EXPERIMENTAL STUDY

Continuous s.c. infusion rather than twice-daily injections of IGF-I more effectively increases serum IGF binding protein-3 in female monkeys

M E Wilson and S L Lackey
Yerkes Primate Research Center, Emory University, 2409 Taylor Lane, Lawrenceville, GA 30043, USA
(Correspondence should be addressed to M E Wilson, Yerkes Primate Research Center of Emory University, Field Station, 2409 Taylor Lane, Lawrenceville, Georgia 30043, USA; Email: markw@rmy.emory.edu)

Abstract

Objective: In order to better understand how the IGF-I axis is affected by exogenous IGF-I, this study compared the effects of a constant s.c. infusion of IGF-I with that of twice-daily injections of IGF-I in young adult female rhesus monkeys. Clinical studies suggest that circulating concentrations of insulin-like growth factor binding protein-3 (IGFBP-3) are decreased or unaffected by IGF-I administration, whereas acute increases in IGF-I may increase serum IGFBP-1. However, studies in monkeys indicate that acute or continuous infusion of IGF-I effectively increases serum IGFBP-3.

Design and methods: Female monkeys were studied for 5 days with no IGF-I supplementation (baseline) and for 5 days of IGF-I treatment by either constant infusion (120 μg/kg per day s.c., n = 5) or twice-daily injections of IGF-I (60 μg/kg per injection s.c., n = 5). Serum samples were collected daily at 0800 h and at 0800, 0900, 1100, 1500, and 2000 h on days 1 and 4 for each condition. Samples were assayed for IGF-I, IGFBPs-1 and -3, insulin, and glucose.

Results: Serum IGF-I was consistently increased above baseline within 24 h of the initiation of constant infusion, but was delayed until the second day of treatment in the injection group. Serum IGFBP-3 followed the pattern of IGF-I, with concentrations increased by day 1 during constant infusion and by day 2 during intermittent injections. Although both treatments effectively increased serum IGFBP-3, the increase was greater during constant infusion (31% above baseline) compared with injection (17%). Immunoblotting revealed that the constant infusion of IGF-I resulted in quantitatively more lower-molecular-mass fragments of IGFBP-3 than were observed during baseline or intermittent injections. Size-exclusion chromatography and ultrafiltration indicated that most IGFBP-3 was found in the ternary complex, with a greater percentage found in the ternary complex during baseline (90%) than during constant infusion (86%) or intermittent injections of IGF-I (87%). In contrast, serum concentrations of IGFBP-1 were increased on day 1 of both treatments, but declined towards baseline values as treatment progressed. Serum concentrations of insulin and glucose were unaffected by either mode of IGF-I treatment.

Serum concentrations of IGF-I and IGFBP-3 were increased within 3 h of the injection, before declining towards the pre-injection level. In contrast, the daily pattern of serum hormone concentrations was similar between the baseline condition and during constant infusion of IGF-I. Although higher during the treatment phase, serum IGF-I and IGFBP-3 concentrations decreased significantly from 0800 h until the afternoon meal, reaching a nadir in the evening before increasing again the next morning. Serum insulin decreased also after the morning meal and increased significantly immediately after the afternoon meal. Although serum IGFBP-1 also decreased initially after the morning meal, concentrations reached a peak before the afternoon meal as serum insulin reached its nadir.

Conclusion: The results of the present analysis indicate that the constant infusion of IGF-I more effectively sustains serum concentrations of IGF-I and IGFBP-3 than do twice-daily injections. Although the percentage of IGF-I and IGFBP-3 in the ternary complex was similar during both treatments, the constant infusion regimen produced lower-molecular-mass fragments of IGFBP-3. In addition, serum IGF-I and IGFBP-3 appeared to be regulated diurnally, even during IGF-I infusion, whereas IGFBP-1 and insulin were affected by the timing of food intake. Taken together, these data suggest that, in the monkey, IGFBP-3 is regulated by factors in addition to GH, and that IGF-I can affect its own bioavailability by increasing circulating concentrations of IGFBP-3.
Introduction

