Randomized Cross-Over Trial of Insulin Glargine Plus Lispro or NPH Insulin Plus Regular Human Insulin in Adolescents With Type 1 Diabetes on Intensive Insulin Regimens

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OBJECTIVE — To compare blood glucose control and incidence of nocturnal hypoglycemia in adolescents with type 1 diabetes on multiple injection regimens managed with either an insulin analog combination or NPH insulin plus regular human insulin.

RESEARCH DESIGN AND METHODS — In a randomized cross-over study, 28 adolescents with type 1 diabetes on multiple injection therapy received either insulin glargine prebedtime plus lispro preprandially (LIS/GLAR) or NPH insulin prebedtime plus regular human insulin preprandially (R/NPH). During each 16-week treatment arm, subjects completed home blood glucose profiles, and at the end of each treatment arm, they were admitted for an overnight metabolic profile. A total of 25 subjects completed the study.

RESULTS — Compared with R/NPH therapy, LIS/GLAR was associated with lower mean blood glucose levels (LIS/GLAR versus R/NPH): fasting (8.0 vs. 9.2 mmol/l, $P < 0.0001$), 2 h postbreakfast (8.1 vs. 10.7 mmol/l, $P < 0.0005$), prelunch (8.9 vs. 10.1 mmol/l, $P < 0.01$), and 2 h postlunch (8.0 vs. 9.5 mmol/l, $P < 0.002$). However, there was no difference in mean blood glucose levels before or after the evening meal. Incidence of nocturnal hypoglycemia on overnight profiles was 43% lower on LIS/GLAR compared with R/NPH therapy; however, there was no difference in rates of self-reported symptomatic hypoglycemia. Total insulin dose required to achieve target blood glucose control was lower on LIS/GLAR (1.16 IU/kg) compared with R/NPH therapy (1.26 IU/kg, $P < 0.005$), but there was no significant difference in HbA_{1c} levels (LIS/GLAR versus R/NPH: 8.7 vs. 9.1%, $P = 0.13$).

CONCLUSIONS — Combination therapy with insulin glargine plus lispro reduced the incidence of nocturnal hypoglycemia and was at least as effective as R/NPH insulin therapy in maintaining glycemic control in adolescents on multiple injection regimens.

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Good blood glucose control reduces the risk of long-term diabetes complications (1), and the therapeutic challenge in type 1 diabetes is to achieve near-normal glycemia with minimal risk of hypoglycemia. Tight metabolic control is particularly difficult during adolescence due to endocrine, behavioral, and social factors (2). Intensive insulin therapy using multiple injection regimens (MIR) has been recommended to improve metabolic control (1) but is associated with increased risk of daytime and nocturnal hypoglycemia, particularly in adolescents (3). Recurrent hypoglycemic episodes reduce self-confidence and, in this age group, are a greater concern than long-term complications (4). A major factor responsible for nocturnal hypoglycemia is overinsulinization during the early nighttime (5), which has been attributed to the pharmacokinetic properties of conventional insulin preparations.

Endogenous insulin secretion is characterized by continuous basal insulin secretion and meal-related peaks (6). Basal–bolus insulin therapy aims to mimic physiological insulin secretion and requires both a basal insulin with a stable 24-h serum insulin profile and premeal administration of fast-acting insulin (7). However, conventional intermediate and long-acting human insulin preparations for basal therapy have insulin peaks 4–6 h postinjection, durations of action <24 h, and large variability in absorption (8,9). Regular insulin given premeal has a slower onset and more prolonged action than endogenous insulin secretion. Consequently, the combination of conventional human insulins results in high postprandial blood glucose excursions and risk of hypoglycemia between meals and overnight (10,11).

Insulin analogs have been genetically engineered to produce more physiologi-

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Abbreviations: CV, coefficient of variation; FBG, fasting blood glucose; LIS/GLAR, prebedtime insulin glargine plus preprandial lispro; MIR, multiple injection regimens; R/NPH, prebedtime NPH insulin plus preprandial regular human insulin.

