belgrade, Serbia), whereas the concentration of IGFBP-1 was measured by IGFBP-1 ELISA kit (BIOSERV Diagnostics, Rostock, Germany).

Immunoaffinity Chromatography (IgY-C)
Immunoaffinity chromatography was performed using Proteomelab IgY-12 High Capacity Proteome Partitioning kit (Beckman Coulter, Fullerton, CA, USA). The matrix with immobilized antibodies enables binding of albumin, IgG, IgA, IgM, α₂-antitrypsin, haptoglobin, transferrin, orosomucoid, α₂M, HDL (apo A-I and apo A-II), and fibrinogen. IgY column was loaded with 20 μL of serum diluted with 480 μL of dilution buffer: 10 mMTris-HCl/1 M NaCl, pH 7.4, and incubated for 15 min at room temperature using rotator. Unbound proteins were separated by centrifugation for 30 s at 2,000 x g and the column was washed three times with dilution buffer. Bound proteins were eluted with 500 μL of the elution buffer (0.1 M glycine-HCl, pH 2.5) for 3 min at room temperature using rotator. They were separated from the gel by centrifugation and neutralized with 50 μL of 1 M Tris-HCl buffer, pH 8.0. The column was washed three times with the elution buffer, neutralized with 600 μL of 2 M Tris-HCl buffer, pH 8.0, and washed with a dilution buffer before the next chromatographic cycle.

Immunoprecipitation (IP)
Immunoprecipitation was performed using Pierce Coimmunoprecipitation Kit (Pierce Biotechnology, Rockford, IL, USA). 50 μL of AminoLinkPlus Coupling Resin (50% slurry) was loaded into a spin column and 10 μg of polyclonal affinity-purified goat anti-IGFBP-1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was immobilized onto a resin according to manufacturer’s instructions. 40 μL of serum was diluted in 160 μL of dilution buffer (2.5 mMTris/0.15 M NaCl/1 mM EDTA/1% NP-40/5% glycerol, pH 7.4, and incubated overnight at 4 °C. The resin was washed three times with dilution buffer. Bound immunoreactive proteins were eluted with 110 μL of the elution buffer, pH 2.8 and neutralized with 2 μL of 2 M Tris-HCl buffer, pH 8.9.

Electrophoresis and Immunoblotting
Electrophoresis under reducing conditions (SDS-PAGE, 12% gel) and immunoblotting were used for protein detection in sera (Lagundžin et al., 2010). Polyclonal affinity-purified goat anti-IGFBP-1 antibody (Santa Cruz Biotechnology) and HRP-conjugated swine antigoat antibody (Biosource, Camarillo, CA, USA) were used for the IGFBP-1 detection. Rabbit polyclonal antio₂M antibody (AbDSerotec, Oxford, UK) and HRP-conjugated donkey antirabbit antibody (Pierce Biotechnology, Rockford, IL, USA) were used to detect o₂M. Immunoreactive proteins were visualized with enhanced chemiluminescence (ECL) reagents (Pierce, Minneapolis, MN, USA).

Gelatin Zymography
Expression of the gelatinase MMP-9 in serum samples was examined by standard gelatin zymography (Snoek-van Beurden et al., 2005), using 12% SDS-PAGE gels containing 0.1% gelatin (DrOetker, Zagreb, Hrvatska).
Gels were stained in 0.4% Coomassie brilliant blue R-250 solution. Protease activity was observed as a clear band in a gel due to proteolyzed gelatin.

**Surface-Enhanced Laser Desorption/Ionization-Time of Flight Mass Spectrometry (SELDI-TOF MS)**

Serum samples and protein fractions after IgY-C and IP were analyzed by ProteinChip SELDI system, Series 4000 (BioRad Laboratories, Hercules, CA, USA). ProteinChip PS20 array (preactivated surface-epoxide) was used to immobilize polyclonal affinity-purified goat anti-IGFBP-1 antibody (Santa Cruz Biotechnology), and analyze immunoreactive proteins from sera and IgY-bound fractions. Protein fractions after IP were analyzed using three chip arrays: IMAC30 (immobilized metal affinity capture), Q10 (strong anion exchanger) and CM10 array (weak cation exchanger).

