Characterization of Insulin-like Growth Factor (IGF) Binding Proteins and Their Role in Modulating IGF-I Action in BHK Cells*

David Hsu and Jerrold M. Olefsky

From the Division of Endocrinology and Metabolism, Department of Medicine, University of California at San Diego, La Jolla, California 92039 and the Veterans Administration Medical Center, San Diego, California 92161

We have found that over one-half of the total cell surface 125I-insulin-like growth factor I (IGF-I) binding to BHK cells represents binding to IGF binding proteins (IGFBPs) rather than to the IGF-I receptor. In addition to a number of secreted IGFBPs, we have now characterized two cell-associated IGFBPs with unique characteristics. The cell-associated IGFBPs have molecular weights of 30,000 (30K) and 25,000 (25K), as determined by the Western ligand blot technique. IGFBP-30K is located at the cell surface and can be readily labeled by affinity cross-linking with 125I-IGF-I. Surface expression of IGFBP-30K increases 5.4 ± 1.2-fold (n = 11) with serum starvation. This induction is fully evident by 4 h, plateauing by 24 h, and is completely inhibitable by cycloheximide. The fasting-induced increase in IGFBP-30K is inhibited by IGF-I and by des-IGF-I and, to a lesser extent, by insulin. Unlike cell-associated IGFBP-30K, secretion of IGFBP was stimulated (6.8 ± 0.5-fold, n = 2) by IGF-I, whereas IGFBP secretion was inhibited 54% by insulin. These results demonstrate coordinate regulation of IGFBP by serum starvation and IGF-I, such that at low concentrations of IGF-I, cell surface binding protein increases whereas binding protein secretion decreases. At high concentrations of IGF-I, IGFBP secretion increases and cell surface IGF-I receptor, as well as IGF binding, decreases. Taken together, these regulatory events regulate the availability of IGF-I for biologic signalling.

Expression of each individual IGFBP appears to be regulated by different hormones, as well as nutritional status (2). These IGFBPs bind IGF-I and can modulate its function by: 1) prolonging the half-life of IGF-I; 2) altering the distribution of IGF-I between body fluids and the cell surface; and 3) affecting the transport and distribution of IGF-I to different tissues. The IGFBPs can either enhance or inhibit cellular responses to IGF-I in vitro (3–17). These reports indicate that the cellular actions of IGF-I could be directly modified by IGFBPs. The general consensus of these various reports is that the cell surface-associated IGFBPs can enhance IGF-I action, while the IGFBPs in the medium inhibit IGF-I action. These observations also suggest that the distribution of the IGFBPs between the cell membrane and the medium could determine their effects on IGF-I action.

In the current studies, we have characterized two cell-associated IGFBPs in BHK cells with molecular weights of 30,000 and 25,000 (IGFBP-30K and IGFBP-25K), respectively. IGFBP-30K is expressed on the cell surface. Its expression is regulated by IGF-I and insulin and is markedly induced with serum starvation. The cell surface-associated IGFBP-30K may play a role in modulating IGF-I action. IGFBP-25K is localized intracellularly and secreted into the medium. It is not significantly affected by serum starvation or hormone treatment. A number of IGFBPs are secreted into the medium by BHK cells. The major species is a 30K IGFBP (IGFBP-30K-M) which was stimulated by IGF-I and inhibited by insulin.

**EXPERIMENTAL PROCEDURES**

Materials—131I-Labeled and unlabeled IGF-I were kindly provided by Eli Lilly and Co. Des-IGF-I was purchased from GroPep Pty Ltd (Adelaide, Australia). Disuccinimidyl suberate was purchased from Pierce Chemical Co. Nitrocellulose filter paper was purchased from Schleicher and Schuell. Fetal bovine serum was purchased from Gemini Bioproducts, Inc. (Carlabasa, CA).

Cells and Culture Conditions—The Syrian hamster fibroblast cell line BHK-21 with defective thymidine kinase (BHK TK-) was used in this study (18). The cells were grown in DME medium supplemented with 10% bovine fetal serum at 37°C and 10% CO2.

Binding Assay—BHK cells were grown to confluence in DME medium supplemented with 10% fetal bovine serum. The monolayer was washed twice with phosphate-buffered saline (PBS) and once with Krebs-Ringer phosphate-Hepes binding buffer (pH 7.5). Binding was carried out in 1 ml of binding buffer with 0.2 ng/ml 125I-IGF-I, with or without 500 ng/ml unlabeled IGF-I or des-IGF-I. After incubation at 12°C for 3 h, the monolayer was washed with ice-cold PBS three times and solubilized in 0.4 N NaOH. Radioactivity was then determined.