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

cal insulin pharmacokinetics. Insulin lispro is a rapid-acting insulin analog that reduces both postprandial hyperglycemia and between-meal hypoglycemia risk. The insulin analog glargine differs from the human insulin peptide by a glycine-for-asparagine substitution in position A21 and the addition of two arginine residues at the NH_2-terminal of the B-chain. These modifications result in a shift in the isoelectric point from pH 5.4 to 6.7, making insulin glargine less soluble at neutral pH. Insulin glargine is supplied as a clear solution at acidic pH. After subcutaneous injection, the acid in the vehicle is neutralized and insulin glargine precipitates, thereby delaying absorption and prolonging its action (12–15). We hypothesized that the long-acting profile of insulin glargine without a pronounced peak (16) would reduce nocturnal hypoglycemia and improve blood glucose control.

Therefore, we compared the combination of insulin analogs insulin glargine plus lispro with human NPH plus regular human insulin by home blood glucose monitoring and overnight metabolic profiles in adolescents with type 1 diabetes who were already on MIR.

RESEARCH DESIGN AND METHODS

Subjects

Of 47 eligible patients attending the Pediatric Diabetes Clinics at the John Radcliffe Hospital (Oxford, U.K.), 28 patients (13 male, 15 female) entered the screening phase, 26 of whom were randomized to this phase III, active-controlled, two-way, cross-over trial conducted between March 2000 and April 2001. Inclusion criteria were age between 12 and 20 years, currently in puberty (Tanner stage B2/G2 or higher), duration of diabetes longer than 1 year or C-peptide negative, and already using a basal–bolus insulin regimen. Exclusion criteria included renal or hepatic impairment, evidence of diabetic complications, or unstable metabolic control (defined as HbA_{1c} >12%). Two patients were recruited but subsequently withdrew from the study before randomization because they were taking school examinations and believed they would not have the time to attend the clinic for overnight profiles. A third patient withdrew from the study 3 weeks after randomization (on the treatment arm) because she wished to discontinue multiple injection therapy. A total of 25 patients completed the study, and our results are based on these subjects; their clinical characteristics are described in Table 1. The study was approved by the Local Research Ethics Committee; written informed consent was obtained from all patients and, where appropriate, from parents.

Table 1—*Subject characteristics*

Total number of patients in study	25
Male:female ratio	11:14
Age (years)	14.8 (12–18)
Duration of type 1 diabetes (years)	7.3 (1.8–15)
HbA_{1c} (%)	9.3 (7.1–12)
BMI (kg/m^2)	23.2 (18.1–30.4)
Insulin dose (IU/kg)	1.2 (0.3–1.7)

Data are *n* or means (range).

Study design

The study design is summarized in Fig. 1. All patients were already receiving four injections per day (preprandial regular human insulin or lispro and prebedtime NPH or glargine) before enrollment in the study. No additional lispro or regular human insulin was given with the bedtime snack. During the 4-week run-in period (weeks −4 to 0), patients were instructed on use of a glucose meter and insulin doses were individually titrated to achieve optimal blood glucose control using their usual insulin therapy (target premeal and fasting blood glucose (FBG) levels 4.5–9.0 mmol/l while maintaining 0300 blood glucose levels >3.5 mmol/l. At visit 2 (week 0), subjects were randomized to one of the two 16-week treatment regimens: prebedtime insulin glargine plus preprandial lispro (LIS/GLAR) or prebedtime NPH insulin plus preprandial regular human insulin (R/NPH). Subjects were advised to administer their insulin 15–30 min preprandially when on R/NPH and 5–10 min preprandially when on LIS/GLAR. During the first 4 weeks of each treatment arm (active titration phase: weeks 0–4 and weeks 16–20), insulin doses were titrated to achieve the blood glucose targets. Metabolic control was maintained for the following 12 weeks with ongoing adjustment of insulin dose. At the end of each 16-week treatment arm

