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# The Combination of Insulin-Like Growth Factor I and Insulin-Like Growth Factor-Binding Protein-3 Reduces Insulin Requirements in Insulin-Dependent Type 1 Diabetes: Evidence for *in Vivo* Biological Activity*

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**ABSTRACT**

Insulin-like growth factor-I (IGF-I) enhances insulin action in normal subjects and in patients with both type 1 and 2 diabetes; however, its administration is associated with significant side effects in a high percentage of patients. The coadministration of IGF binding protein-3 (IGFBP-3, the predominant IGF binding protein in serum) with IGF-I limits IGF-I inducible side effects, but it does not attenuate the ability of IGF-I to enhance protein synthesis and bone accretion; therefore, we determined whether IGF-I/IGFBP-3 would retain biological activity in type 1 DM and limit side effects associated with free IGF-I administration.

Twelve patients received recombinant human IGF-I plus IGFBP-3 (2 mg/kg·day) by continuous sc infusion for 2 weeks. Each subject served as his own control; and, during a paired 2-week period, each received a placebo infusion. The order of the treatments was randomized. Subjects were placed on a constant caloric intake but were allowed to adjust insulin doses to maintain appropriate levels of glycemic control. Subjects measured blood glucose four times per day at home and kept a log of their insulin use. Frequent sampling for glucose, insulin, and GH was conducted during four inpatient study periods, one at the beginning and one at the end of each 2-week study interval.

During IGF-I/IGFBP-3, insulin doses were reduced by 49%, and mean serum glucose was reduced by 23%. Free insulin levels obtained during frequent sampling in hospital fell 47% on IGF-I/IGFBP-3, compared with control, but showed no change with placebo. Concomitant glucose measurements did not differ in the two treatment groups. There was no change in body weight. Fructosamine levels decreased by 12%, but this was not significant ($P < 0.1$). Fasting triglyceride was unchanged, but cholesterol declined from 170 ± 24 to 149 ± 31 mg/dL ($P < 0.05$). IGFBP-2 (an IGF-I-dependent responsive variable) rose from 141 ± 56 to 251 ± 98 ng/mL ($P < 0.01$) on IGF-I/IGFBP-3. To analyze the mechanism by which IGF-I/IGFBP-3 might reduce insulin requirements, the change in serum GH was quantified. Mean GH levels were reduced by 72%, from 2.48 to 0.55 ng/mL ($P < 0.001$). An equal number (40%) of drug- and placebo-treated subjects had minor hypoglycemic episodes at home that required adjustment of insulin doses. No episode was classified as severe. In contrast to previous studies with free IGF-I, there were no cases of edema, headache, jaw pain, retinal edema, or Bell's palsy. No subject withdrew because of drug complications. These findings indicate that IGF-I/IGFBP-3 is biologically active on carbohydrate metabolism, as measured by a decrease in insulin requirements in patients with type 1 diabetes. Further studies will be required to determine the long-term safety and efficacy of this combination in patients with insulin resistance and diabetes. (*J Clin Endocrinol Metab* **85:** 1518–1524, 2000)

INSULIN-LIKE growth factor-I (IGF-I) is a polypeptide hormone that has 48% amino acid sequence identity with proinsulin. Although the affinity of IGF-I for the insulin receptor is 0.5% that of insulin *in vitro*, when it is infused into animals and human subjects, IGF-I has approximately one twelfth the glucose-lowering capacity of insulin (1–4). Therefore, it has been hypothesized that IGF-I, working through its own receptor, is functioning to enhance insulin sensitivity and that this accounts for part of its glucose-lowering activity (5). Further verification of this hypothesis has come from studies in patients with extreme insulin resistance and in patients with type 2 diabetes, wherein analysis by either steady-state plasma glucose infusion or the frequently sampled IVGTT (Bergman model) has shown that there is a substantial (3.4-fold) improvement in insulin sensitivity during recombinant human (rh) IGF-I administration (6–9). Patients with extreme insulin resistance, treated for periods as long as 12 months, have shown substantial reduction in hemoglobin $A_1c$, indicating that IGF-I not only improves insulin sensitivity but also improves glycemic control (10). Large trials of several hundred patients with type 2 diabetes show that when IGF-I is given for 3 months, either as monotherapy (11) or with insulin, there is substantial improvement in hemoglobin $A_1c$ (12). Administration of IGF-I to patients with type 1 diabetes resulted in a 10% reduction in mean daily glucose, while reducing insulin dosage by 28% (13). Additional studies have confirmed this finding and

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reported a decrease in the degree of fluctuation in insulin requirements during IGF-I therapy (14, 15).

