# Normalization of the IGF-IGFBP Axis by Sustained Nightly Insulinization in Type 1 Diabetes

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**OBJECTIVE** — We sought to test the hypothesis that start of insulin glargine with sustained nightly insulin action results in changes in circulating concentrations of IGF-I and IGF binding proteins (IGFBPs) in adolescents with type 1 diabetes—changes that may support improvement of A1C.

**RESEARCH DESIGN AND METHODS** — Twelve pubertal adolescents with type 1 diabetes and initially on NPH insulin were studied during 12 weeks of intensified treatment with glargine.

**RESULTS** — Subnormal IGF-I SD scores on NPH ($-1.8 \pm 0.4$) rapidly increased and remained $54 \pm 9\%$ elevated ($P < 0.001$) after 12 weeks on glargine. A1C decreased from $8.3 \pm 0.6\%$ to a nadir of $6.9 \pm 0.3\%$ ($P = 0.002$) at 6 weeks and correlated with changes in IGF-I ($r = -0.64$, $P < 0.05$). The increase in IGF-I did not suppress the mean overnight growth hormone (GH) secretion at 6 weeks. The mean overnight IGFBP-1 levels decreased ($P = 0.035$), supporting the hypothesis that the nightly hepatic insulin action was increased. Circulating IGF-I increased in the absence of changes in both GH secretion and GH receptor numbers (assessed by growth hormone binding protein), indicating that postreceptor mechanisms are involved. IGFBP-3 proteolysis was decreased.

**CONCLUSIONS** — Increased hepatic insulin action after start of glargine was evident from a decrease in night time IGFBP-1 concentrations. This may improve GH postreceptor signaling, resulting in increased circulating IGF-I. We suggest that even in the absence of changes in GH, increased IGF-I and decreased IGFBP-1 support the improvement of metabolic control.

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In type 1 diabetes, metabolic control often deteriorates during puberty. Increased GH secretion is thought to contribute by impairing insulin sensitivity (1). GH-induced lipolysis, stimulation of hepatic glucose output, and induction of suppressors-of-cytokine-signaling (SOCS) expression may be involved (2–4). Even healthy adolescents develop insulin resistance when GH and IGF-I increase during the period of rapid longitudinal growth, suggesting that GH effects are not balanced by the insulin-like effects of IGF-I (5). IGF-I improves insulin sensitivity directly by stimulating glucose uptake in human muscle ex vivo with the same potency as that of insulin (6), and IGF-I administration reduces insulin needs in healthy subjects (7). Although direct IGF-I effects are significant (8), IGF-I also reduces GH secretion by feedback inhibition and thereby indirectly increases insulin sensitivity (9).

During puberty, patients with type 1 diabetes have GH hypersecretion (10,11) and markedly subnormal IGF-I levels (12), which further augment their insulin resistance during the period of rapid growth. The relative GH hypersecretion is secondary to decreased negative IGF-I feedback on pituitary GH secretion (13). The subnormal IGF-I levels are thought to be due to portal insulinopenia and GH receptor (GHR) resistance (14). The starting of subcutaneous insulin therapy in newly diagnosed type 1 diabetic patients increases IGF-I (15) although direct portal insulin infusion (16) or intraperitoneal insulin delivery (17) is required to completely restore normal IGF-I concentrations. The molecular mechanisms by which insulin promotes GH signaling are not well understood. GH binding protein (GHBP) is the extracellular part of the GHR that is shredded into the bloodstream after proteolytic degradation. GHBP may reflect to some extent the hepatic GHR number and function. GHBP is reduced in pubertal type 1 diabetic patients (18) and increases during intraperitoneal insulin delivery (17).

