Additional Lunchtime Basal Insulin During Insulin Lispro Intensive Therapy in a Randomized, Multicenter, Crossover Study in Adults

A real-life design

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OBJECTIVE — This study was performed to evaluate whether an additional dose of NPH insulin at lunchtime might overcome the deleterious effects of waning basal insulinemia on pre-dinner and evening glucose values during insulin lispro intensive therapy with once daily basal insulin at night.

RESEARCH DESIGN AND METHODS — The study was a 10-month multicenter, randomized, crossover trial. After a 2-month run-in period, subjects injected NPH insulin once (1 × NPH) or twice (2 × NPH) daily for 4 months in a randomized order. Adult patients were included if they had HbA_{1c} levels <8.5%. Efficacy measures were HbA_{1c} levels, 8-point glucose profiles, and the frequency of hypoglycemia. The statistical analysis included a within-patient comparison for crossover trials.

RESULTS — In all, 104 patients completed the trial. The mean HbA_{1c} level before randomization was 7.1 ± 0.85%. The HbA_{1c} levels did not change significantly within patients (*t* test, mean difference = 0.06%; 95% confidence interval [CI] −0.073 to 0.20). The pre-dinner blood glucose values were significantly lower during the 2 × NPH daily protocol, with a mean difference of 0.76 mmol/l (*t* test, $P = 0.004$; CI 0.25 to 1.3). In the evening, the frequency of hypoglycemia increased significantly during the 2 × NPH daily protocol with a median difference of 0.56 mild episodes/30 days ($P = 0.001$) and 6.9 severe episodes/patient year ($P = 0.007$), respectively.

CONCLUSIONS — Equal HbA_{1c} levels and increasing frequencies of hypoglycemia in the evening overshadow the slight improvement of the evening glucose profiles during a regimen with 2 × NPH daily insulin. Therefore, generalized use of a second injection of NPH insulin at lunchtime cannot be recommended to all adult patients with type 1 diabetes using intensive insulin lispro therapy.

Diabetes Care 25:712–717, 2002

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Received for publication 20 June 2001 and accepted in revised form 30 December 2001

Abbreviations: CI, confidence interval; RMANOVA, repeated measures ANOVA.

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

Insulin analogs have been developed to obtain physiological insulin levels resulting in near normal glycemia with minimal frequencies of hypoglycemia. A regimen using the rapid-acting insulin analog insulin lispro (Humalog; Eli Lilly, Indianapolis, IN) combined with once daily bedtime NPH insulin decreases the frequency of severe hypoglycemia, especially during the night (1,2), and improves postprandial glucose excursions (3). However, in most studies, insulin lispro with once daily basal insulin has failed to reduce HbA_{1c} levels (4), perhaps because the waning basal insulinemia in the afternoon resulted in increased pre-dinner and evening glucose values.

Only few comparative studies are available to determine which basal insulin injection regimen is superior for insulin lispro intensive therapy. In one study comparing short-acting regular human insulin regimen with 1–2 daily NPH insulin versus mealtime insulin lispro in combination with multiple doses (3–4 times daily) of NPH insulin injections, the latter combination resulted in improved overall metabolic control (5). However, that study did not address whether once daily NPH insulin in combination with insulin lispro is actually insufficient.

Still, the optimum number of basal insulin injections for insulin lispro intensive therapy has not been determined because studies have not been randomized (6,7) or have not had control subjects using a rapid-acting insulin (5,8). Consequently, we performed this crossover study to determine whether an additional lunchtime dose of basal insulin in patients with type 1 diabetes using insulin lispro intensive therapy had beneficial effects on glycemic control, as reflected in improved HbA_{1c} and blood glucose profiles and equal frequencies of hypoglycemia. Patients with well-controlled diabetes suffer the most from the limitations imposed by

hypoglycemic events. Therefore it was decided to study only patients with HbA_{1c} <8.5%.

RESEARCH DESIGN AND METHODS

Patients

We recruited adults with type 1 diabetes from 15 outpatient diabetes clinics in the Netherlands. Their diagnosis was based on C-peptide criteria (plasma C-peptide <0.30 nmol/l 6 min after 1 mg glucagon i.v.). All eligible patients gave written informed consent and met the following inclusion criteria: an HbA_{1c} <8.5%, using insulin lispro intensive therapy for >3 months combined with once or twice daily NPH insulin, age 18–65 years, and the ability and willingness to do regular home blood glucose monitoring. Patients were excluded if they lived alone; had hypoglycemia unawareness, determined from the history taken by the investigator or evidenced by having needed assistance for hypoglycemia in the previous 3 months; had a BMI >29 kg/m^2; had decreased insulin sensitivity (daily dosage of insulin >1.5 IU/kg); had major microvascular complications, such as proliferative retinopathy, proteinuria (>300 mg/24 h), or angina pectoris New York Health Academy (NYHA) class ≥ III; was pregnant; was breastfeeding; had childbearing potential without adequate contraception; abused drugs or alcohol; or used systemic corticosteroids or oral antidiabetic drugs.

Design

The study was a 10-month randomized, multicenter trial with an open-label crossover design. After a 2-month run-in period, subjects injected NPH insulin once (1 × NPH) or twice (2 × NPH) daily for 4 months in a randomized order. An independent person made a block randomization for each investigator. The investigator disclosed the assignment of the allocated schedule during the participant's second (baseline) visit to the outpatient clinic.