The molecular and physiological mechanisms by which IGF-I induces changes in carbohydrate metabolism and in insulin sensitivity have not been discerned. However, it has been shown that IGF-I infusions will acutely suppress glucagon and GH, two counter-regulatory hormones. Short-term infusion of IGF-I enhances lipid oxidation (16), and long-term administration results in reduction in body fat (11, 12), particularly intraabdominal fat. Each of these changes could contribute to the change in insulin sensitivity.

A major problem associated with administration of free IGF-I has been the induction of side effects (17), particularly at doses $\geq$ 80 $\mu$g/kg twice daily. Mild side effects, such as edema, jaw pain, and headaches, occur in more than 80% of subjects, and serious side effects, such as Bell's palsy and retinal edema, in 10–15% of such patients. Free IGF-I, administered sc, induces a 2.4-fold increase in IGF binding protein (IGFBP)-2, suppresses IGFBP-3, and increases free IGF-I levels (18–20). These changes that occur in the protein binding of IGF-I in plasma are not physiologic and raise the question of whether they may be related to the development of complications. In contrast, when IGF-I was administered with IGFBP-3 to normal volunteers at higher doses of IGF-I than were tolerated in previous studies, side effects were not observed (21). Similarly, when the combination of IGF-I/IGFBP-3 was administered to a group of 12 elderly, osteoporotic women, status post hip fracture, for 2 months at a dose of 1 mg/kg·day, there were no side effects (22). These findings suggest that IGFBP-3 is acting to limit the side effect profile, probably by changing free IGF-I levels and the distribution of IGF-I among the various binding proteins in serum. Although this regimen seems to limit IGF-I-associated side effects, the question remains whether IGFBP-3 also will reduce the efficacy of IGF-I. Administration of IGF-I/IGFBP-3 to elderly, osteoporotic subjects with hip fractures improved bone accretion (22). Similarly, infusion of the IGF-I/IGFBP-3 complex into patients with severe burns stimulated protein synthesis (23). Infusion of the IGF-I/IGFBP-3 complex into animals reveals that the anabolic and osteotrophic effects of IGF-I can be retained, even if equimolar concentrations of IGFBP-3 are infused simultaneously (24). Because the presence of IGFBPs in plasma has been presumed to limit the hypoglycemic response to IGF-I (25), the current studies were undertaken to determine whether the known effects of IGF-I on carbohydrate metabolism could be retained if it was administered with IGFBP-3.

## Subjects and Methods

This was a proof-of-concept, randomized, cross-over, double-blinded, placebo-controlled, trial of IGF-I/IGFBP-3 *vs.* placebo in insulin-treated patients with type 1 DM. Fifteen patients with type I diabetes were recruited from the clinics of the University of North Carolina at Chapel Hill, NC; Beth Israel Deaconess Medical Center, Boston, MA; and Northwest Kinetics, Tacoma, WA. Three subjects withdrew during the study. One was dismissed because of failure to adhere to the protocol, and the other 2 subjects voluntarily withdrew because of inability to adhere to the testing regimen. The 12 subjects that completed the study had the following characteristics: they were between 19 and 40 yr of age, and body mass indices were between 23.1 and 29.4, with weights between 58.1 and 109 kg. All subjects had type 1 diabetes confirmed by an absolute requirement for insulin to remain free of ketosis. C peptide values were less than 0.1 ng/mL in 10 subjects and 0.2 and 0.6 ng/mL, respectively, in 2 subjects. The subjects had maintained stable weight during the 3 months before treatment. Mean hemoglobin $A_1c$ in these subjects averaged 8.6% and ranged from 6.1–11.6% (normal range, 4.9–5.9%) (Table 1). Baseline IGF-I values ranged from 81–268 ng/mL, with a mean of 152 ng/mL. No subject had evidence of significant diabetic retinopathy, as defined by the presence of microaneurysms. All were screened for proteinuria, and all excreted less than 50 $\mu$g/day protein. None had significant diabetic neuropathy. Subjects with a history of any endocrinopathy or those who were taking corticosteroids were excluded.