**ProteinChip (Profiling On-Spot) Protocol.**

**ProteinChip PS20 Array.** 5 μL (200 μg/mL) of anti-IGFBP-1 antibody (Santa Cruz Biotechnology) were added to the spot and incubated in a humid chamber at 4°C overnight. The spot was washed with 5 μL of 50 mM PBS, pH 7.2 three times and blocked with 5 μL of 0.5 M Tris-HCl, pH 8.0 for 1 hr. After washing with PBS three times, 5 μL of sample was added and incubated in a humid chamber at room temperature for 3 hr. The spot was washed with PBS three times and distilled water twice, then air-dried for 20 min.

**ProteinChip IMAC30 Array.** 5 μL of 0.1 M FeCl3 was added to the spot and incubated for 10 min. The spot was washed with 5 μL of distilled water, 5 μL of 0.1 M Na-acetate (pH 4.0) for 5 min and then again 5 μL of distilled water and 5 μL of binding buffer (0.1 M PB/0.5 M NaCl, pH 7.0) for 5 min. The sample (5 μL) was added and incubated in a humid chamber at room temperature for 3 hr. The spot was washed with binding buffer three times and distilled water twice, then air-dried for 20 min.

**ProteinChip Q10 Array.** The spot was prewet with 5 μL of binding buffer (50 mM Na/NH4PB, pH 7.0 for 5 min; twice). The sample (5 μL) was added and the spot was further treated as described for IMAC30 Array.

**ProteinChip CM10 Array.** The spot was prewet with 5 μL of binding buffer (50 mM Na/NH4-acetate, pH 5.0 for 5 min; twice). The sample (5 μL) was added and the spot was further treated as described for IMAC30 Array. After drying, 1 μL of 50% sinapinic acid in acetonitrile/distilled water/trifluoroacetic acid (50:0:49.9:0.1) was added to each spot, dried and reapplied.

Mass spectra were acquired in 25 kV positive ion acquisition mode, mass range 2.5–300 kDa, and with 8,815 laser shots per spot. The laser energy was 6,000 nJ. The calibration was performed with ProteinChip all-in-one protein standards II (BioRad Laboratories, Hercules, CA, USA). All spectra were analyzed using Ciphergen Express 3.0 software (BioRad Laboratories, Hercules, CA, USA).

**Densitometric Analysis**

Densitometric analysis of protein bands on zymograms and immunoblots was done using Image Master Total Laboratory v2.01 software (Amersham BioSciences, Buckinghamshire, UK). Results were expressed in arbitrary densitometric units (ADU).

**Statistical Data Analysis**

Concentrations of biochemical parameters, IGF-I and IGFBP-1, body fat percentage, relative abundance of different IGFBP-1 molecular forms, and relative enzyme activity of MMP-9 were expressed as mean values ± SD. Statistical data analysis was performed using SPSS 16.0 software. The difference between soccer players and controls was assessed by student t test.

**Results**

Concentrations of biochemical parameters, IGF-I, and body fat percentage in athletes and control subjects are given in Table 1. Concentrations of IGFBP-I were higher in athletes compared with controls. Concentrations of IGFBP-1, determined by ELISA, were not statistically different between groups (Table 2), although the difference within the group was more pronounced for the soccer players. Immunoblotting was employed to investigate the IGFBP-1 profile. Representative IGFBP-1 profiles are given in Figure 1a (three samples from each group). Three immunoreactive protein bands were distinguished at approximately 30 kDa (monomer) and at 21 and 9 kDa (fragments). Intensities of the bands representing IGFBP-1 fragments were significantly greater in athletes compared with controls, whereas intensities of bands representing monomer IGFBP-1 were similar (Table 2). Thus, the total amount of IGFBP-1 (intact and fragmented) was significantly greater in athletes than in nontrained subjects.

In gelatin zymography, two forms of MMP-9 (92 and 82 kDa) were distinguished (Figure 1b; three samples from each group). The activity of both MMP-9 forms was significantly greater in athletes than in controls (Table 2). In immunoblotting with antiα2M antibody, proteins at masses close to 200 kDa, corresponding to α2M monomer subunits, were recognized. Representative results are shown in Figure 1c. Densitometric analysis did not reveal differences between two study groups.