Insulin-like growth factor (IGF)-I is not stored in granules but, like steroid hormones, circulates bound to proteins, a number of which have been identified and found to bind IGF-I with variable affinity (1). The majority of IGF-I circulates bound to IGF-binding protein (IGFBP)-3, which subsequently binds to an acid-labile subunit (ALS) to form the 150 kDa ternary complex (2). IGFBP-3 also has a broad tissue distribution but hepatic secretion determines its circulating concentrations (1). As for IGF-I, the synthesis and release of IGFBP-3 are dependent upon GH (3–6). However, IGF-I stimulates IGFBP-3 mRNA (6, 7) and secretion (8) and decreases IGFBP-3 mRNA degradation in rats (9). Although evidence of an increase in serum IGFBP-3 during IGF-I treatment of patients with GH receptor deficiency, obtained from Western ligand blotting (10) and immunoenzyme assay (11, 12), has been presented, reports typically state that twice-daily injections of IGF-I do not increase serum IGFBP-3 (13–16).

Indeed, repeated daily s.c. (17) or i.v. (18) injections of IGF-I to normal adults suppresses serum concentrations of IGFBP-3. Conversely, serum IGFBP-3 was increased in children receiving an s.c. infusion of IGF-I for 10 h on 3 consecutive days (19). Recently, it was suggested that some of these inconsistencies may be due to the small number of subjects studied at different ages, differences in treatment regimens, and different methods of analysis for IGFBP-3 (20). In contrast to these data from humans, observations in monkeys have shown that a constant s.c. infusion of IGF-I increases serum IGFBP-3 in adolescent (21) and adult females (22), and acute IGF-I produces a brief but significant increase in serum IGFBP-3 (21). IGFBP-3 binds IGF-I to form the binary complex and, in the presence of an ALS, forms the ternary complex that increases the half-life of IGF-I and acts as a slow-release reservoir for IGF-I to interact with its receptor (1). Consequently, an increase in serum IGFBP-3 by IGF-I may occur only if IGF-I is present in large concentrations, resulting in the formation of IGF-I–IGFBP-3 complexes and slowing the degradation of IGFBP-3. In contrast, IGFBP-1 is believed to increase when IGF-I is acutely increased (1), in order to block the hypoglycemic effects of IGF-I (23), particularly when insulin secretion is low (24). Although produced primarily in the reproductive tract and liver (25), serum IGFBP-1 is inversely related to insulin secretion (26), and any effects of IGF-I on serum IGFBP-1 may be due to IGF-I-induced reductions in insulin secretion (17). However, the differential effects of continuous versus intermittent IGF-I administration on insulin–IGFBP-1 dynamics have not been investigated. Furthermore, if a particular mode of IGF-I administration more effectively increases IGFBP-3, thereby diminishing the acute, hypoglycemic effects of IGF-I, increases in serum IGFBP-1 may be inversely related to changes in serum IGFBP-3.

In order to define more fully how IGF-I affects the serum patterns of IGFBPs-1 and -3, the mode of IGF-I treatment was evaluated in young adult, female rhesus monkeys. We tested the hypothesis that a continuous rather than an intermittent IGF-I treatment regimen would be more efficacious at elevating serum IGFBP-3. Conversely, we predicted that an intermittent mode of IGF-I administration would more effectively decrease serum insulin and, consequently, increase serum IGFBP-1.

Materials and methods

Subjects were young adult, female rhesus monkeys (50 months of age; Macaca mulatta) born and raised at the Yerkes Primate Research Center. Females were housed indoors in pairs or small groups under a 12-h light:12-h darkness photoperiod and constant temperature (22°C) as described previously (27). All procedures were approved by the Emory University Institutional Animal Care and Use Committee in accordance with USDA and NIH policy and standards.