### *Lead-in phase*

During a 2-week lead-in phase, the subjects were evaluated thoroughly, by history and physical exam, to exclude other major medical problems. They were then placed on a constant food intake to match their home caloric intake. No attempt was made to improve dietary control, and the objective was to maintain a consistent caloric intake throughout the study. Subjects were also instructed in the use of a continuous sc infusion pump (CADD-1, Sims-Deltec, St. Paul, MN) and in obtaining glucoses 4 times per day by glucose reflective meter (Accu-check Advantage, Roche Molecular Biochemicals, Indianapolis, IN). The glucose values were down-loaded into a computerized recall system. Home blood glucose monitoring was timed to coincide with fasting in the morning, 30 min after lunch and dinner, and at bedtime. Meal times were standardized to occur at 0800, 1200, and 1800 h. No subject lost significant weight during this 2-week lead-in phase, and the degree of improvement in glucose control during this phase was modest (*e.g.* <5% reduction in mean daily glucose). All subjects provided written, informed consent, which was approved by the Institutional Review Board of each institution involved in the study.

### *Treatment phase*

After the 2-week lead-in phase, subjects were randomized to one of two treatments, either IGF-I/IGFBP-3 at a dosage of 2 mg/kg·day (IGF-I and IGFBP-3 were prepared as a complex containing equimolar concentrations, *i.e.* 400 $\mu$g/kg of IGF-I and 1600 $\mu$g/kg IGFBP-3) or vehicle (saline) by continuous sc infusion. The order of treatment was randomized, and both the investigators and subjects were blinded as to what was being administered. The final analysis showed that seven subjects initially received placebo, and five received IGF-I/IGFBP-3. Subjects were hospitalized for 2 days before the start of the infusion. During this time, they received further instruction in diet and underwent frequent sampling (36 samples in 24 h) on day 2 for insulin and glucose. GH was measured every 30 min, from 2000 to 0800 h. At the beginning of day 3, the sc infusions were initiated; and, after 4 h, the subjects were discharged to home. Home glucose monitoring and insulin treatment continued during a 12-day outpatient treatment interval. During the home glucose monitoring interval, the subjects measured glucoses four times per day and maintained a log of insulin consumption. No attempt was made to alter the formulation of insulin that was being administered, and adjustments were made through daily phone contact to achieve reasonable glycemic control and to avoid hypoglycemia. At the completion of each treatment phase, the subjects were rehospitalized for 2 days and underwent the same testing protocol that was performed during the first 2 study days. They were discharged to a 2-week re-equilibration period (wash-out) at home. During this time, the subjects were counseled by the dietitian and encouraged to continue the same dietary intake. They continued to monitor glucose levels and docu-

**TABLE 1.** Patient characteristics

| | Mean | Range |
|---|---|---|
| Age (yr) | 27 | (19–40) |
| Insulin dose (U) | 59 | (26–92) |
| $HgbA_1c$ (%) | 8.6 | (6.1–11.6) |
| Weight (kg) | 81.9 | (58.1–109.1) |
| Fructosamine ($\mu$mol/L) | 417 | (246–602) |
| IGF-I (ng/mL) | 152 | (81–268) |
| C peptide (ng/mL) | <0.3 | (<0.3–0.6) |

mented insulin consumption as before. After this 2-week interval, the subjects were rehospitalized and restudied in a manner identical to the first hospitalization. On the morning of the third hospital day, the subjects received a continuous, sc infusion of the paired treatment, with each subject serving as his or her own control. After the second 12-day outpatient study interval, the subjects were rehospitalized for 2 more days, during which time all of the previous measurements were repeated.

In addition to those measurements listed previously, each subject had a total serum cholesterol, fructosamine, body weight, triglycerides, and hemoglobin $A_1c$ at the beginning and end of each 2-week treatment interval. Safety studies were conducted with a complete serum chemistry analysis, including liver and renal function tests, as well as uric acid and serum phosphorous determinations. Laboratory measurements of glycosylated hemoglobin and fructosamine (normal range, 190–270 mmol/L) were determined using standard methods. Insulin and C peptide were determined, by double-antibody RIAs, by Quest Diagnostics, Inc., San Juan Capistrano, CA. Total serum IGF-I levels were determined by Quest Diagnostics, Inc., using the Nichols assay kit. IGFBP-2 values were determined by RIA, as previously described (25a). GH values were determined by a sensitive immunochemiluminescence assay by Quest Diagnostics, Inc., with a detection limit of 20 pg/mL.

### *Statistics*

All data are expressed as the mean ± SD. The significance of the effects of IGF-I/IGFBP-3 on the various outcome parameters was determined using the paired Student's *t* test with a Bonferroni correction for multiple comparisons using SAS Institute, Inc. (Cary, NC). Significant changes were expressed as a value of $P < 0.05$.