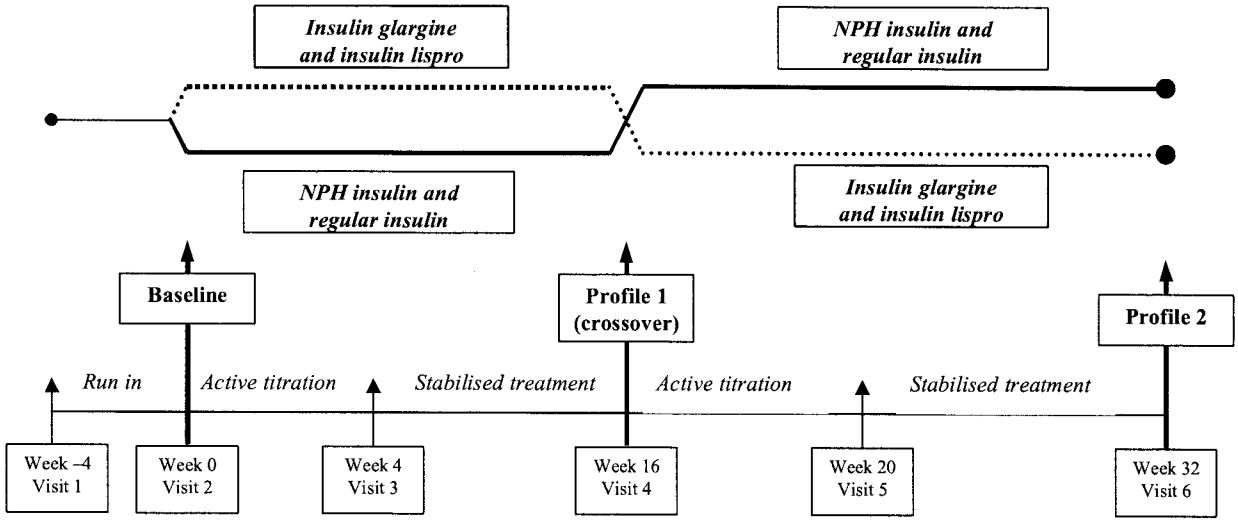

Figure 1—*Study design.*

(weeks 16 and 32), subjects were admitted to the hospital for an overnight metabolic profile. One clinical investigator (N.P.M.) kept in close telephone contact with the patients throughout the study. All subjects had received dietary education as part of routine diabetes care and adjustments in short-acting insulin were made by patients and parents to allow for exercise, food portion size, and blood glucose readings.

Lispro, insulin glargine, NPH, and regular insulins were supplied in 3-ml pen cartridges and self-administered using Optipen (Aventis, Kansas City, MO) or HumaPen (Lilly, Indianapolis, IN) insulin pens.

Monitoring of blood glucose control

Patients recorded home blood glucose measurements on 7 consecutive days before randomization, on each day of the 4-week active titration periods, and on 7 consecutive days every 4 weeks during each treatment arm using the One Touch Profile system (LifeScan, Milpitas, CA). In addition, patients performed an eight-point blood glucose profile (at 0300; immediately before and 2 h after breakfast, lunch, and dinner; and at bedtime) for 1 day in each of the above weeks. HbA_{1c} was measured at each visit (weeks −4, 0, 4, 16, 20, and 32).

Subjects were also asked to record self-monitored blood glucose levels at the time of any episode of symptomatic hypoglycemia throughout the study period. These episodes were classified as symptomatic (symptoms consistent with hypoglycemia and confirmed by a blood glucose reading <2.8 mmol/l), nocturnal (hypoglycemia occurring during the sleep period, after the bedtime injection and before the morning determination of FBG), or severe (hypoglycemia requiring assistance of another person and associated with a blood glucose level <2.8 mmol/l or with prompt recovery after oral administration of carbohydrate or intravenous administration of glucose or glucagon).