All the monkeys used in these studies had been previously ovariectomized. Studies were initiated after they had been acclimated to the sampling procedures, as described previously (28), to enable the collection of blood samples from unanesthetized subjects (29). The monkeys were randomly assigned to one of two groups (n = 5 each) and were studied during a 5-day baseline phase (no IGF-I treatment) and a 5-day treatment phase of either continuous s.c. infusion or twice-daily s.c. injections of IGF-I: a 2-week washout period separated the two phases. Intermittent treatment was achieved by the twice-daily injection (0800 and 2000 h) of 60 μg/kg s.c. IGF-I (Genentech Inc., South San Francisco, CA, USA), yielding a daily total of 120 μg/kg; this dose is in the range that effectively stimulates growth in GH-receptor-deficient children (30). Continuous treatment was achieved by infusing, s.c., 120 μg/kg per day IGF-I (Genentech Inc.) with an osmotic mini-pump (2 ML1, Alza Corp., Palo Alto, CA, USA), implanted s.c. between the scapulae while the animals were anesthetized. This dose has been shown to consistently increase serum IGF-I and IGFBP-3 to 80% and 25% respectively above values in untreated monkeys (21). Serum samples were collected daily at 0800 h, which, on day 1 of treatment occurred 12 h after the initial IGF-I injection and 18 h after the pumps were implanted. In addition, on days 1 and 4, samples were collected at 0800, 0900, 1100, 1500, and 2000 h. Animals were fed commercial monkey chow (Harlan Monkey Diet, Madison, WI, USA) at 0700 and 1530 h. In addition, animals were fed fresh fruit daily at the 1530 h feeding. Thus the initial morning sample was obtained 1 h after the morning meal, whereas the early afternoon sample was obtained before the afternoon meal. Water was available at all times. This design enabled us to assess whether continuous s.c. infusion produced results
different from those obtained with repeated twice-daily injections.

**Assays**

IGF-I was determined by a previously validated RIA in which the IGFBPs are neutralized with acid–glycine (31). The assay uses rhIGF-I (Peninsula Labs, Belmont, CA, USA) as the reference and the iodinated ligand and a polyclonal IGF-I antibody (National Hormone and Pituitary Program). The assay has a sensitivity of 10 nmol/l (using a molecular mass of 7.5 kDa) with inter- and intra-assay coefficients of variation (CV) of 6.9% (n= 7) and < 5% respectively. IGFBP-1 was determined with a commercially available IRMA (Diagnostic Systems Laboratory (DSL), Webster, TX, USA). The assay has a sensitivity of 0.01 nmol/l (using a molecular mass of 25.5 kDa) and inter- and intra-assay CV of 11% (n = 7) and < 5% respectively. IGFBP-3 was determined with a commercially available IRMA (DSL). The assay has a sensitivity of 6 nmol/l (using a molecular mass core of 29 kDa) and inter- and intra-assay CV of 18% (n = 7) and < 5% respectively. Insulin was determined with a commercially available RIA (Diagnostic Products Corporation, Los Angeles, CA, USA) having a sensitivity of 2.5 IU/l and inter- and intra-assay CV of 14% (n = 20) and < 5% respectively. Serum glucose was determined enzymatically using a colorimetric assay (Sigma, St Louis, MO, USA). All the samples were assayed in the same run for each of the hormones.