Affinity Cross-linking—BHK cells were incubated with 1 ng/ml 125I-IGF-I as described above. After 3 h at 12°C, the cells were washed three times with ice-cold PBS and overlaid with 1 ml of bovine serum albumin-free Krebs-Ringer phosphate-Hepes binding buffer. Disuccinimidyl suberate was added to a final concentration of 0.1 μM and
incubated at 4 °C for 20 min. One ml of quench buffer, 0.1 M Tris, 1 mM EDTA (pH 7.5), was added to inactivate DSS, and the cells were incubated at 4 °C for 10 min. Finally, the quench buffer was removed and cells were washed once with fresh quench buffer, followed by solubilization in Laemmli’s sample buffer and analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Western Ligand Blot—The procedures described by Hossenlopp et al. (19) were followed with minor modification. To analyze the cell-associated IGFBPs, BHK cell monolayers were washed with PBS two times and solubilized in Laemmli’s sample buffer without reducing agents. To analyze the secreted IGFBPs, 1 ml of conditioned medium was adjusted to a concentration of 7% trichloroacetic acid and 0.02% bovine serum albumin. The mixture was incubated at 4 °C overnight before the precipitated proteins were pelleted by centrifugation. The pellets were washed twice with cold PBS and redissolved in 200 μl of Laemmli’s sample buffer without reducing agents. Samples were heated at 60 °C for 10 min and subjected to SDS-PAGE analysis. Proteins separated on the gel were transferred to nitrocellulose filters and probed with 125I-IGF-I (106 cpm) for 3 h at 12 °C or overnight at 4 °C. The filter was washed three times with Tris-buffered saline (pH 7.5), air-dried, and exposed to x-ray film.

Serum Starvation—2.0 × 105 BHK cells were seeded in each well (35 mm) of 6-well plates. After 24 to 36 h of growth in DME medium supplemented with 10% fetal bovine serum, the medium was replaced with DME medium without serum (serum-free medium). The cells were further incubated for another 12 to 24 h before they were studied.

RESULTS

Cell Surface IGF-I Receptors and IGFBPs—During standard cell surface binding assays, greater than 95% of the total cell-associated 125I-IGF-I could be displaced by unlabeled native IGF-I. On the other hand, des-IGF-I, a derivative of IGF-I that binds with normal affinity to the IGF-I receptor but very poorly to IGFBPs, could displace 42 ± 4% (n = 4) of the cell-associated 125I-IGF-I. This value is higher than the des-IGF-I resistant binding of other cell lines which is approximately 5%, suggesting that a large portion of the surface IGF-I binding to BHK cells involves cell-associated IGFBPs. Affinity cross-linking of 125I-IGF-I to the cells reveals two components of cell surface IGF-I binding. One component is the IGF-I receptor as visualized by the 135-kDa α subunit on SDS-PAGE (Fig. 1, lane 1). The other components involve cell-associated IGFBPs indicated by bands at 31, 37, and 41 kDa (Fig. 1, lane 1). Cross-linking to the 135-kDa α subunit could be completely inhibited by IGF-I and des-IGF-I, as seen in Fig. 1, lanes 2 and 4, whereas insulin only partially inhibited this band (Fig. 1, lane 3). These results confirm that the 135-kDa band represents the α subunit of the IGF-I receptor. In contrast, the bands in the 31–41-kDa range were inhibited by native IGF-I (Fig. 1, lane 2), but not by des-IGF-I or insulin (Fig. 1, lanes 4 and 3), consistent with the conclusion that these bands represent IGFBPs.

Effect of Serum Starvation on IGFBPs—Fig. 2a shows that IGF-I binding competition curves have a comparable shape in fed and starved cells with ED50 values of 6.5 and 3.8 ng/ml, respectively. However, the serum-starved cells display much higher total binding compared to fed cells (Fig. 2a). When cells were serum-starved for 12 h, both the total surface binding and the des-IGF-I resistant binding were markedly increased (4.4 ± 0.6- and 5.9 ± 1.3-fold, respectively) compared to fed cells (Fig. 2b, lanes 1 and 2 compared to 4 and 5). This indicated that the amount of surface IGFBPs, as represented by des-IGF-I resistant binding, was strikingly increased with serum starvation. IGF-I receptor binding, represented by the difference between total and des-IGF-I resistant binding, increased 2.2 ± 1.1-fold (n = 7). A similar increase in receptor binding was also observed in NIH/3T3 and CHO cells (data not shown). Since the IGFBP increased dramatically, whereas the increase in IGF-I receptor is only modest, the ratio of IGFBP to IGF-I receptor is increased to approximately 4:1.