## Results

During the 14-day treatment interval with IGF-I/IGFBP-3, mean insulin dosage was significantly less, 27 ± 13 U/day, compared with the 2 weeks of placebo treatment, 53 ± 18 U/day (49% decrease, $P < 0.01$). In spite of this reduction, mean blood glucose, based on four-times-per-day measurements, decreased from 187 ± 95 on placebo to 144 ± 73 mg/dL on IGF-I/IGFBP-3 (23%, $P = 0.02$) (Fig. 1). Analysis of the time of day of glucose reduction revealed no reproducible pattern of the decrease. All subjects reported some reduction in insulin dosage (range, 38–61%). No subject reported significant reduction in food intake, and weights were constant during this phase of the study. Frequent insulin and glucose measurements that were obtained during the inpatient study intervals confirmed these findings. Measurement of free insulin confirmed reduced insulin dosage, because after 2 weeks of treatment, mean serum free insulin decreased from 37.5 ± 16.9 to 20.1 ± 9.0 μU/mL (47% decrease, $P < 0.01$), whereas mean blood glucose did not change [216 ± 88 to 204 ± 76 (*P*, not significant, N.S.)] (Figs. 2 and 3). In contrast, after placebo treatment, the mean free insulin concentrations were unchanged at 40.1 ± 14.0, compared with 38.2 ± 14.1 μU/mL, after 2 weeks (*P*, N.S.), and mean glucose values were 202 ± 61 before and 207 ± 81 mg/dL during treatment (*P*, N.S.).

To determine the effect of IGF-I/IGFBP-3 administration on GH secretion, GH was measured at 30-min intervals from 2000 to 0800 h. Mean GH decreased 77% from 2.48 ± 1.73 to 0.55 ± 0.23 ng/mL during IGF-I/IGFBP-3 treatment ($P < 0.001$), whereas on placebo, the values were 2.29 ± 1.68 and 2.33 ± 1.41 ng/mL, respectively (*P*, N.S.) (Fig. 4). In addition, the frequency of GH peaks greater than 3 ng/mL was reduced from a mean of 4.2 to 1.7 in the IGF-I/IGFBP-3 treatment group. These findings indicate that sufficient IGF-I was available to significantly suppress endogenous GH secretion. Total IGF-I values rose from a mean of 167 ± 65 to 625 ± 186 ng/mL after 1 week and declined to 493 ± 143 ng/mL ($P < 0.001$) after 2 weeks of IGF-I/IGFBP-3. There was no change in total IGF-I levels during the placebo phase (Fig. 5). Neither hemoglobin $A_1c$ nor fructosamine changed significantly during either treatment; however, fructosamine values trended downward even after only 2 weeks (Table 2). Analysis of total triglycerides and cholesterol revealed a significant reduction in total cholesterol, from 170 ± 24 to 149 ± 31 mg/dL ($P < 0.05$), during IGF-I/IGFBP-3 administration. In contrast, triglycerides, which have been reported to decrease 40% when IGF-I alone is given to subjects with type 2 diabetes, were not reduced significantly. Because IGFBP-2 has been shown to be very responsive to IGF-I, we determined the IGFBP-2 response to IGF-I/IGFBP-3. After 2 weeks of IGF-I/IGFBP-3, the mean IGFBP-2 rose from 141 ± 56 to 251 ± 98 ng/mL ($P < 0.01$), whereas it remained unchanged on placebo [148 ± 50, compared with 143 ± 46 ng/mL (*P*, N.S.)] (Fig. 6).

The study patients were analyzed for the appearance of side effects. The only problems noted were some irritation and redness at the site of continuous sc infusion that occurred in 3 of the 12 subjects. This resolved spontaneously when the infusion site was moved. Mild hypoglycemic events (defined as a glucose value <60 ng/dL) were noted in 40% of the subjects. Both of these problems also occurred with the placebo treatment with similar frequency.