The functional importance of low IGF-I in type 1 diabetes is suggested by direct IGF-I effects on glucose metabolism in type 1 diabetes (8). Adjunctive administration of IGF-I (19) or IGF-I/IGFBP (IGF binding protein)-3 (13) in type 1 diabetic patients decreases insulin requirements and improves insulin sensitivity in short-term studies. The association of increased IGF-I and improved insulin sensitivity with decreased GH secretion in these studies has not been found in longer-term studies demonstrating improved glycemic control (20,21). Direct IGF-I effects on glucose metabolism and its negative feedback on GH are modulated by a family of six IGFBPs. Circulating IGFBPs reduce the glucose-lowering potency of systemically administered IGF-I (22). Insulin deficiency impairs the suppression of hepatic IGFBP-1 production in type 1 diabetes, and increased IGFBP-1 may attribute to attenuation of the insulin-like effects of circulating IGF-I. Hepatic production and circulating levels of IGFBP-1 are decreased during hyperinsulinemic clamps in type 1 diabetic patients (23)

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**Abbreviations:** GH, growth hormone; GHBP, growth hormone binding protein; GHR, growth hormone receptor; IGFBP, IGF binding protein; IGFBP-3-PA, IGFBP-3 proteolysis; MIT, mealtime insulin therapy; SOCS, suppressors of cytokine signaling.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

and even in surgical patients with peripheral insulin resistance (24). Thus, IGFBP-1 is considered to be a marker of hepatic insulin actions. IGFBP-3 binds 95% of circulating IGF-I in a circulating ternary complex with an acid labile subunit. Proteolysis of IGFBP-3 decreases IGF-I affinity and has been shown to increase circulating IGF-I activity (25). In adolescent boys with type 1 diabetes, proteolysis of IGFBP-3 is increased, and this may partly counteract the effects of increased IGFBP-1 on circulating IGF-I activity (26).

Intensive insulin treatment is frequently based on a basal bolus regimen with NPH combined with mealtime insulin therapy (MIT) with a rapid-acting insulin analog. It has been shown that the long-acting insulin analog glargine, with sustained delivery of insulin over 24 h (27), improves A1C (28,29) and reduces the incidence of nocturnal hypoglycemia compared with NPH (30). The rather short duration of the effect of NPH given at bedtime contributes to the dawn phenomenon, with increasing B-glucose levels in the early morning. It is likely that early-morning insulinopenia further contributes to low IGF-I levels, GH hypersecretion, and insulin resistance. In the present study, changes in the GH-IGF-IGFBP axis, not previously reported, were assessed in adolescents with type 1 diabetes changing basal insulin from NPH to glargine. We report that the increase in IGF-I and decrease in IGFBP-1 are associated with improvement of A1C, while GH secretion is unaffected.

## RESEARCH DESIGN AND METHODS

— We recruited 12 adolescents, 4 boys and 8 girls, with type 1 diabetes from our outpatient clinic. Their mean age was 12.7 years (range 11.1–15.0), diabetes duration 3.1 years (1.0–6.0), BMI 18.6 kg/m$^2$ (15.9–24.7), and A1C 8.3 ± 0.6% (Mono S standard). They were all in puberty, and, according to Tanner staging (31), the girls were B2–B3 and boys G2–G3. The reported total insulin dose was 1.1 IU · kg$^{-1}$ · day$^{-1}$ (0.5–2.1). One female patient had a suspected viral infection at the 6-week admission and was excluded from calculations at that time point.

### Study protocol

Patients underwent a physical examination for Tanner staging ~4 weeks before the start of the study. They were instructed to change their current insulin doses to optimize metabolic control. Each subject was admitted to our clinical research unit during 26 h (1600 to 1800 h plus 1 day) before the start of glargine (0 week) and after 6 weeks on glargine (Lantus; Sanofi-Aventis). In addition, they were studied in the morning fasted state after 1, 2, 4, 8, and 12 weeks on glargine. At the first admission, all subjects were on MIT/NPH with NPH insulin twice daily and the rapid-acting insulin analog Lispro or Aspart at meal- and snack times at 1800, 2100, 0900, 1200, and 1430 h. Bedtime was from 2400 to 0830 h. Following admission, two peripheral venous catheters (antecubital or dorsal hand), as well as gluteal probes for continuous glucose monitoring and microdialysis, were inserted on preanesthetized (EMLA) locations. Blood samples were drawn at cannulation (1800 h) and then every 30 min from 2100 to 1700 h the next day. Hormone determinations between 0000 and 1000 h were defined as overnight and the remaining ones as daytime. Blood samples were allowed to clot (5°C, 2.0 h), and serum was stored at −20°C. All samples were analyzed in the same batch and all samples from each individual within the same assay run.