To further confirm and explore these observations, the cell surface binding sites were analyzed by affinity cross-linking and Western ligand blot techniques. As seen in Fig. 3, cross-linking studies revealed that IGFBPs were increased 5.3 ± 0.9-fold (n = 4) following 12 h of serum starvation, whereas cross-linking to the IGF-I receptor was increased 2.0 ± 0.4-fold (n = 5). This is consistent with the 2.2 ± 1.1-fold increase predicted by the binding assay. Using the Western ligand blot method (Fig. 4), only two species of cell-associated IGFBPs were observed at molecular weights of about 30,000 and 25,000 (IGFBP-30K and IGFBP-25K) (Fig. 4, lane 1). IGFBP-30K is localized to the cell surface, as evidenced by the fact that about 80% of IGFBP-30K was trypsin-sensitive, whereas IGFBP-25K was not affected by trypsin treatment. 20% of the IGFBP-30K is resistant to trypsin even after prolonged treatment (20 min), suggesting a small portion of IGFBP-30K is localized intracellularly. IGFBP-30K and the 3 IGFBPs identified by cross-linking (M, = 31,000, 37,000, and 41,000, Fig. 1) most likely represent the same protein species, since they share the same features regarding responses to serum starvation, IGF-I, des-IGF-I, and insulin treatment. The size heterogeneity in Fig. 1 may represent an artifact of the cross-linking procedure. Therefore, for purposes of this discussion, all the cell surface-associated IGFBPs are assumed to represent a single species termed IGFBP-30K. According to Western ligand blot studies, the amount of cellular IGFBP-25K was not influenced by serum starvation, whereas the cell surface-associated IGFBP-30K was enhanced by 4.9 ± 1.8-fold (n = 3). Thus, results of cross-linking and Western ligand blot studies confirmed our observations based on binding studies.

Time course studies (Fig. 5) revealed the induction of IGFBP-30K was evident at 4 h of starvation (1.7 ± 0.5-fold, n = 4) and proceeded in a relatively linear fashion through 12 h.

During serum starvation, the morphological appearance of cells was strikingly altered. The cells changed from a rounded and condensed shape to a flatter, more spindle-shaped appearance (Fig. 6). Based on this, it is likely that the cell surface area was increased after starvation, raising the possibility that the starvation-induced increase in IGFBP-30K at
Binding assays were performed on cell monolayers with (lanes 1-3) or without (lanes 4-6) serum starvation. Data represent the mean ± S.E.M. of four separate experiments. Lanes 1 and 4, 125I-IGF-I tracer only; lanes 2 and 5, 125I-IGF-I plus 500 ng/ml unlabeled des-IGF-I; lanes 3 and 6, 125I-IGF-I plus 500 ng/ml unlabeled IGF-I.

Fig. 3. Affinity cross-linking and fed and starved cells. Monolayers of BHK cells, with or without serum starvation, were cross-linked with 125I-IGF-I to identify surface binding sites. Cross-linked samples were fractionated on 8% SDS-PAGE. Lane 1 represents data from fed cells and lane 2 from 12-h serum-starved cells. Lanes were loaded with equal amounts of cell extracts. Arrows at 135 kDa and 31-41 kDa indicate IGF-I receptor α subunit and IGFBPs, respectively. The relative counts/min of the IGFBPs is displayed in the bar graph. Data represent the mean ± S.E.M.

Fig. 4. Western ligand blot of IGFBPs. Western ligand blot was performed in fed (lane 1), serum-starved (lane 2), and trypsin-treated starved cells (lane 3). Cell-associated IGFBP-30K and IGFBP-25K were identified by Western ligand blotting. Trypsin treatment was used to identify the IGFBPs located at the cell surface. The cell surface is a result of redistribution of this protein to the cell surface. However, this seems unlikely since the Western ligand blot studies demonstrated a net increase of total cellular IGFBP-30K. To address this possibility, cycloheximide was used to determine whether IGFBP induction was dependent on ongoing protein synthesis. As seen in Fig. 7, the starvation-induced increase in IGFBP was completely inhibited by 50 µg/ml cycloheximide. These results indicated that the induction of IGFBP-30K was most likely due to new protein synthesis rather than redistribution of IGFBPs from an occult site to a cell surface location. Concomitantly, the starvation-induced increase in IGF-I receptor binding was also inhibited by cycloheximide (Fig. 7, lane 2). This inhibitory effect of cycloheximide was also confirmed by binding studies (data not shown).