Because the IRMA for IGFBP-3 detects the intact molecule and fragments of IGFBP-3, serum IGFBP-3 was also subjected to electrophoresis, and immunodetection followed procedures previously described (32). Briefly, sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed on serum samples (3 ml diluted 1:2 in Laemmli buffer and heated at 95°C for 4 min) under non-reducing conditions on 12% gels at 100 V until the dye ran off the gel (Ready Gel Cell, BioRad, Hercules, CA, USA). Biotin-labeled molecular mass standards (BioRad) were run with each gel. After electrophoresis, gels were soaked (3 × 5 min) in transfer buffer (48 mmol/l Tris, 39 mmol/l glycine and 20% methanol, pH 9.2). Size-separated proteins were electrotransferred to nitrocellulose membranes (Hyperbond, Amersham Life Sciences, Arlington Heights, IL, USA) with 15 V for 1 h (Mini Trans-Blot Semi-dry Electrophoretic Transfer Cell, BioRad). After the membranes had been allowed to air dry, non-specific binding sites were blocked by incubating the membranes in blocking buffer (TBS-T: 20 mmol/l Tris–HCl, 137 mmol/l NaCl and 0.1% Tween-20 with 3% BSA) for 1 h, with gentle agitation. Membranes were probed with a polyclonal biotin-labeled IGFBP-3 antisera in TBS-T with 3% BSA (1: 20 000; DLS) overnight at 4°C. Membranes were again washed (4 × 5 min, 1 × 15 min with agitation), followed by incubation with a streptavidin–horseradish peroxidase solution in TBS-T with 3% BSA (1:1500; Amersham Life Sciences) for 1 h, with gentle agitation. After washing in TBS-T (3 × 15 min), bands were visualized by exposing the membranes to equal volumes of enhanced chemiluminescence detection reagents 1 and 2 (Amersham Life Sciences), placing the membranes on imaging film (X-OMAT LS, Kodak), and developing the film for periods of from 30 s to 5 min. Photographs of the gel films were scanned (Afga ArtLine) into a Macintosh computer and the resulting images were digitized using software (Silk Scientific; Orem, UT, USA) that determines band density, migration distances, and molecular mass of each band based on molecular mass standards.

Size-exclusion chromatography was carried out with HPLC (Biologic HR System, BioRad) using a Bio-Prep SE 1000/17 column (BioRad). The elution buffer was a 0.05 mol/l sodium phosphate buffer with 0.15 mol/l NaCl (pH 7.4). The column was calibrated by eluting molecular mass markers (range 1.35–670 kDa) before each run. Samples collected on day 5 from each subject were pooled for each of the baseline, pump, and injection phase and were injected (0.040 ml on to the column. Fractions were collected (0.1 ml) and were assayed for IGFBP-3 as described above. These same pools were also subjected to size-exclusion ultrafiltration, following the procedure described previously (33). Pools were diluted 1:4 in 0.05 mol/l sodium phosphate buffer and applied to a unit with a 100 kDa cut off (Centricon-100, Amicon, Inc, Beverly, MA, USA) and centrifuged at 1000 g for 30 min. The retentate, containing the ternary complexes, and the filtrate, containing the binary complexes, were assayed for IGFBP-3 as described above.

**Analyses**

Data were expressed as means ± S.E.M. for each treatment condition. Answers to two questions were being sought: 1) do intermittent injections or continuous infusions change the pattern of hormone secretion compared with baseline, and 2) is one mode of treatment more efficacious than another for inducing these changes? Consequently, data were analyzed with analysis of variance models for repeated measures. Contrasts to identify significant main or interactional effects of the categorical or repeated variable were made using post hoc contrasts in which each mean was compared with the other means in the series (SPSS, Version 6 · 1; Chicago, IL, USA). In order to control for differences in baseline hormone secretion, data were converted to percent change from that time point during baseline. Linear relationships among variables were evaluated with simple and stepwise multiple regression. All statistical tests for which P<0.05 were considered significant.
Results

**Basal hormone secretion**

The mode of IGF-I administration had a significant effect on serum concentrations of IGF-I and IGFBP-3 (Fig. 1). Before treatment, IGF-I concentrations were similar in the two groups ($F_{1,8} = 1.65$) across the 5-day baseline period ($F_{4,32} = 0.95$). Although treatment with IGF-I produced an overall significant increase in serum IGF-I for both groups ($F_{1,8} = 15.82$), the absolute increase in serum IGF-I concentrations were not significantly different between the injection and the pump groups ($F_{1,8} = 4.12$). However, when data were expressed as percent change from baseline, the increase in serum IGF-I was significantly greater throughout the 5-day treatment period for the pump compared with the injection group ($F_{1,8} = 5.23$). For both groups, the percent increase was greater on treatments days 2–5 compared with day 1 ($F_{4,32} = 4.25$). Serum IGFBP-3 concentrations were also similar between groups before treatment ($F_{1,8} = 1.02$; Fig. 1). As can be seen, both injection and pump infusion of IGF-I significantly increased serum IGFBP-3 overall compared with baseline values ($F_{1,8} = 58.31$). However, when expressed as percent change from baseline, serum IGFBP-3 was significantly increased in the pump group compared with the injection group ($F_{1,8} = 10.02$). As observed with IGF-I, the percent increase in IGFBP-3 was greater during treatment days 2–5 than during day 1 ($F_{4,32} = 16.43$).