Safety monitoring and other adverse events

All treatment-emergent adverse clinical events during the study period were recorded. Serious adverse events were defined as those deemed medically important or those that resulted in hospitalization. Serum transaminase levels, serum creatinine level, and complete blood count were measured at baseline, after active titration, and at the end of each treatment arm. Height and weight were measured using a wall-mounted stadiometer and electronic scales at baseline and at the end of each treatment arm. Age-related SD scores were calculated by comparison with the U.K. 1990 growth reference.

Overnight glucose and insulin profiles

On the day of each overnight metabolic profile, patients were asked to undertake their usual activities, meals, and insulin regimen until admission to the hospital overnight (1800 to 0800). Using ethyl chloride local anesthetic spray, an intravenous cannula was inserted into a hand vein for blood sampling. Subjects had their short-acting insulin before a standard evening meal (50% carbohydrate, 15% protein, and 35% fat) and had a snack before their prebed insulin at 2200. Evening meals and snacks were adjusted to their usual intake of carbohydrate established by a dietitian. Subjects went to bed at 2230 and were encouraged to sleep.

Venous blood samples were collected every 15 min for determination of blood glucose level (measured the next day). Nocturnal hypoglycemia was defined as two consecutive blood glucose values <3.5 mmol/l occurring between 2230 and 0800. Episodes of symptomatic hypoglycemia occurred overnight in only two patients (once in each treatment arm at 0245 and 0300). These episodes were treated with 20 g oral carbohydrate, and biochemical data from the remainder of these nights were excluded from analyses.

Assays

Blood glucose was measured using a YSI model 2300 Stat Plus analyzer (YSI, Yellow Springs, OH) on whole blood collected into fluoride oxalate bottles. The intraassay coefficient of variation (CV) at 4.4 mmol/l was 2.6% and the equivalent interassay CV was 3.1%.

HbA_{1c} was measured by high-performance liquid chromatography (Clinserv Laboratories, Hamburg, Germany). The intra-assay CVs were 1.53 and 0.94% at 5.9 and 14.4%, respectively, and interassay CVs were 1.3 and 1.8% at 5.4 and 9.7%, respectively.

Calculations and statistics

The primary outcome variable, nocturnal hypoglycemia on metabolic profiles, was analyzed using McNemar's test for difference in proportions, and unpaired analysis was performed using χ^2 test. The continuous variables HbA_{1c} and blood glucose were compared between insulin treatment regimens using paired Student's *t* tests and ANCOVA to compare within-subject treatment difference. Positively skewed data such as blood glucose levels were log-transformed to normal distributions to allow use of parametric tests. Overnight profile data were divided into "postprandial," until the bedtime snack and insulin injection at 2230, and "overnight," between 2230 and 0800. Data were analyzed using SPSS version 10.0 (SPSS, Chicago, IL).

RESULTS

Blood glucose home monitoring

FBG levels were lower on LIS/GLAR (mean 8.0 mmol/l) compared with either baseline (9.8 mmol/l, $P < 0.0001$) or R/NPH (9.2 mmol/l, $P < 0.0001$). Ascertainment of self-monitored FBG data was high (97% of all expected data were recorded). The rate of achievement of target FBG (4.5–9.0 mmol/l) was similar on LIS/GLAR (38%) and R/NPH therapies (35%).

LIS/GLAR also resulted in lower blood glucose levels than R/NPH at 2 h postbreakfast (mean 8.1 vs. 10.7 mmol/l, $P < 0.0005$), prelunch (8.9 vs. 10.1 mmol/l, $P < 0.01$), and 2 h postlunch (8.0 vs. 9.5 mmol/l, $P < 0.002$) on the self-monitored eight-point profiles (Fig. 2), but there were no differences predinner, 2 h postdinner, prebedtime, or at 0300.

There was no significant difference between LIS/GLAR and R/NPH in numbers of self-recorded symptomatic hypoglycemic episodes (294 vs. 250, respectively; $P = 0.27$) or nocturnal hypoglycemic episodes (29 vs. 41, respectively; $P = 0.17$). No severe hypoglycemic events were reported during the study period.