In SELDI-TOF MS, three types of interactions between our IGFBP-1 samples and reactive phases were investigated: immunoreactivity and reactivity with ion exchangers and with metal ion (Fe3+). The spectral interval between 6 and 10 kDa was the region where differences in signal profiles were noted. Representative SELDI-TOF MS profiles for both examined groups are shown in Figure 2.
Table 1 Concentrations of Biochemical Parameters, IGF-I, and Body Fat Percentage in Athletes and Control Subjects (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Athletes, (n = 30)</th>
<th>Controls, (n = 25)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>22 ± 1.5</td>
<td>23 ± 1.4</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>22.3 ± 1.35</td>
<td>22.8 ± 2.54</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.6 ± 0.23</td>
<td>4.4 ± 0.32</td>
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<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.7 ± 0.35</td>
<td>0.7 ± 0.35</td>
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<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.8 ± 0.74</td>
<td>3.5 ± 0.45</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>14.9 ± 2.73</td>
<td>13.2 ± 3.01</td>
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<tr>
<td>Hemoglobin (g/L)</td>
<td>145 ± 8.3</td>
<td>159 ± 6.5</td>
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<tr>
<td>Albumin (g/L)</td>
<td>48 ± 5.3</td>
<td>47 ± 2.2</td>
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<tr>
<td>Aspartat aminotransferase, AST (U/L)</td>
<td>79 ± 7.4*</td>
<td>17 ± 4.5</td>
</tr>
<tr>
<td>Alanin aminotransferase, ALT (U/L)</td>
<td>29 ± 6.2</td>
<td>20 ± 3.5</td>
</tr>
<tr>
<td>Creatine kinase, CK (U/L)</td>
<td>433 ± 163.4*</td>
<td>84 ± 25.5</td>
</tr>
<tr>
<td>Lactate dehydrogenase, LDH (U/L)</td>
<td>348 ± 56.1*</td>
<td>277 ± 37.9</td>
</tr>
<tr>
<td>Iron (mmol/L)</td>
<td>21.0 ± 4.62</td>
<td>20.6 ± 4.53</td>
</tr>
<tr>
<td>Total iron-binding capacity (mmol/L)</td>
<td>56.4 ± 5.33</td>
<td>57.6 ± 6.52</td>
</tr>
<tr>
<td>Ferritin (pmol/L)</td>
<td>173 ± 36.1</td>
<td>168 ± 27.2</td>
</tr>
<tr>
<td>IGF-I (nmol/L)</td>
<td>48.5 ± 16.20*</td>
<td>31.8 ± 7.33</td>
</tr>
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</table>

*Statistically significant differences between two groups (p < .05).

Firstly, serum samples were examined using PS20 chip array with immobilized anti-IGFBP-1 antibody (Figure 2a). Triply protonated IGFBP-1 form was observed in all samples at m/z 7,800; three fragments were detected at m/z 6,400, 6,600, and 6,800, while the peak at m/z 9,300 was clearly seen only in the spectra of sera from the athletes. The mass of triply protonated IGFBP-1 form was slightly lower than the mass expected for the native IGFBP-1, implying possible influence of the method itself. Tendency to detect masses slightly lower...
than expected was present to the same extent in spectra of all samples, so the shift did not affect the comparison between two sets of data. Statistical analysis indicated a significant decrease in signal intensity at m/z 6,600 and 6,800 in athletes compared with controls and all signals between 6 and 8 kDa were slightly shifted to the lower masses.

Immunoaffinity chromatography with immobilized antiα2M antibody separated IGFBP-1/α2M complexes. Proteins eluted from IgY matrix were allowed to interact with anti-IGFBP-1 antibody immobilized onto PS20 chip array (Figure 2b). Three different fragment ions were detected at m/z 8,200, 8,600, and 8,900 in all samples. A signal corresponding to triply protonated IGFBP-1 form at m/z 7,800 was not observed. There was no significant difference in signal intensities of the fragments between two study populations.