The first dose of glargine was given before dinner immediately after the first 24 h of admission. The initial once-daily glargine dose equaled ~80% of the total NPH dose. After 6 weeks on glargine, the 24-h blood sampling procedure was repeated. At the visits at 1, 2, 4, 8, and 12 weeks after starting glargine, morning fasting samples were obtained and subjects were examined and advised to change insulin doses to optimize their metabolic control.

### Ethics

All participants and their parents gave written informed consent. The study was approved by the local ethics committee of Karolinska Institute, Stockholm, Sweden, No. 2003-006.

### Biochemical methods

**IGF-I.** Serum IGF-I was determined after acid-ethanol extraction in a radioimmunoassay using des(1-3)IGF-I as radioligand (32). The intra- and interassay coefficients of variation (CVs) were 5 and 11%, respectively.

IGF-I SD scores were calculated by adjusting for sex and Tanner stage as previously described (33). IGF-I was measured three times during the 24-h study periods (1800, 0600, and 1700 h) at 0 and 6 weeks and at 0800 h at 2, 4, 8, and 12 weeks.

**GH.** GH was analyzed in serum by a commercial dissociation-enhanced lanthanide fluorescence immunoassay from Perkin Elmer (Turku, Finland) according to the manufacturer's directions. Standards were prepared against World Health Organization Reference Preparation 80/505. The intra- and interassay CVs were 3.4 and 5.3%, respectively. GH profiles were assessed by a peak detection program (Pulsar) from determinations every 30 min.

**IGFBP-1.** The total IGFBP-1 serum levels were determined by a radioimmunoassay elsewhere described (34), modified from ref. 35. The detection limit was 6 μg/l, and the intra- and interassay CVs were 5.6 and 11.8%, respectively. IGFBP-1 was analyzed hourly from 2100 to 1700 h.

**IGFBP-3 proteolysis.** Serum IGFBP-3 proteolysis (IGFBP-3-PA) was determined by in vitro degradation of $^{125}$I-IGFBP-3 as previously described (34). Briefly, serum was incubated with $^{125}$I-labeled glycosylated recombinant human IGFBP-3 (Upstate, Charlottesville, VA) for 5 h (37°C). Proteolysis was stopped by adding SDS sample buffer, and samples were processed overnight (50 V) on 12% SDS-PAGE. Gels were dried, exposed to hyperfilm (Amersham Biosciences, Umeå, Sweden), and the optical density of the bands was quantified using Image J (National Institutes of Health). IGFBP-3-PA was expressed as a percentage of all IGFBP-3 fragments relative to the total intensity. IGFBP-3-PA was determined every 4 h from 2100 to 1700 h.

**GHBP.** Serum GHBP was analyzed by means of a commercial ELISA (enzyme-linked immunoassay) kit (DSL-10-48100; Diagnostic Systems Laboratories) according to the manufacturer's instructions. GHBP was determined at 0600 h.

**A1C.** Blood collected on filter paper was analyzed for A1C by high-performance liquid chromatography (Variant II; Bio-Rad Laboratories, Hercules, CA). The normal reference value is <5.2%. The intraassay CV was 2.9%. All values are Swedish Mono-S standard (Mono-S = DCCT [Diabetes Control and Complications Trial] standard × 1.0678 − 1.341).

### Data analysis

Results are expressed as means ± SE if not otherwise stated. Data distribution

was checked for normality and analyzed by repeated-measures one-way ANOVA with pairwise multiple comparison procedures (Student-Newman-Keul method), or Student's paired *t* test was used when appropriate, and correlations were analyzed by linear regression (Sigma Stat 2.0; SYSTAT Software, London, U.K.). $P < 0.05$ was considered significant.

## RESULTS

### Auxology

During intensified treatment with glargine, body weight increased from 46.8 ± 2.5 to 48.3 ± 2.5 kg ($P = 0.002$), height from 157.8 ± 2.0 to 158.7 ± 2.0 cm ($P = 0.002$), and BMI from 18.7 ± 0.8 to 19.1 ± 0.9 kg/m$^2$ ($P = 0.03$) in the 11 patients studied at 6 weeks.