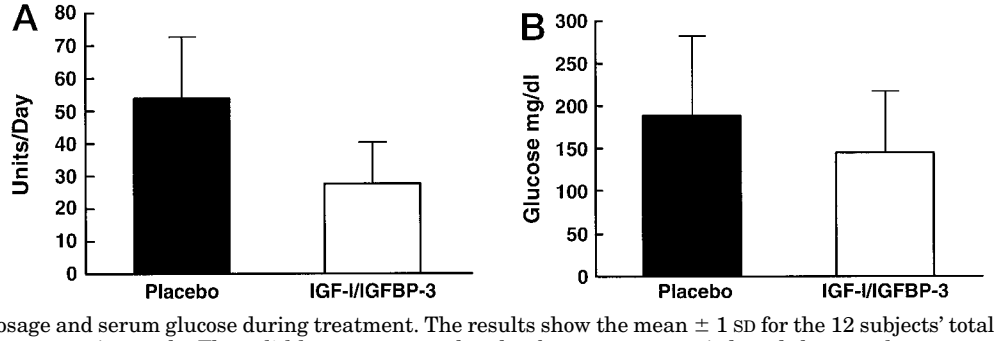


FIG. 1. A, Insulin dosage and serum glucose during treatment. The results show the mean ± 1 SD for the 12 subjects' total daily insulin dosage during the 2-week treatment intervals. The *solid bar* represents the placebo treatment period, and the *open bar* represents the combination of IGF-I/IGFBP-3. B, The data are expressed as the mean glucose ± 1 SD. The *solid bar* represents placebo for the 12 subjects, and the *open bar* represents IGF-I/IGFBP-3.

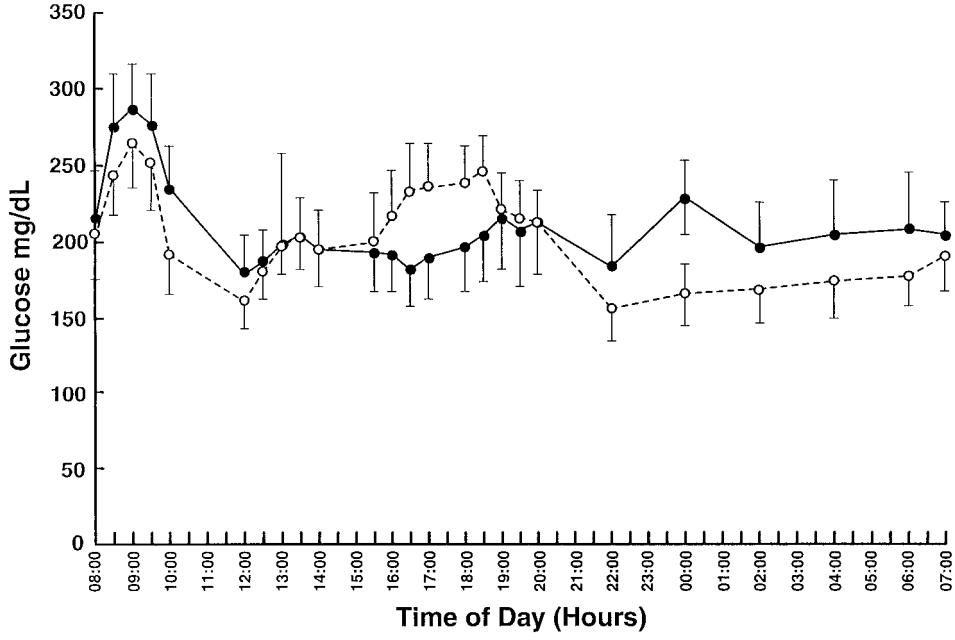


FIG. 2. Serum glucose values during in-hospital monitoring. The mean ± SE of the 12 subjects for IGF-I/IGFBP-3 (○–○) and for placebo (●–●) for blood glucose are shown. Samples were obtained at the times listed on the x-axis.

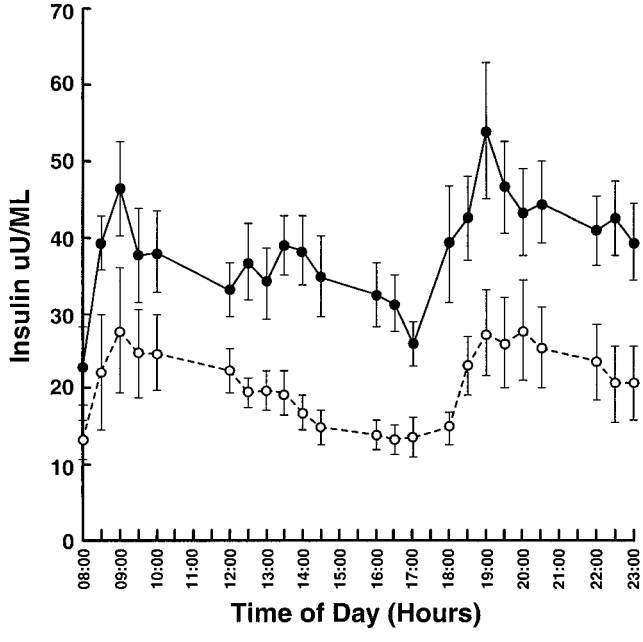


FIG. 3. Serum free insulin during IGF-I/IGFBP-3 in-hospital monitoring. The data points represent the mean ± 1 SE for insulin values obtained at frequent times during the in-patient hospital admissions at the end of the treatment period with IGF-I/IGFBP-3 (○–○) or placebo (●–●).