Effect of Insulin and IGF-I on IGFBP-30K—Inclusion of 500 ng/ml IGF-I in the medium inhibits the starvation-induced increase in IGFBP-30K by 75% (Fig. 8, lane 3). Interestingly, des-IGF-I, which binds fully to the IGF-I receptor, also inhibits IGFBP-30K induction but by only 50% (lane 4). Induction is inhibited 38% by insulin. As seen in Fig. 9, the inhibitory effect of IGF-I is dose-responsive, with about 25% inhibition seen at an IGF-I concentration as low as 1 ng/ml. IGF-I receptors are increased after serum starvation, but down-regulated at increasing IGF-I concentrations (Fig. 9) in a dose-responsive fashion. To confirm that the IGF-I-induced decrease in IGFBP-30K and the IGF-I receptor is
Fig. 5. Time course of IGFBP-30K induction. Cells were serum-starved for various lengths of time before cross-linking to $^{125}$I-IGF-I. Lanes 1, 2, 3, and 4 were serum-starved for 0, 4, 8, and 12 h, respectively. The relative intensity of the 30-kd bands is displayed in bar graph form.

Fig. 6. Morphology of fed (a) and serum-starved (b) cells.

Fig. 7. Induction of IGFBP-30K is inhibited by cycloheximide. Cells were serum-starved in the presence or absence of 50 µg/ml cycloheximide. The cells were cross-linked to $^{125}$I-IGF-I and analyzed on 8% SDS-PAGE. The induction of IGFBP was completely inhibited by cycloheximide. Lane 1, fed; lane 2, serum-starved in the presence of cycloheximide; lane 3, serum-starved for 12 h. Relative counts/min of IGFBPs is displayed in the bar graph.

not due simply to competition by residual IGF-I not removed during the washing procedures, a high concentration of IGF-I was added to cells followed 5 min later by washing and cross-linking. This acute treatment by IGF-I did not affect the level of either IGFBP-30K or the IGF-I receptor (data not shown), indicating that the observed down-regulation was not caused by residual IGF-I. Furthermore, studies using the Western ligand blot technique also show that IGFBP-30K is down-regulated by IGF-I in a dose-responsive way (data not shown), and this technique would not be influenced by residual IGF-I. The dose-response study of insulin indicated that it was effective only at higher concentrations suggesting insulin might act through IGF-I receptor. The concentration of cell-associated IGFBP-25K is not significantly affected by serum starvation or any of these hormones (Fig. 8).

Secreted IGFBP—IGFBPs with molecular weights of about 30,000 and 25,000, termed IGFBP-30K-M and IGFBP-25K-M, can be detected in the conditioned media from BHK cells. Interestingly, both IGF-I and des-IGF-I stimulate the secretion of IGFBP-30K into the medium (6.8 ± 0.5- and 3.8 ± 0.4-fold, respectively), although the effect of des-IGF-I is not as great as that of native IGF-I (Fig. 10a). Thus, IGF-I and des-IGF-I inhibit the starvation-induced increase in cell-associated IGFBP-30K, whereas they stimulate the appearance of IGFBP-30K-M in the medium. In contrast, a divergent effect is seen with regard to insulin. Thus, insulin partially inhibits the starvation-induced increase in surface IGFBP-30K (Fig. 8), whereas it also markedly inhibits the appearance of IGFBP-30K-M in the medium in a dose-responsive manner with a maximal inhibitory effect of ~54% (Fig. 10b). At this point, it is not clear whether IGFBP-30K-M is simply the secreted form of IGFBP-30K or whether this is a different
species altogether. In this regard, Fig. 11 demonstrates that IGF-I and des-IGF-I do not simply promote the dissociation, or release of cell surface IGFBP-30K, so that it appears in the medium as IGFBP-30K-M. Thus, treating serum-starved cells acutely with 500 ng/ml IGF-I for 10 min did not lead to any increase in IGFBP-30K in the medium. Similarly, cell surface IGFBP-30K was not significantly reduced after 12 h of serum starvation followed by prolonged IGF-I treatment (data not shown). In addition, as shown in Fig. 10a, des-IGF-I, which presumably only binds to the IGF-I receptor and not IGFBPs, also increases IGFBP-30K-M appearance into the medium. Taken together, these results argue against the possibility that increases in IGFBP-30K-M are due to simple IGF-I-mediated dissociation of IGFBP-30K from the cell surface.