Insulin doses

Subjects on LIS/GLAR therapy required significantly lower total insulin doses (1.16 IU/kg) to achieve home blood glucose targets compared with those on R/NPH therapy (1.26 IU/kg, $P < 0.005$). On LIS/GLAR and R/NPH therapies, re-

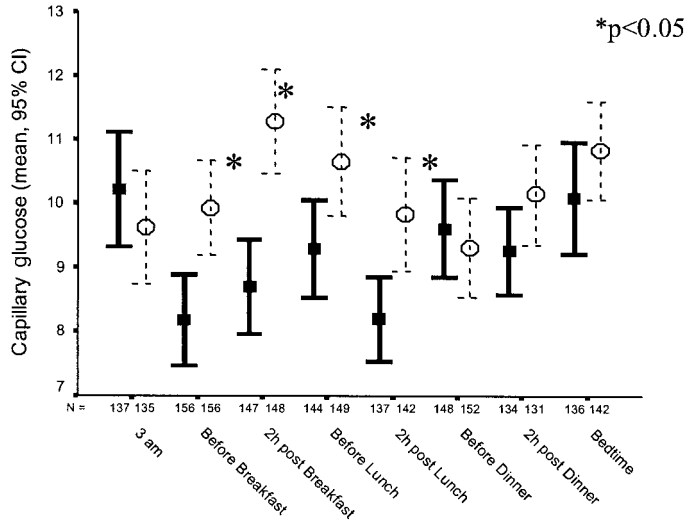

Figure 2—*Geometric mean blood glucose levels during 24-h (eight-point) profiles on LIS/GLAR and R/NPH regimens. Bold error bars and black squares = LIS/GLAR; dashed error bars and white circles = R/NPH therapy.*

ductions were seen in both long-acting insulin doses (0.56 vs. 0.62 IU/kg, respectively; $P = 0.01$) and short-acting insulin doses (0.60 vs. 0.64 IU/kg, respectively; $P = 0.01$), and the ratio of short- to long-acting insulin doses was similar on each regimen ($P = 0.3$).

HbA_{1c} levels

Despite the lower insulin doses on LIS/GLAR therapy, there was a trend toward lower HbA_{1c} levels, although this difference was not significant (overall mean for both study periods on each treatment: LIS/GLAR versus R/NPH, 8.7 vs. 9.1%; $P = 0.13$). HbA_{1c} levels by both treatment arm and study period are shown in Table 2. The slight initial improvements in HbA_{1c} levels during period 1 in both treatment arms were not significant (LIS/GLAR −0.6%, R/NPH −0.7%). During study period 2, HbA_{1c} levels increased by 1.0% (+0.5% compared with baseline) in patients who changed from LIS/GLAR back to R/NPH, whereas patients who were randomized to receive LIS/GLAR second maintained their lower HbA_{1c} levels (−0.5% compared with baseline) (Table 2).

Overnight blood glucose profiles

LIS/GLAR was associated with fewer episodes of nocturnal hypoglycemia (8 of 25 nights, 32%) compared with R/NPH (14 of 25 nights, 56%). Paired analysis using McNemar's test did not reach statistical significance ($P = 0.1$), but an unpaired comparison (χ^2 test) showed that this reduction in nocturnal hypoglycemia with LIS/GLAR was significant ($P < 0.05$). Mean overnight blood glucose levels were significantly lower on R/NPH insulin therapy (mean 5.6 mmol/l, range 4.1–10.8) compared with LIS/GLAR (mean 7.5 mmol/l), range 5.4–12.9; $P = 0.02$; Fig. 3), despite no differences in blood glucose levels on admission at 1800 (LIS/GLAR versus R/NPH, 7.3 vs. 7.1 mmol/l; $P = 0.9$) or on fasting the next morning at 0800 (LIS/GLAR versus R/NPH, 6.6 vs. 6.2 mmol/l; $P = 0.6$; Fig. 3). Time at onset of nocturnal hypoglycemia was later on LIS/GLAR (median time 0345) than on R/NPH treatment (0145), but there was no difference in duration of hypoglycemia (1.75 vs. 1.87 h, respectively).