Figure 2 — IGFBP-1 SELDI-TOF MS fragmentation analysis in a gel view mode of the serum on PS20 chip array with immobilized anti-IGFBP-1 antibody (a), IgY-bound protein fraction (containing IGFBP-1/α2M complex) on PS20 chip array with immobilized anti-IGFBP-1 antibody (b), and IP fraction (containing isolated IGFBP-1 forms) on Q10 chip array (c), IMAC30 chip array (d) and CM10 chip array (e) of samples obtained from the athlete (A) and the control subject (C). The negative control for the PS20 chip is given at the bottom (f). Numbers on the top indicate the most intensive signals.
To examine chemical reactivity, IGFBP-1 molecules were isolated by immunoprecipitation and subjected to mass spectrometry using chip arrays with different reactive surfaces. Q10 chip array was used for binding and characterization of the negatively charged IGFBP-1. The most pronounced signal in the protein fraction from the athletes was triply protonated IGFBP-1 form and only one fragment ion was seen at m/z 8200 (Figure 2c). More extensive fragmentation of IGFBP-1 was observed in fractions from the controls. Beside the very abundant triply protonated IGFBP-1 ion, three other fragment ions were distinguished at m/z 7,900, 8,100, and 8,200. Signals of triply protonated IGFBP-1 form and fragment ions at m/z 8,200 and 8,600 were present in IMAC30 chip array spectra from both groups and in similar relative ratios (Figure 2d). A weak signal at m/z 6,800 was seen only in fractions from the athletes. Positively charged ions at m/z 7,800, 8,100, 8,200, 8,600, and 9,300 were detected using CM10 chip array (Figure 2e). The intensity of the signal at m/z 9,300 was markedly higher in samples from the athletes than from the controls.

### Discussion

Intensive physical activity is accompanied by an increased rate of glucose metabolism, which is tightly connected to insulin secretion and, consecutively, to the IGF-I/IGFBP-1 axis (Manetta et al., 2002). Accurate measurement of IGFBP-1 is highly dependent on antibodies used in the formulation of assay, as phosphorylation of IGFBP-1 significantly alters its immunoreactivity (Khosravi et al., 1997; Khosravi et al., 2007). The group of Khosravi has developed several ELISAs and concluded that concentrations of IGFBP-1 determined by these assays were different several fold, depending on the state of phosphorylation of IGFBP-1. Some ELISAs measure only intact IGFBP-1, whereas others detect both intact and fragmented species. The difference in the amount of intact IGFBP-1 (assessed both by ELISA and immunoblotting) between soccer players and the matched controls was not found in our study, but athletes had significantly greater amount of fragmented IGFBP-1. When both intact and fragmented species were taken into account, the total amount of IGFBP-1 was calculated to be greater in athletes.

IGFBP-1 degradation by MMPs was observed in different (patho)physiological conditions, including inflammation and infection. A high-intensity exercise promotes the increase of MMPs expression (Carmeli et al., 2005), and MMPs participate in remodeling of the extracellular matrix (ECM) by degrading ECM proteins. MMP-9 was found to regulate the bioavailability of growth factors. Different MMPs generate IGFBP-1 fragments of different masses. Coppock et al. (2004) have found that MMP-3 and MMP-9 cleave IGFBP-1 at Lys145/Lys146, generating a 9 kDa C-terminal fragment. Our results are in accordance with these findings, as we have also detected increased activity of MMP-9 in professional soccer players. An IGFBP-1 fragment at 9 kDa most likely resulted from the activity of MMP-9, which was also higher in athletes than in controls.

It is known that IGFBP-1 in complex with α2M is protected from proteolysis. The concentration of α2M was not found to be altered in soccer players, the amount of IGFBP-1/α2M complexes was not changed due to physical exercise, and the fragmentation pattern of IGFBP-1/α2M complexes in athletes and controls was the same. It may be postulated that adaptive response to exercise excludes prevention of IGFBP-1 proteolysis, which could be mediated by increased binding of IGFBP-1 to α2M.

There are limited data on structural changes of IGFBP-1 due to exercise and even less on their possible influence on IGF-1 availability. Berg et al. (2006) have found that only highly phosphorylated IGFBP-1 increased significantly during physical activity. Knowledge regarding the phosphorylation status of IGFBP-1 in the circulation due to professional training is lacking.