Serum concentrations of IGFBP-1 were also similar between the two groups before treatment (Fig. 2; $F_{1,8} = 1.40$). The mode of administration did not differentially affect serum IGFBP-1 concentrations ($F_{1,8} = 0.16$) or the change in serum IGFBP-1 from baseline ($F_{1,8} = 0.68$). However, IGFBP-1 concentrations were significantly increased at the initiation of IGF-I treatment (Fig. 2, $F_{1,8} = 9.25$), with values significantly greater on day 1 of treatment compared with day 5 of baseline and day 5 of treatment (post hoc tests). Serum IGFBP-1 decreased to baseline values by day 4 of treatment in the pump group and day 5 of treatment in the injection group (post hoc tests). Serum insulin concentrations were significantly higher before IGF-I treatment in the monkeys that eventually received pump treatment ($F_{1,8} = 27.70$; Fig. 2). Neither continuous infusion nor intermittent injections affected serum insulin ($F_{1,8} = 0.23$) or the change in insulin from baseline.

![Figure 1](image-url)  
**Figure 1** Serum IGF-I (top panel) and IGFBP-3 (bottom panel) during 5 days of baseline (open bar) and treatment with IGF-I (shaded bar) by either twice-daily injections (○) or constant infusion (pump, ●). The left panels illustrate serum concentrations and the right panels illustrate percent change from baseline (values are means ± S.E.M.). Injections of IGF-I were made 12 h before and immediately after the collection of each sample.
Finally, serum concentrations of glucose were similar during baseline between animals that eventually received IGF-I by injection (3.7 ± 0.2 mmol/l) and those that received the IGF-I by pump infusion (3.8 ± 0.2 mmol/l: $F_{1,8} = 0.26$). Concentrations were also unaffected by the mode of IGF-I treatment (injection: 3.6 ± 0.1 mmol/l; pump: 4.0 ± 0.1 mmol/l; $F_{1,7} = 5.27$).

Regression analysis indicated that serum IGFBP-3 was significantly predicted from serum IGF-I during baseline ($r_8 = 0.88$) and during treatment ($r_8 = 0.82$). Addition of either IGFBP-1 or insulin values to the multiple regression equation did not significantly increase the amount of variance in IGFBP-3 accounted for by IGF-I. Mean serum values of IGF-I were unrelated to serum IGFBP-1 during baseline ($r_8 = 0.25$) or treatment ($r_8 = 0.11$). In addition, the linear relationship between serum insulin and IGFBP-1 was not significant during either baseline ($r_8 = -0.39$) or treatment ($r_8 = -0.38$). In contrast, the inverse relationship between serum IGFBP-1 and IGFBP-3 on day 1 of treatment was significant ($r_8 = -0.67$). However, as IGF-I was significantly correlated with IGFBP-3 on day 1 of treatment ($r_8 = 0.85$), IGF-I significantly predicted the variance in IGFBP-1 ($r = 0.76$), with IGFBP-3 values not contributing to the equation – that is, greater concentrations of IGF-I on day 1 of treatment were associated with lower IGFBP-1 and greater IGFBP-3 concentrations.