IGFBP-25K appears to be synthesized and secreted into the medium at a constitutive rate (Fig. 10, a and b), and cells maintain a relatively constant intracellular pool of this protein (Fig. 4). Furthermore, the secretion of this IGFBP is not affected by serum starvation, IGF-I, des-IGF-I, or insulin.

**DISCUSSION**

In the current studies, we have found that the majority of IGF-I binding to BHK cells is due to cell surface IGFBPs. Specifically, greater than 95% of the $^{125}$I-IGF-I binding can be inhibited by saturating concentrations of native IGF-I, whereas only about 45% of the binding is inhibited by des-IGF-I, an analog with normal affinity for the IGF-I receptor and very poor affinity for IGFBPs. With this approach, the des-IGF-I-inhibitable $^{125}$I-IGF-I binding component is taken as an estimate of receptor binding, and the des-IGF-I-resistant component is attributed to IGFBPs. This analysis agrees quite well with $^{125}$I-IGF-I cross-linking studies which showed that about 40% of the IGF-I was cross-linked to the IGF-I receptor and about 60% to the IGFBPs. IGF-I binding studies demonstrated that total surface binding and des-IGF-I-resistant binding are both markedly increased (5-fold) by serum starvation. Additionally, binding to the IGF-I receptor is increased about 2-fold. These observations were confirmed by affinity cross-linking and Western ligand blot studies.

The induction of IGFBP-30K is rapid and is evident after 4 h of serum starvation. The increase in IGFBP-30K and the receptor are dependent on protein synthesis, since treatment with cycloheximide prevents the induction of both proteins. The starvation-mediated increase in IGFBP-30K is inhibited by IGF-I even at concentrations as low as 1 ng/ml. Insulin can also inhibit the induction, but to a much lesser degree compared to IGF-I; i.e. 35% inhibition is achieved by 500 ng/ml insulin, suggesting that insulin might be acting, at least in part, through IGF-I receptors. These observations indicate that induction of IGFBP-30K by serum starvation is probably due to deprivation of IGF-I.

Contrary to IGFBP-30K, secretion of IGFBP-30K-M is increased by both IGF-I and des-IGF-I, 6.8- and 3.8-fold, respectively. The results indicate that IGFBP-30K and IGFBP-30K-M are differentially regulated by IGF-I. When the IGF-I concentration is low, the level of IGFBP-30K-M in the medium is decreased. When the IGF-I concentration is low, the level of IGFBP-30K-M in the medium is decreased.
cells were serum-starved in the presence of IGF-I, des-IGF-I, or insulin. Conditioned medium was collected after 12 h. Secreted IGFBPs were precipitated with 7% trichloroacetic acid and analyzed by Western ligand blotting. a: lane 1, serum-starved; lane 2, starved plus 100 ng/ml IGF-I; lane 3, starved plus 100 ng/ml des-IGF-I. b: lanes 1 and 2, starved; lanes 3 and 4, starved plus 1 ng/ml insulin; lanes 5 and 6, starved plus 10 ng/ml insulin; lanes 7 and 8, starved plus 100 ng/ml insulin; lanes 9 and 10, starved plus 500 ng/ml insulin. Sample equivalent to 200 μl of conditioned medium was loaded in each lane. The relative counts/min of the 30-kDa band is displayed in the bar graph.

**Fig. 10. Effect of IGF-I and insulin on secretion of IGFBPs.** Cells were serum-starved in the presence of IGF-I, des-IGF-I, or insulin. Conditioned medium was collected after 12 h. Secreted IGFBPs were precipitated with 7% trichloroacetic acid and analyzed by Western ligand blotting. a: lane 1, serum-starved; lane 2, starved plus 100 ng/ml IGF-I; lane 3, starved plus 100 ng/ml des-IGF-I. b: lanes 1 and 2, starved; lanes 3 and 4, starved plus 1 ng/ml insulin; lanes 5 and 6, starved plus 10 ng/ml insulin; lanes 7 and 8, starved plus 100 ng/ml insulin; lanes 9 and 10, starved plus 500 ng/ml insulin. Sample equivalent to 200 μl of conditioned medium was loaded in each lane. The relative counts/min of the 30-kDa band is displayed in the bar graph.