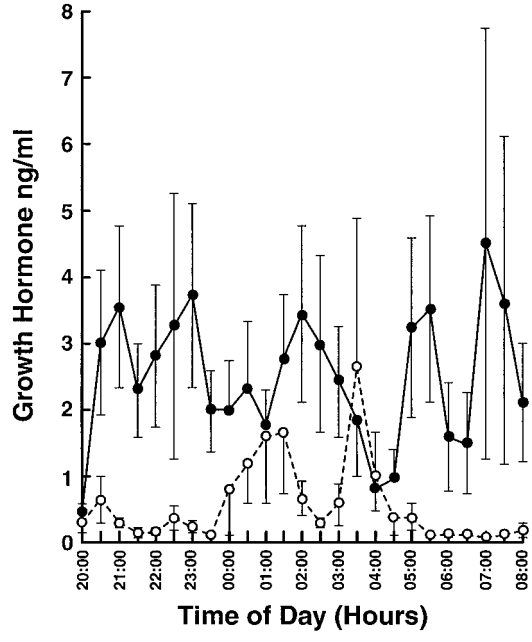


FIG. 4. Serum GH during in-hospital monitoring. The results represent the mean ± SE after 2 weeks of IGF-I/IGFBP-3 (○–○) or 2 weeks of placebo (●–●).

## Discussion

These results demonstrate that the combination of recombinant IGF-I and IGFBP-3 lowered exogenous insulin requirements while improving glycemic control, as measured by home glucose monitoring. Because these patients had minimal or no insulin secretory capacity, their ability to maintain improved glycemic control for a period of 2 weeks with a 49% reduction in insulin requirement is strong evidence of either an improvement in insulin sensitivity or a direct glucose-lowering effect of IGF-I. The validity of the observation of self-reported reduction in insulin dosage was confirmed by showing a similar degree of reduction in free insulin levels measured during the hospitalization. Although there was not a statistically significant change in fructosamine, there was a trend toward lower levels, suggesting that longer-term treatment might have resulted in a statistically significant improvement.

Administration of IGF-I alone has been shown to result in improved glycemic control and a 3.4-fold improvement in insulin sensitivity in patients with type 2 diabetes (6). A larger study (*i.e.* 204 patients), in which IGF-I was used as monotherapy (40 $\mu$g/kg BID), showed a 1.7% reduction in

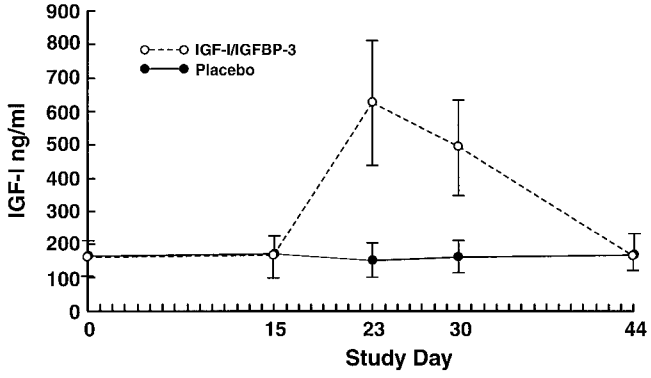


FIG. 5. Serum IGF-I levels. The IGF-I levels obtained on the 12 subjects are shown as a mean ± SD The values are plotted for the study days listed for IGF-I/IGFBP-3 (○–○) and for placebo (●–●).

hemoglobin $A_1c$ compared with placebo (11). This degree of improvement also was demonstrated in a study in which IGF-I was administered with insulin to subjects with type 2 diabetes. Quattrin *et al.* were able to demonstrate a 28% reduction in insulin dosage with no significant deterioration in glycemic control during a 4-week trial in patients with type 1 diabetes using 80 $\mu$g/kg·day of free IGF-I (13). Because our study also showed a significant reduction in total daily insulin dose and mean glucose, this suggests that the administration of this binding protein with IGF-I did not result in a loss of efficacy.