### A1C

Intensified treatment with glargine significantly decreased A1C (Mono-S standard) from 8.3 ± 0.6% to a nadir of 6.9 ± 0.3% at 6 weeks ($P < 0.002$, $n = 11$). The improvement in A1C was already significant at 2 weeks (7.5 ± 0.4%, $P < 0.008$) and was sustained at the end of the 12-week study period (7.3 ± 0.3%, $P < 0.008$) in all patients (Fig. 1*B*).

### IGF-I

The mean total IGF-I level increased as early as after 1 week from 231 ± 19 to 309 ± 17 μg/l ($P < 0.001$) and was increased by 44 ± 7% at 4 weeks ($P < 0.001$) (Fig. 1*A*). At 6 weeks, on the fasting morning, IGF-I was 274 ± 25 μg/l ($P = 0.022$) or 17 ± 9% over the starting value. Subsequently, the increase consolidated with a peak value of 347 ± 25 μg/l ($P < 0.001$) or 54 ± 9% over baseline at 12 weeks. A1C at 0 weeks was positively correlated with the percentage increase in IGF-I at all time points ($r = 0.93$, $P < 0.001$ at 6 weeks). The changes in IGF-I mirrored A1C changes as shown in Fig. 1, and the percentage changes in IGF-I and A1C were inversely correlated at 6 weeks ($r = -0.64$, $P = 0.035$), at 4 weeks ($r = -0.64$, $P = 0.026$), and at 8 weeks ($r = -0.81$, $P = 0.001$). The individual mean IGF-I SD score was markedly subnormal on NPH at 0 weeks (−1.8 ± 0.4) with five patients having values below −2 and only one patient above 0 (1.2). Individual IGF-I SD scores correlated inversely with A1C ($r = -0.64$, $P = 0.025$) at 0 weeks. Although 12 weeks of treatment markedly increased the mean IGF-I SD score by 54 ± 9% to −0.55 ± 0.3 ($P > 0.001$), the IGF-I SD score was in the lower normal range in all patients except the one with a 1.2 score at basal.

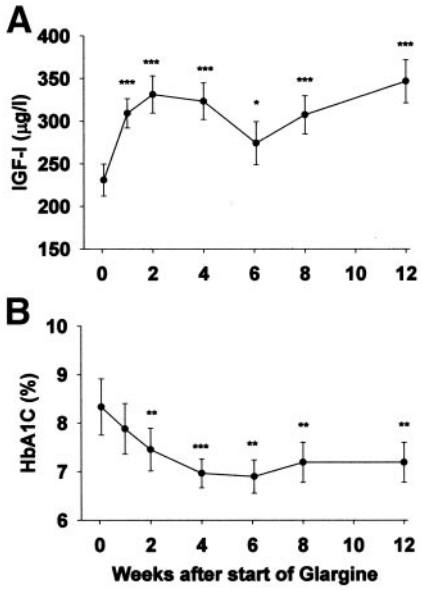


**Figure 1**—*IGF-I* (A) *and A1C* (B) *on MIT using basal NPH insulin at baseline (0 weeks) and during 12 weeks on MIT/glargine treatment in 12 adolescent subjects with type 1 diabetes.* *P < *0.05,* **P < 0.01, *and* ***P < *0.001.*

During both admissions, a significant diurnal variation in IGF-I levels was observed, with the lowest values in the morning samples. On NPH insulin, IGF-I was 249 ± 24 μg/l at 1800 h, 231 ± 19 μg/l at 0600 h, and 248 ± 23 μg/l at 1700 h ($P < 0.05$). After 6 weeks on glargine, IGF-I was 307 ± 27, 274 ± 25, and 286 ± 24 μg/l, respectively ($P = 0.002$).

### IGFBP-1

The individual IGFBP-1 levels during the admission periods at 0 and 6 weeks are shown in Fig. 2*A*. In the majority of patients, IGFBP-1 displayed a diurnal rhythm with higher levels during the night and early morning. On glargine, the excursions of IGFBP-1 to higher levels in the early morning were suppressed. The mean overnight IGFBP-1 concentration decreased significantly from 127 ± 21 to 90 ± 12 μg/l ($P = 0.035$) but did not reach significance when evaluated over the total admission periods ($P = 0.065$). There were no correlations between the percentage of change in IGF-I and IGFBP-1 or in A1C and IGFBP-1 from 0 to 6 weeks.