Molecular mass distribution of IGFBP-3

Immunodetection after SDS–PAGE and Western blotting revealed that constant s.c. infusion produced a different distribution of molecular mass IGFBP-3s than did intermittent injections. Illustrated in Fig. 3 are results of immunodetection and subsequent image analysis from representative female monkeys during baseline and on the 4th day of treatment. Samples from each phase corresponded to the 0, +1 h, and +3 h bleeds on those days. During baseline periods, there were two distinct bands, at 44 and 31 kDa. During twice-daily injections, the 42 and 31 kDa bands were more prominent than during baseline and a band in the range of 27 kDa was evident. In contrast, during the constant infusion of IGF-I, these bands were not only more prominent, but additional bands were evident, at approximately 21 and 14 kDa.
Size-exclusion chromatography (Fig. 4) revealed that the distribution of complexed IGFBP-3 was similar during baseline (combined for all females), constant infusion and intermittent injections and that most (>85%) IGFBP-3 was found in the ternary complex, regardless of treatment condition. The percentage of IGFBP-3 in chromatographic fractions corresponding to >100 kDa, representing the ternary complex, was compared with fractions <100 kDa representing the binary complex or unbound IGFBP-3 (inset table in Fig. 4). A slight reduction in the percent of IGFBP-3 found in the ternary complex was observed during both modes of IGF-I treatment, compared with baseline. This pattern was also confirmed when the samples were applied to size-exclusion ultrafiltration (see table in Fig. 4).

Daytime hormone patterns
In addition to the single daily samples, more frequent samples were collected on days 2 and 4 during both baseline and treatment. The data for days 2 and 4 have been combined within each phase for illustration (Fig. 5). Analysis of variance revealed that IGF-I ($F_{5,40} = 9.95$), IGFBP-3 ($F_{5,40} = 9.58$), IGFBP-1 ($F_{5,40} = 25.70$), and insulin ($F_{5,40} = 18.17$) varied significantly throughout these 24-h periods. The monkeys were fed 1 h before the first sample obtained each morning (time 0) and again after the sample obtained at +7 h. During baseline, serum IGF-I ($-29 \pm 8\%$), IGFBP-3 ($-8 \pm 1\%$), and insulin ($-59 \pm 7\%$) declined after the morning meal (Fig. 5). IGF-I and IGFBP-3 remained low until +12 h, whereas insulin increased significantly after the afternoon meal. In contrast, serum IGFBP-1 decreased after the morning and afternoon meals, but was significantly increased before the afternoon meal ($80 \pm 20\%$), when insulin was at its nadir.

This pattern of hormone secretion observed throughout the day during constant infusion of IGF-I was similar to that of baseline (Fig. 5). However, the pattern was significantly different in the injection group for IGF-I ($F_{5,40} = 6.71$) and IGFBP-3 ($F_{5,40} = 5.25$) but not for IGFBP-1 ($F_{5,40} = 1.12$) or insulin ($F_{5,40} = 2.25$). This difference was due to the significant increase from time 0 within the 3 h after the IGF-I injection in serum IGF-I ($t_4 = 2.81$) and IGFBP-3 (Fig. 3: $t_4 = 4.86$). Nevertheless, serum concentrations of IGF-I ($-16 \pm 9\%$) and IGFBP-3 ($+4 \pm 1\%$) were also decreased by +12 h relative to time 0 in the injection group, before the next injection. However, it is evident that the intermittent injection of IGF-I did not alter the post-feeding changes in serum IGFBP-1 or insulin.

Discussion
Data from rodents indicate that IGF-I stimulates IGFBP-3 hepatic mRNA (6, 7) and secretion (8) and decreases IGFBP-3 mRNA degradation (9). Although IGF-I increases concentrations of IGFBP-3 in human fibroblast cultures (34), IGF-I replacement therapy given to GH-receptor-deficient patients fails consistently to increase serum IGFBP-3 (13–16). Furthermore, acute s.c. administration of IGF-I to normal humans produces a transitory increase in IGFBP-3, but overall concentrations are reported to be lower than during saline treatment (17). In contrast to these data, an acute IGF-I injection produces a brief yet significant increase in
serum IGFBP-3 in adolescent monkeys (21), whereas the continuous s.c. infusion of IGF-I maintains a significant increase in serum IGFBP-3 in adolescent and adult monkeys (21, 22, 35, 36). In support of these observations, serum IGFBP-3 was increased in children receiving an s.c. infusion of IGF-I for 10 h on 3 consecutive days (19). Consequently, we hypothesized that a continuous infusion, rather than twice-daily injections, of IGF-I would more effectively increase serum IGFBP-3. The results of the present study support this hypothesis, as serum IGFBP-3 was consistently increased above baseline values by the first day of IGF-I infusion. However, unlike the situation observed in GH-receptor-deficient patients receiving twice-daily IGF-I injections (13–16), a sustained increase in serum IGFBP-3 above baseline concentrations was achieved by the second day of twice-daily injections and continued for the remaining 3 days of treatment. Nevertheless, the increase in serum IGFBP-3 was significantly greater during constant infusion compared with that achieved with twice-daily injections. Furthermore, the results of the Western immunobLOTS indicate that the constant s.c. infusion of IGF-I not only results in a more prominent doublet band in the 42–31 kDa range, but also increases the presence of smaller molecular mass, immunoreactive fragments of IGFBP-3. Thus the overall increase in serum IGFBP-3, determined by the IRMA assay, induced by the constant infusion of IGF-I probably reflects the sum total of the immunoreactive IGFBP-3 fragments.