Previous studies using the combination of IGF-I/IGFBP-3 have shown that it preserves muscle protein and bone mineral content in oophorectomized rats (24). Osteoporotic females with hip fractures have responded with a 1.5% decrease in bone mineral density, as compared with an 6.3% loss in age-matched hip fracture subjects who received placebo (22). Similarly, catabolic patients with severe burns responded to the IGF-I/IGFBP-3 complex with a significant improvement in net protein balance (23). These results suggest that the combination of IGF-I/IGFBP-3 does not attenuate the anabolic effects of IGF-I if the two are administered in equimolar concentrations. Taken together, these findings suggest that when IGF-I is administered with IGFBP-3, the material that is bound to the ternary complex in blood equilibrates with other binding proteins within the vascular space and with extravascular tissues so that IGF-I receptors are exposed to a greater concentration of IGF-I over an extended period of time (26). The exact mechanism by which this occurs is undefined, but the biologic activity of IGF-I clearly can be preserved. That this maintenance of a strong anabolic effect is caused by a change in the distribution and clearance of IGF-I and not by direct additive effects of IGFBP-3 acting at the cellular level is suggested by the observation that infusion of an anti-IGF-I antibody with IGF-I to experimental animals results in potentiation of IGF-I's anabolic effects (27).

Although the growth-promoting effects of IGF-I might have been predicted to be preserved based on these animal studies, IGFBP-3 has been proposed previously to be an inhibitor of the glucose-lowering actions of IGF-I. Indeed, some *in vitro* studies have shown that IGFBP-3 will inhibit IGF-I-stimulated glucose transport (28). However, in contrast to IGFBP-1, no *in vivo* study has shown an attenuation of glucose tolerance to occur after IGFBP-3 infusion or overexpression (29, 30).

The mechanism(s) by which IGF-I induces its effects on carbohydrate metabolism is unknown. Previous studies with IGF-I in type 2 diabetics have shown that it lowers triglycerides by as much as 40% in patients with diabetes (5). In one study, IGF-I infusion decreased free fatty acid (FFA) levels, suggesting that the reduction in triglycerides and FFA might enhance insulin sensitivity by facilitating glucose use and decreasing the competitive effect of FFA on the cellular uptake of glucose (31). However, in the current study in type 1 diabetics, there was no change in the triglycerides when IGFBP-3 was administered with IGF-I, suggesting that changes in triglyceride metabolism were probably not responsible for the short-term changes that were observed.

IGF-I administration to normal humans and to diabetic animals has been associated with glucagon suppression, which could alter insulin sensitivity (32, 33). Similarly, IGF-I administration to type 1 diabetics has been shown to suppress GH secretion (33, 34), and several studies have hypothesized that suppression of GH significantly improves insulin sensitivity (12–15, 33–35). In keeping with that finding, in this study, we found a 77% reduction in overnight GH secretion, suggesting that eliminating the counterregulatory effect of GH on gluconeogenesis and hepatic glucose output may have been an important determinant of the change in insulin requirements. GH also may act to directly antagonize insulin action in the liver; thus, IGF-I/IGFBP-3 administration, by lowering GH levels (which are elevated in some patients with type 1 diabetes), restores more normal physiology.

Because this was a short-term study and there was no reduction in body weight, this suggests that body fat did not change significantly during the 2-week period. Therefore, it is unlikely that changes in visceral fat contributed to the change in insulin sensitivity. Another possible mechanism by which an IGF-I could induce changes in insulin requirements is through insulin/IGF-I hybrid receptors, which have been shown to be increased in skeletal muscle in type 2 diabetics (36). The peak total IGF-I levels achieved in this study (*e.g.* 540 ng/mL) remain primarily protein bound; and therefore, it is difficult to extrapolate that high-enough free IGF-I levels were achieved to directly stimulate the insulin receptor. However, the IGF-I/insulin receptor signaling pathways use many of the same components. It is also possible that alteration in stimulation of IGF-I signaling results in downstream enhancement of insulin receptor-mediated signaling, particularly in skeletal muscle, where IGF-I receptors are abundant.

A major concern in previous studies with IGF-I has been toxicity. Those studies have demonstrated that severe edema, arthralgias, temporomandibular joint pain, and headaches are extremely common (*i.e.* 25–80%) in patients receiving ≥40 $\mu$g/kg sc BID of free IGF-I (11, 12, 17). Other more unusual (but nevertheless severe) side effects include Bell's palsy and optic nerve edema (4–8% of patients). None of these side effects were noted in this study, in spite of the fact that total IGF-I levels were comparable with peak levels that have induced side effects in previous studies (6, 15). This suggests that, in the short-term studies, IGF-I/IGFBP-3 is