### GH and GHBP

The individual GH levels during the admission periods at 0 and 6 weeks are shown in Fig. 2*B*. In general, the individual patterns of GH pulses were similar before and after glargine. The GH peaks appeared to be equally distributed during the day and night, although some patients displayed more normal rhythms with more peaks during the night. There were no significant changes in measures of GH secretion (mean, mean overnight, or AUC) or the GH secretory pattern (peak number, peak height, peak length, or interpeak interval) after 6 weeks on glargine (Pulsar analysis, data not shown). The mean GHBPs determined at 0 and 6 weeks did not significantly differ (523 ± 95 vs. 488 ± 80 pmol/l).

### IGFBP-3-PA

The individual IGFBP-3-PA profiles during the admission periods at 0 and 6 weeks are shown in Fig. 2*C*. The mean IGFBP-3-PA on glargine was significantly lower than that on NPH (35.2 ± 1.2 vs. 33.3 ± 1.3%, $P < 0.001$). Day mean values were significantly higher than night mean values on both NPH and glargine ($P = 0.003$ and 0.002, respectively).

### Insulin dosage

The mean total insulin dose during the first admission was 1.21 ± 0.12 IU · kg$^{-1}$ · day$^{-1}$. After 6 weeks, the mean total insulin dose was 1.05 ± 0.11 IU · kg$^{-1}$ · day$^{-1}$ or 89 ± 6% of the initially given total dose, although this did not reach significance ($P = 0.13$). Interestingly, the change in total insulin dose and in IGF-I were positively correlated ($r = 0.61$, $P = 0.046$).

**CONCLUSIONS** — This is the first study to report that start of intensified treatment using glargine as basal insulin in adolescents with type 1 diabetes on MIT resulting in improved A1C is associated with markedly increased circulating IGF-I and decreased nightly IGFBP-1 secretion. Previous studies of newly diagnosed patients with type 1 diabetes have demonstrated increased IGF-I levels after the initiation of insulin treatment (15). However, portal delivery of insulin (16,17) is required to restore normal circulating IGF-I by improving hepatic GH sensitivity. The study design of the present study does not allow us to separate the effects of the intensified treatment with frequent visits and supervision from a direct role of glargine on improved A1C