**Fig. 11. Acute treatment with IGF-I.** Following serum starvation, cells were treated with 500 ng/ml IGF-I for 10 min before cells were analyzed with Western ligand blotting. Binding to the IGFBP-30K was not significantly reduced by the treatment. Lane 1, serum-starved; lane 2, serum-starved and treated with IGF-I for 10 min.

High, the level of IGFBP-30K on the surface is decreased and IGFBP-30K-M in the medium is increased. This would represent an integrated system to regulate the availability of IGF-I to cells and, therefore, regulate the biologic effects of IGF-I. When IGF-I levels are high, secreted IGFBP is increased, reducing the availability of the free ligand to the cell surface; low IGF-I levels decrease secreted IGFBP, enhancing ligand availability. We also speculate that at low IGF-I levels surface IGFBP-30K is induced to increase the IGF-I concentration available to the IGF-I receptor by reversibly binding IGF-I at the cell membrane. Alternatively, IGFBP-30K could bind IGF-I and in some way transfer the ligand to the IGF-I receptor, or perhaps occupied IGFBP-30K can participate in signal transduction directly or by modulating IGF-I receptor function. This latter speculation is supported by our findings that IGF-I is more effective than des-IGF-I in decreasing the IGFBP-30K level and increasing the IGFBP-30K-M level. Thus, regulation of IGFBP-30K represents a biologic effect of IGF-I. Since native IGF-I binds to receptors and IGFBPs, whereas des-IGF-I binds primarily to receptors, the greater effect of native IGF-I would indicate that binding to IGFBPs in some way enhances this biologic effect. These ideas are in agreement with previous reports demonstrating that membrane-associated IGFBPs can enhance IGF-I action and IGFBPs in the medium can inhibit IGF-I action.

It is interesting to note that insulin inhibits IGFBP-30K-M secretion, and this is opposite to the effect of IGF-I. The inhibitory effect of insulin is seen at concentrations as low as 1 ng/ml. These results argue that this effect (inhibition of IGFBP-30K-M secretion) is probably mediated through insulin receptors. The identities of the IGFBP-30K and IGFBP-30K-M are unknown. It is possible that IGFBP-30K-M is the secreted form of IGFBP-30K, and its secretion is induced by IGF-I. However, the following evidence suggests this is not the case: 1) cycloheximide can completely inhibit the induction, indicating the induced IGFBP is newly synthesized, 2) the Western ligand blot studies, which measure total IGFBPs, show a net increase of IGFBP after induction, and 3) in the presence of IGF-I, the induction is completely blocked despite the morphological change of the cells.

It is interesting to note that cell morphology is markedly altered after serum starvation. Thus, cells become flatter and more spindleshaped during starvation (Fig. 6), and this leads to an increase in surface area. This raises the possibility that the actual membrane concentration of IGFBP-30K might remain relatively constant, despite the large increase in IGFBP-30K per cell.

The IGF-I receptor appears to be up-regulated when IGF-I is removed during serum starvation and is down-regulated when IGF-I is included. This is the typical response of the IGF-I receptor to its ligand and is another way to modulate the cellular response to IGF-I. In theory, increased IGF-I receptor binding could be due to increased receptor number or affinity. IGF-I binding competition studies using native IGF-I (Fig. 2a) and des-IGF-I (data not shown) showed that the ED50 was not changed after serum starvation, despite the large increase in total binding, indicating that the increased receptor binding was not due to an increase in affinity. It is also possible that increased receptor binding is due to increased IGFBP on the cell surface. However, this is unlikely, since IGF-I binding is also increased in NIH/3T3 cells after serum starvation, even though there is little or no IGFBP on the cell surface (data not shown).

In summary, at high IGF-I concentrations, the IGF-I receptor is down-regulated, cell-associated IGFBP-30K is down-regulated, and secreted IGFBP-30K-M is increased. As a result, cells would be less sensitive to IGF-I. When IGF-I concentration is low, the receptor is up-regulated, the cell-associated IGFBP-30K is up-regulated, and the secreted IGFBP-30K-M is decreased. Thus, cells are more sensitive to IGF-I stimulation. Since IGF-I was more effective than des-IGF-I, the portion of IGF-I which binds to IGFBP may lead.
to biologic signalling directly by the IGFBP or indirectly through IGF-I receptors.

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