Adverse events

A total of 50 treatment-emergent adverse events were recorded during the study: 21 events in 13 patients on LIS/GLAR and 29 events in 15 patients on R/NPH. Most of these events were mild and unrelated to insulin therapy (e.g., mild viral upper respiratory tract infections). Only one event was classified as serious: a patient on R/NPH insulin therapy required a 15-h hospital admission during an episode of gastroenteritis. The only potential causally related adverse event was transient pain in the injection site reported by one patient on LIS/GLAR. This pain was mild and did not necessitate discontinuation of the study insulin.

No clinically relevant treatment-related changes in biochemical or hematological variables were observed, and there were no changes in age-adjusted weight SD scores between baseline (0.95) and the end of LIS/GLAR (0.96) or R/NPH treatment (0.96), indicating normal weight gain for age.

CONCLUSIONS — This 32-week, two-way, cross-over, randomized, open-label study demonstrated that the analog combination LIS/GLAR reduced the incidence of asymptomatic nocturnal hypoglycemia in adolescent patients on MIR compared with conventional R/NPH. In addition, LIS/GLAR lowered blood glucose levels from prebreakfast to 2 h postlunch, with no differences in blood glucose levels pre–evening meal or prebedtime or in frequency of daytime hypoglycemic episodes. Both long- and short-acting insulin doses required to achieve target blood glucose levels were lower on LIS/GLAR than on R/NPH therapy, and the LIS/GLAR combination was at least equally effective as R/NPH therapy at maintaining glycemic control as reflected by HbA_{1c} levels. Weight gain is a major disincentive to teenagers on intensive insulin regimens; however, age-adjusted body weight did not increase during this study.

This is the first study to examine the combination of insulin glargine and lispro in adolescents on MIR. In a recent randomized prospective 28-week study of adults with type 1 diabetes on MIR with

Table 2—*HbA_{1c} and change from baseline during each study period*

	1) LIS/GLAR 2) R/NPH			*1*) R/NPH 2) LIS/GLAR		
Sequence	*n*	Mean HbA_{1c} (%)	Change (%)	*n*	Mean HbA_{1c} (%)	Change (%)
Baseline	12	9.4	—	13	9.1	—
Period 1	12	8.9	−0.6	13	8.4	−0.7
Period 2	12	9.9	0.5	13	8.6	−0.5

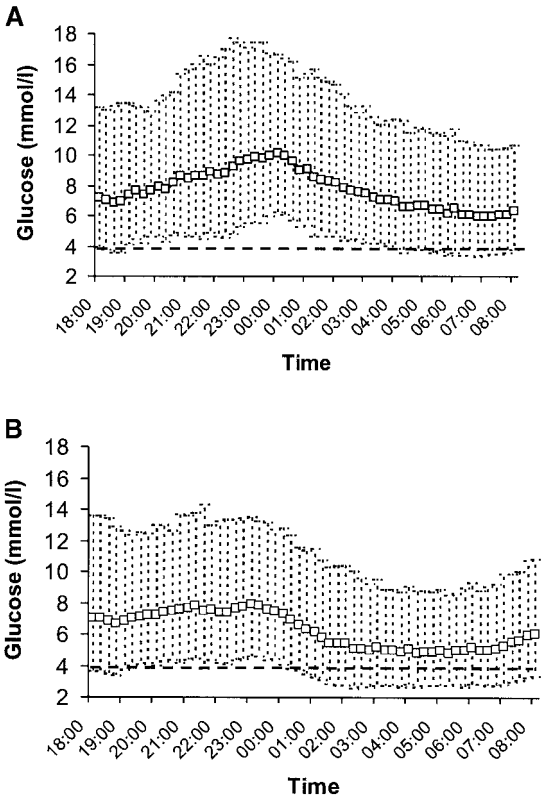

Figure 3—*Geometric mean blood glucose levels (plus 1-SD confidence intervals) on overnight metabolic profiles from 1800 to 0800: LIS/GLAR* (A) *and R/NPH* (B).