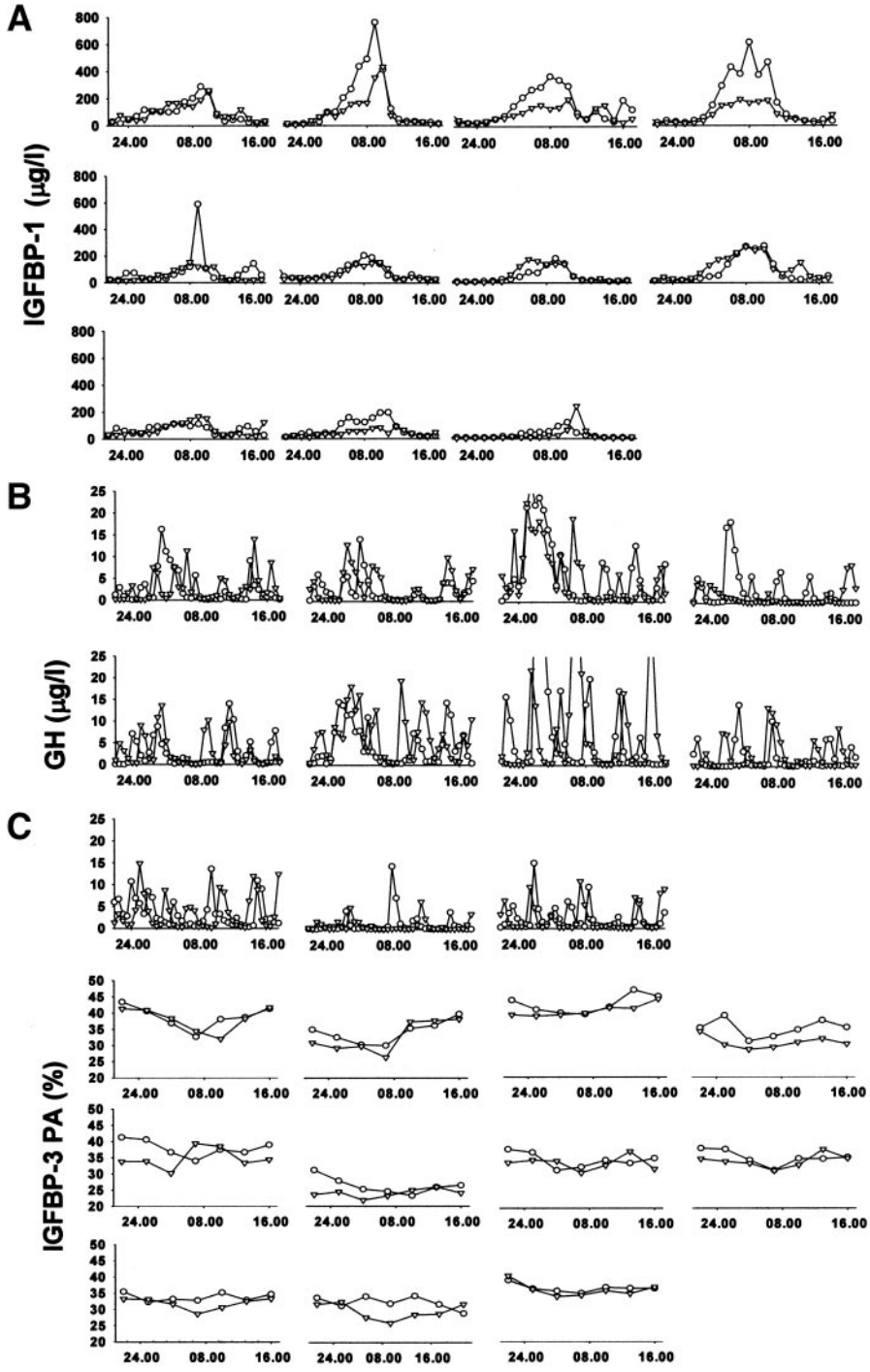


**Figure 2—***Individual IGFBP-1* (A) *, GH* (B)*, and IGFBP-3 proteolysis* (C) *profiles during the first admission period on MIT/NPH at 0 weeks (○) and during the second admisson period 6 weeks after start of MIT/glargine (▽).*

and increased IGF-I. Data from adolescent type 1 diabetic patients on insulin pumps suggest that an intensified treatment regime improves A1C by reducing the number of omitted meal doses (36). Therefore, intensified treatment per se may be expected to predominantly increase daytime insulin action. In favor of a specific effect of glargine in our study is the decrease in the overnight mean IGFBP-1 but not 20-h mean IGFBP-1 after 6 weeks. This finding suggests that glargine, through sustained hepatic delivery, promotes insulin action during the night. The use of IGFBP-1 as a marker of hepatic insulin effects is based on the fact that circulating IGFBP-1 is liver derived and that insulin suppression of IGFBP-1 production is the major determinant of circulating IGFBP-1 levels. This is also true in subjects with peripheral insulin resistance (24), which may be expected in our patients. At the present time, measurements of the insulin concentration in a sample containing several insulin analogs are not possible. The role of a sufficient insulin delivery for GH induction of

circulating IGF-I is further emphasized by the positive correlation between the change in the total insulin dose and IGF-I, which suggests that if the insulin dose is markedly decreased, an IGF-I increase cannot be supported. The diurnal rhythm of IGF-I with low levels in the morning is a new finding in adolescents with type 1 diabetes and has not been found with older IGF-I assay protocols (37). Whether diminished nightly delivery of insulin contributes to the decreasing IGF-I levels should be further investigated. Even in healthy subjects, the lower insulin secretion during overnight fasting may contribute to their diurnal rhythm (38), and the nightly absence of meal insulin in our patients may explain that depressed morning IGF-I persists on glargine. The lack of significant changes in GHBP in our study is in contrast with the finding of increased GHBP in patients on continuous peritoneal insulin delivery (17). Given the lack of significant changes in the total insulin dose, GH secretion, and GHBP on glargine, the improved hepatic insulin action appears to increase GH sensitivity and IGF-I generation by postreceptor mechanisms. Whether decreases in cytokines such as interleukin-6 or tumor necrosis factor-α via decreased activation of SOCS or other hormonal changes are involved in improved GH receptor signaling is currently under investigation.