**TABLE 2.** Responses of other variables

| | Placebo | | IGF-I/IGFBP-3 | |
|---|---|---|---|---|
| | Baseline | After 2 weeks | Baseline | After 2 weeks |
| Fructosamine ($\mu$mol) | 355 ± 58 | 368 ± 68 | 347 ± 54 | 328 ± 48[a] |
| Cholesterol (mg/dL) | 161 ± 27 | 163 ± 16 | 170 ± 24 | 149 ± 31[b] |
| Triglycerides (mg/dL) | 112 ± 66 | 118 ± 86 | 106 ± 55 | 109 ± 81 |
| Body weight (kg) | 83.1 ± 14.7 | 82.9 ± 14.7 | 82.9 ± 14.8 | 83.2 ± 14.3 |
| Hemoglobin $A_1c$ (%) | 8.6 ± 1.7 | 8.5 ± 1.8 | 8.6 ± 1.8 | 8.3 ± 1.9 |

[a] $P < 0.1$, compared with the baseline value.
[b] $P < 0.05$, compared with the baseline value.

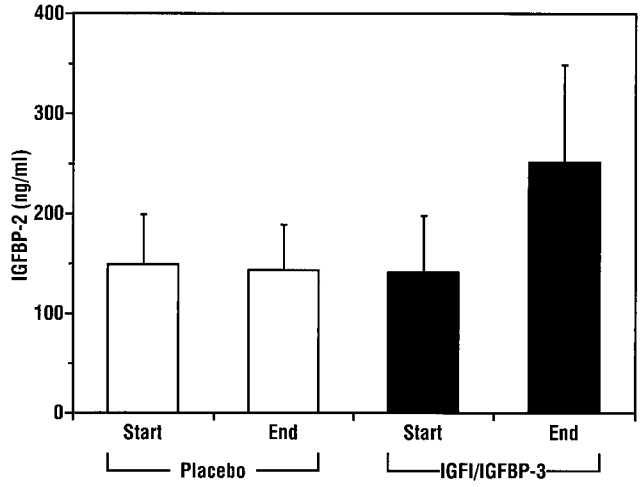


FIG. 6. Serum IGFBP-2 levels. The serum IGFBP-2 at the beginning or end of 2 weeks of IGF-I/IGFBP-3 (*solid bars*) or placebo (*open bars*) are shown as the mean ± SD for the 12 subjects.

safe. Longer studies must be undertaken in order to determine whether patients remain free of these side effects for periods of 2 months, as they did when IGF-I/IGFBP-3 was administered to elderly female subjects with osteoporosis. Similarly, longer studies will be required to conclude that there is no progression of diabetic retinopathy.

The findings also suggest that side effects induced by IGF-I are not a continuum of biologic response. In this study, we noted a significant biologic response, but no significant side effects were present. This strongly suggests that the side effect profile noted previously with free IGF-I administration probably is attributable to transient increases in free IGF-I that are much greater than those that occur when it is administered by continuous sc infusion with IGFBP-3, wherein both proteins form a stable ternary complex with the acid labile subunit. The pharmacodynamics of IGF-I when administered with IGFBP-3 were not analyzed. However, we did note a 1.8-fold increase in IGFBP-2 that was substantially less than the 3.2-fold increase noted previously with administration of free IGF-I (6). This suggests that relatively more IGF-I would be bound to IGFBP-3 and less to IGFBP-2 in our patients, compared with patients receiving free IGF-I. Because IGFBP-2 does not form the ternary complex and crosses the intact capillary barrier, this suggests that, in patients who are receiving free IGF-I, more of the peptide may enter the extravascular compartment in the period of time immediately after IGF-I administration. Therefore, improvement in the side effect profile may be closely related to a reduction in the rate at which IGF-I enters the extravascular compartment. It will be interesting, in future studies, to determine whether side effects are more closely related to the temporal pattern of changes in free IGF-I, a time-dependent change in the rate of equilibration with the extracellular space, or to changes in the type of binding protein that binds to IGF-I. Direct measurements of these pharmacokinetic parameters need to be undertaken to determine which of these parameters bear the closest relationship to side-effect development.

In summary, the results of this study show clearly that IGF-I can be administered with IGFBP-3 and retain biological activity for carbohydrate metabolism, as reflected by a decrease in requirements for exogenous insulin. The combination was safe for 14 days, and many of the short-term side effects that have been noted with free IGF-I did not occur. This suggests that longer-term studies can be safely undertaken to determine the efficacy of IGF-I/IGFBP-3 in improving glycemic control in diabetes and to determine the mechanism by which IGF-I enhances insulin sensitivity in these patients.

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