### Table 2

<table>
<thead>
<tr>
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<th>Athletes, (n = 30)</th>
<th>Controls, (n = 25)</th>
</tr>
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<tbody>
<tr>
<td>Concentration of IGFBP-1 (nmol/L)</td>
<td>0.3 ± 0.16</td>
<td>0.2 ± 0.13</td>
</tr>
<tr>
<td>Monomer IGFBP-1, 30 kDa (ADU)</td>
<td>71 ± 9</td>
<td>73 ± 3</td>
</tr>
<tr>
<td>IGFBP-1 fragment 1, 21kDa (ADU)</td>
<td>28 ± 2*</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>IGFBP-1 fragment 2, 9 kDa (ADU)</td>
<td>119 ± 4*</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>Total IGFBP-1 (ADU)</td>
<td>218 ± 14*</td>
<td>119 ± 8</td>
</tr>
<tr>
<td>Pro MMP-9, 92 kDa (ADU)</td>
<td>55 ± 4*</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>MMP-9, 82 kDa (ADU)</td>
<td>36 ± 4*</td>
<td>12 ± 3</td>
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*Statistically significant differences between two groups (p < .05).
Conclusions

Our investigation of the exercise-related changes of IGFBP-1 revealed increased fragmentation of IGFBP-1, which was most likely promoted by increased activity of MMP-9, unaltered binding to protease inhibitor α2M — and thus unaltered protection against degradation — and slight changes in chemical reactivity of the IGFBP-1 (most likely induced by minor structural changes). We have used multiple methods to detect IGFBP-1-linked molecular events and relations that seem to be involved in metabolic adaptation to physical exercise, but their physiological implications on the IGF system and, more specifically, on the IGF-I availability are unknown at this time. It can be hypothesized that IGFBP-1 proteolysis leads to altered distribution of IGF-I among IGFBPs, which may affect the final IGF-associated response.

Acknowledgment

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References


Supplementary Material

Investigation of the Anti-IGFBP-1 Antibody Specificity

Materials and Methods

Gel filtration (GF) was performed to analyze the specificity of anti-IGFBP-1 antibody. Amniotic fluid, which is known to have IGFBP-1 as the only IGFBP, served as a sample. Three experiments were conducted.

Amniotic fluid (10 μl, IGFBP-1 concentration was 5.4 μM) diluted to 1 ml with 50 mM phosphate buffered saline (PBS) pH 7.5 was incubated with 5 × 10^5 cpm 125I-IGF-I (1 pmol) at 4 °C overnight and chromatographed on the sephadex G-100 column (1.8 x 60 cm). Proteins were eluted with PBS buffer and the radioactivity of the collected fractions (2 ml) was measured.

In the second experiment, unlabeled IGF-I (100 pmol) was added to the incubation mixture and it was chromatographed as described.

In the third experiment, a sample of amniotic fluid was incubated with anti-IGFBP-1 antibody (2.5 nmol IgG) at 4 °C overnight. Complexes formed between IGFBP-1 from the sample and anti-IGFBP-1 antibody were precipitated using immobilized protein A (Ey et al.,
1978, p. 429–436). The supernate left after centrifugation was incubated with $^{125}\text{I}-\text{IGF-I}$ in a standard manner and chromatographed.

Molecular mass markers (IgG, 150 kDa and ovalbumin, 45 kDa) were from Pharmacia Biotech (Uppsala, Sweden).

Results

Using GF, it was shown that $^{125}\text{I}-\text{IGF-I}$ interacted with proteins in amniotic fluid forming two types of complexes; the major fraction corresponded to the binary $^{125}\text{I}-\text{IGF-I}/\text{IGFBP-1}$ complex (approximately 40 kDa) and the minor had the molecular mass $\geq 150$ kDa (eluted in $V_0$). Their relative abundance was 10:1.

When unlabeled IGF-I was added in the incubation mixture, it completely inhibited binding of $^{125}\text{I}-\text{IGF-I}$ in the binary complex, while $^{125}\text{I}-\text{IGF-I}$ still interacted to some extent with proteins of the higher molecular mass.

When IGFBP-1 was depleted from the amniotic fluid by immunoprecipitation, remaining proteins did not bind $^{125}\text{I}-\text{IGF-I}$ in a complex of 40 kDa.