The present study does not allow us to determine the causal relationship between the increase in circulating IGF-I and the improved A1C. An improvement in glycemic control was obtained despite the lack of change in insulin dose. It is possible that the action profile of glargine leads to improved nightly glucose homeostasis and, through reduced glucose toxicity in peripheral tissues, to increased insulin sensitivity and improved A1C. Increased hepatic insulin sensitivity may subsequently result in improved GH generation of IGF-I and increased systemic IGF-I levels. More likely, increased IGF-I levels may depend on a sustained nightly hepatic insulin action of glargine, as demonstrated by decreased nightly IGFBP-1 secretion, and increased hepatic GH sensitivity. In that case, the improvement in glucose homeostasis and A1C is secondary to increased IGF-I effects on glucose homeostasis. Exogenous administration of IGF-I is known to decrease insulin needs in healthy subjects and type 1 diabetic patients (19). However, some of these effects are related to a suppression of GH, particularly in type 1 diabetes. In our study, overnight or 20-h GH secretion did not change despite the marked increase in IGF-I. Therefore, if IGF-I actually improves A1C in our study, it is likely to be independent of GH effects on insulin sensitivity. Acute administration of IGF-I or IGF-I/IGFBP-3 decreases overnight GH secretion and overnight insulin needs (19) (13). IGF-I/IGFBP-3 reduced hepatic glucose output while peripheral glucose uptake remained unchanged (39). In the IGF-I/IGFBP-3 dose-response study, an IGF-I increase of at least 50% was necessary to suppress overnight GH secretion. In our study, IGF-I increased at the most by 50% but only by 17% at 6 weeks when GH was evaluated. In line with this finding, the severe IGF-I deficiency in our patients before glargine treatment was only partially normalized. This may explain why GH secretion was not affected in our study or in other longer-term studies with adjunctive IGF-I treatment of adolescent type 1 diabetic patients (20,21). Thus, IGF-I feedback inhibition of GH secretion may only be significant in individuals with sufficient IGF-I levels.

The direct effects of circulating IGF-I on glucose homeostasis and its ability to suppress GH secretion are regulated by high-affinity binding of IGF-I to six IGFBPs. Proteolytic fragmentation of IGFBP-3, the major determinant of IGF-I turnover in the circulation, is increased in midpubertal male patients with type 1 diabetes (26). We hypothesized that IGFBP-3-PA was upregulated to increase IGF-I bioavailability, and that is further supported by our present finding of decreased IGFBP-3-PA when total circulating IGF-I increases on glargine. On the other hand, the decrease in nighttime IGFBP-1 secretion on glargine may increase circulating IGF-I bioavailability in concert with increased total IGF-I. These changes in IGFBP-3-PA and IGFBP-1 are expected to have opposite effects on the bioavailability of circulating IGF-I. Since data from our laboratory have questioned the validity/relevance of free IGF-I determinations (40), we decided not to perform such determinations. Instead, we have recently developed a microdialysis method to probe tissue interstitial free IGF-I concentrations (41). This method should enable future determinations of local IGF-I activity in tissues involved in diabetes complications (42,43).

The clinical relevance of the present study must await randomized, controlled, long-term trials comparing medium long-acting NPH with long-acting insulin analogs such as glargine and detemir. However, the present study suggests that it will be important to monitor the GH-IGF-IGFBP system, which is associated with changes in A1C and which may play a specific role as a marker of future diabetes complications. It also demonstrates an association between changes in IGF-I and the total insulin dose, suggesting that, after the initial dose titration, one should strive to maintain the total insulin dose. In our study of adolescent subjects with rapid linear growth, minor but significant increases in weight and BMI were observed in accord with previous studies (44). However, the physiological increase in BMI in this age-group should also be considered.

We report that glargine increases circulating IGF-I in the absence of changes in insulin dose. This is conceivable with improved hepatic insulin action as demonstrated by increased overnight inhibition of IGFBP-1 production. The lack of significant changes in GH secretion and GHBP suggests that improved GH induction of hepatic IGF-I production is due to postreceptor mechanisms. Furthermore, our study suggests that IGF-I therapy may be needed to fully normalize IGF-I and prevent adverse effects of GH hypersecretion on peripheral insulin sensitivity and local IGF-I generation.

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