At present, the mechanism by which IGF-I increases serum concentrations of IGFBP-3 in monkeys is open to speculation. The results of the present study indicate that continuous increase in IGF-I more effectively increases serum concentrations of IGFBP-3. The brief, yet significant increase in serum IGFBP-3 after the initial injections of IGF-I was similar to that observed after a single, acute administration of IGF-I in adolescent and adult female monkeys (22). However, with repeated injections, serum IGFBP-3 was significantly increased above baseline values, but not to the extent produced by continuous IGF-I administration. Analysis of the molecular-mass distribution of non-complexed IGFBP-3 indicated that a greater range of fragments are produced during IGF-I treatment, with more lower-molecular-mass species observed during constant infusion. However, confirming data from humans (2), the present results show that most IGFBP-3 is found in the ternary complex during baseline and both modes of IGF-I administration. Furthermore, the size-exclusion chromatography and ultrafiltration analyses suggest a shift in the percentage of total IGFBP-3 from the ternary complex to binary complex or unbound fragments during both modes of IGF-I administration. Although excess unbound ALS circulates in individuals with normal pituitary status (2, 37), enabling the ternary complex to form as IGF-I binds to IGFBP-3, ALS availability may account for the slightly lower estimates of the complex in treated monkeys. Attempts to quantify circulating ALS concentrations have so far proven unsuccessful.

Figure 4 Size-exclusion chromatograph of native pools from baseline (all subjects combined), constant s.c. infusion (Pump), and intermittent injections (Injection). After size-exclusion chromatography, fractions were assayed for IGFBP-3. Molecular masses corresponding to specific fractions are based on molecular mass standards. The inset table illustrates the percentage of IGFBP-3 in fractions corresponding to the ternary complex (TC: >100 kDa) and binary complex (BC: <100 kDa) from the size-exclusion chromatography (SEC) and from size exclusion ultrafiltration (ULF).
Failure of IGF-I to increase serum IGFBP-3 in patients with GH receptor deficiency may be due to a diminution in GH-dependent increases in both the synthesis and release of IGFBP-3 (3–6) and ALS (37). We have found that a single, acute injection of IGF-I produced a brief, yet significant increase in serum IGFBP-3 in female monkeys in which the endogenous GH secretion was blocked by treatment with the somatostatin analog, octreotide, or in which GH activity was antagonized by the GH receptor antagonist, trovert (38). However, we have not yet determined whether continuous infusion of IGF-I can sustain increases in serum IGFBP-3 when GH secretion or action is experimentally compromised.

Furthermore, future studies are required to determine whether this increase in serum IGFBP-3 by IGF-I is a result of an effect on IGFBP-3 biosynthesis, or whether IGF-I perhaps slows the peripheral degradation of IGFBP-3 by forming the binary or ternary complex (20). In support of this view, we have evidence that, unlike IGF-I, the IGF-I variant, Long R^3 IGF-I, which does not readily bind IGFBP-3, does not increase serum concentrations of IGFBP-3 when given acutely or administered by constant s.c. infusion (39). Additional studies must determine whether humans and monkeys are different with respect to the regulation of IGFBP-3 by IGF-I or whether the constant s.c. infusion of IGF-I to normal or GH-receptor-deficient patients can increase serum IGFBP-3. Although the twice-daily injection regimen is clinically effective in stimulating growth in this latter population, treatment strategies that promote the formation of the ternary or, at the least, the binary complex may be more efficacious (2, 40). Despite the statistical difference in serum IGFBP-3 produced by continuous infusion and intermittent injections, it must be emphasized again that serum concentrations were significantly increased above baseline during both modes of treatment.