regular human insulin, insulin glargine lowered FBG levels compared with NPH insulin (17). Another large prospective study of adults with type 1 diabetes on MIR using lispro also reported that insulin glargine consistently lowered fasting glucose levels compared with NPH; again, there were no differences in HbA_{1c} levels (18). Those authors suggested that the failure of lower FBG levels to translate to improved HbA_{1c} might be due to higher blood glucose levels at other times of the day. However, our results do not support this hypothesis, because blood glucose profiles remained lower on LIS/GLAR throughout the daytime until the evening meal. Thereafter, there was no difference in blood glucose levels between LIS/GLAR and R/NPH therapies.

So why were HbA_{1c} levels in our study not lower on LIS/GLAR? One possible explanation may be that the duration of treatment (16 weeks) was too short for the full impact of these improvements to be manifested. Second, our study was not powered to detect a difference in HbA_{1c} levels, and because these levels may be variable during adolescence, much larger numbers may be required. To confirm the observed difference in HbA_{1c} on LIS/GLAR versus R/NPH therapy with 80% power at 5% significance, a study of 85 patients in a similar cross-over design would have been necessary. Third, it is possible that our target FBG was too high; the mean overnight blood glucose level on LIS/GLAR was 7.5 mmol/l and was higher than on R/NPH therapy. However, fear of nocturnal hypoglycemia is a major barrier to optimizing overnight glycemic control, and it remains to be proven whether more aggressive dose titration on LIS/GLAR (e.g., with target FBG of 4.5–6.5 mmol/l) is possible without increasing the incidence of nocturnal hypoglycemia.

Although our HbA_{1c} results are disappointing, even in the Diabetes Control and Complications Trial (1) insulin intensification was less successful in adolescents than in adults. The initial improvement in HbA_{1c} levels that we noted in both treatment groups during the first study period was likely an effect of more intensive surveillance, as also seen in the Diabetes Control and Complications Trial. The maintenance of HbA_{1c} levels in patients who switched from R/NPH to LIS/GLAR therapy, coupled with the deterioration seen in most patients who switched back to R/NPH after 16 weeks of LIS/GLAR therapy, may reflect a preference for the insulin analog combination, and indeed, on study completion, 21 of 25 patients chose to continue with LIS/GLAR. Although this study did not include a formal assessment of quality of life, some subjects liked the convenience of injecting lispro immediately preprandially and the ability to omit snacks. The apparent disadvantages of the standard single daily NPH used in MIR might be reduced by using twice or multiple daily NPH. However, such alterations are often perceived as less convenient than single daily basal insulin.

Nocturnal hypoglycemia is a major adverse effect of and disincentive to achieving good glycemic control (19). The true incidence of hypoglycemia may be underestimated because minor episodes are often not recorded, and nocturnal hypoglycemia is usually asymptomatic (11,20). However, incidence rates up to 70% in children and 50% in adolescents have been reported (20,21). A unique aspect of this study was the use of overnight metabolic profiles to accurately detect nocturnal hypoglycemia. Patients had fewer episodes of nocturnal hypoglycemia on LIS/GLAR than on R/NPH therapy, and this difference was significant on χ^2 testing. Nocturnal hypoglycemia in children and adolescents with diabetes has been shown previously to relate to characteristics of the insulin regimen used, rather than individual susceptibility, which might explain the greater power and appropriateness of this unpaired test than the paired McNemar's test. All but two nocturnal hypoglycemia episodes were asymptomatic. Asymptomatic nocturnal hypoglycemia is important because it may impair counter-regulatory responses and increase the risk of subsequent more prolonged and severe hypoglycemia (22), and it has also been implicated in the increased mortality rates in young adults with type 1 diabetes (23).

In summary, the analog combination of LIS/GLAR was well tolerated with no significant adverse effects, and on completion of the study, most patients chose to stay on this combination. The incidence of nocturnal hypoglycemia was reduced on LIS/GLAR, which was at least as effective as R/NPH therapy in maintaining glycemic control.

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