It has been hypothesized that insulin inversely regulates hepatic production and secretion of IGFBP-1 (24, 38) and that the effects of IGF-I on IGFBP-1 may be mediated through IGF-I-induced decreases in insulin secretion (41). The results of the present study suggest that the relationship between serum IGF-I, insulin, and IGFBP-1 is more complex, as serum insulin was not affected by either mode of IGF-I administration and concentrations of serum IGFBP-1 were not predicted from those of insulin. Both modes of IGF-I administration significantly increased IGFBP-1 during the initial day of treatment, whereas concentrations returned to baseline as treatment progressed. It appears that, in situations in which IGF-I is increased and serum IGFBP-3 does not increase proportionately, IGFBP-1 may be increased. This would account for the significant inverse correlation between IGFBP-1 and -3 on the first day of treatment. This compensatory increase in serum IGFBP-1 may function to inhibit the insulin-like effects of IGF-I after initiation of treatment (1). Indeed, serum concentrations of glucose were similar whether IGF-I was infused continuously or administered twice daily.

Increased concentrations of IGFBP-1 relative to IGFBP-3 are seen in clinical cases of GH deficiency (42), GH receptor deficiency (3) and in short stature (43), in which circulating concentrations of IGF-I are below the normal range. A test of the hypothesis of a compensatory increase in serum IGFBP-1 in response to an increase in serum IGF-I without changes in serum IGFBP-3 could be obtained from patients with GH receptor deficiency receiving IGF-I treatment. However, several studies did not specifically evaluate changes in serum IGFBP-1 (13–16, 30) and the few that did so used Western ligand blotting and demonstrated either
an increase (10) or no change (44) in serum IGFBP-1. However, acute administration of IGF-I increases serum IGFBP-1 to a greater extent during GH receptor antagonism than during baseline in young adult monkeys (38). As IGFBP-1 in equi- or excess-molar concentrations of IGF-I can inhibit somatic growth (45), treatment strategies that favor a greater increase in IGFBP-1 may be counterproductive. Additional work is needed to determine how the mode of IGF-I administration affects the distribution of the IGFBPs and, importantly, the effects on somatic growth in GH-receptor-deficient situations.

Superimposed upon these effects of IGF-I administration was an apparent change in the IGF-I axis throughout the day. In the absence of IGF-I treatment, serum IGF-I and IGFBP-3 declined after the morning meal, reaching nadir concentrations 7–12 h later, unaffected by the afternoon meal. In contrast, serum insulin declined after the morning meal, but increased again after the afternoon meal. Serum IGFBP-1 peaked just before the afternoon meal, as serum insulin reached nadir concentrations. Constant infusion with IGF-I blunted these postprandial changes in serum IGF-I and IGFBP-3, but the pattern was still evident. In contrast, it is not known whether there is some circadian pattern within the IGF-I axis, independent of nutritional status. Future studies must investigate these issues.

Acknowledgements

The technical assistance of Mara Lindsley and Kathy Chikazawa is greatly appreciated. The reagents for the IGF-I assay were a gift from the National Hormone and Pituitary Program, NIDDK, NICHD and the USDA. The IGF-I was a generous gift from Genentech Inc. All assays were performed by Assay Services, Yerkes Primate Research Center. This research was supported by NIH grants HD 16305 and, in part, RR 00165, The Yerkes Primate Research Center is fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

References

8 Gargosky SE, Tapanainen P & Rosenfeld RG. Administration of growth hormone but not insulin-like growth factor (IGF-I) by continuous infusion can induce formation of the 150kDa IGF binding protein-3 complex in growth hormone deficient rats. Endocrinology 1994 134 2267–2276.

Received 4 September 1998
Accepted 28 May 1999