All of the patients maintained mealtime subcutaneous injections of insulin lispro (Humalog; Eli Lilly) throughout the study. Patients entered the run-in period using once daily NPH insulin delivered subcutaneously (Humulin, Eli Lilly or Insulatard, Novo Nordisk, Bagsvaerd, Denmark) at bedtime. The second dose of NPH insulin was injected at lunchtime, since lunchtime is closer to a 12-h time interval after the bedtime dose than breakfast time. Moreover, the aim of decreasing pre-dinner and bedtime glucose concentrations was considered more attainable after a lunch dose than after a morning dose of NPH. Patients switched to 2 × NPH starting with a lunchtime NPH insulin injection of 40% of their last bedtime NPH dose, with a minimum dosage of 4 IU. The lunchtime NPH insulin dose was injected before 1330. The patients were asked to continually optimize glycemic control using the following targets: preprandial blood glucose 4–7 mmol/l, postprandial glucose <8 mmol/l, and bedtime glucose 7–9 mmol/l. The investigators advised patients on the insulin dosage adjustment at each 2-month visit and by telephone 3 weeks after screening/inclusion, randomization time, and crossover time. It was recommended that the lunchtime NPH insulin dosage be increased if the pre-dinner glucose level was >7 mmol/l or the bedtime glucose level was >9 mmol/l dose, and be decreased if hypoglycemia occurred >3 h after the meal. The mealtime insulin lispro dosage was increased if the postprandial glucose excursion was >1 mmol/l and decreased if hypoglycemia occurred up to 3 h postprandial.

The study was conducted in accordance with the Guidelines of Good Clinical Practice and the Declaration of Helsinki 1995 after approval by local ethics committees.

Efficacy measures

Primary efficacy measures were HbA_{1c}, incidence and timing of hypoglycemic episodes, and home blood glucose profiles. Additional efficacy measures included body weight and insulin dosages.

Patients recorded their own glucose profiles twice weekly in a specially designed diary. Patients collected four 8-point glucose profiles before every 2-month visit. Glucose was measured at bedtime, 0430, preprandial, and 2-h postprandial (Accutrend Sensor; Boehringer Mannheim, Mannheim, Germany). In addition, at least two 4-point glucose profiles were required every week throughout the study. Patients recorded details of hypoglycemic events in the same diary. Hypoglycemia was defined as mild when the patient subjectively experienced symptoms associated with hypoglycemia or if glucose readings were ≤ 3 mmol/l. A severe hypoglycemic event was defined as an event that required the assistance of another person. To identify hypoglycemia unawareness, patients recorded if they noticed symptoms of hypoglycemia. Asymptomatic hypoglycemia was defined as an event with a glucose reading ≤3 mmol/l in absence of symptoms. We calculated frequencies of hypoglycemia with the recorded number of hypoglycemic events in the 2 months before crossover and before the end of trial.

Assay methods

Local laboratories measured HbA_{1c} values for clinical use every 2 months during the study period. At randomization, crossover, and the end of the trial, hemolyzed blood was sampled and stored at −70°C (9). At the termination of the study, a central laboratory measured all of the stored HbA_{1c} samples using high-performance liquid chromatography (normal range 4.0–6.0%; Pharmacia LKB, Bromma, Sweden). The values of the stored samples were used for statistical analysis.

Statistical analysis

We calculated the sample size based on the main outcome variable (i.e., the HbA_{1c} level). According to previous studies, we assumed an SD = 1.1. To detect a significant and clinically relevant intra-individual HbA_{1c} difference of 0.3% with a statistical power of 0.8 ($\beta = 0.2$) and a two-sided α of 0.05, and assuming a correlation of 0.5 between the paired values, 106 patients were needed.

Patient treatment was executed on the "intention to treat" principle. However, a crossover analysis requires that outcome data be provided on both study periods. Consequently, outcome data of dropouts were excluded from the analysis.

Crossover statistics were based on within-patient differences. In this study, a within-patient difference was defined as the difference of outcome between treatments (1 × NPH − 2 × NPH) in one patient. The mean difference refers to the average of all the within-patient differences. Interaction tests were used to check data for period and carryover effects (10), which did not occur unless otherwise stated. A period effect was defined as a significant difference in treatment result between the two sequence groups.

Continuous variables, generally pre-

Table 1—*Baseline variables of randomized participants*

	Group 1: 1 ×NPH–2 × NPH	Group 2: 2 × NPH–1 × NPH
n	61	60
Age (years)	39.5 ± 11.4	39.1 ± 12.7
Sex (male/female)	33/28*	44/16*
Duration of diabetes (years)	12 (median) 1–36 (range)	14 (median) 1–45 (range)
BMI (kg/m^2)	24.3 ± 2.59	24.2 ± 2.86
Total daily insulin dosage (IU)	57.4 ± 18.4	61.3 ± 19.3
HbA_{1c} (%)	7.2 ± 0.93	7.0 ± 0.85
Using 2 × NPH before screening (*n*)	9†	2†

Data are means ± SD unless otherwise indicated. *$P = 0.04$; †$P = 0.05$, Fisher's exact test.

sented as means ± SD, were evaluated using a paired *t* test based on a within-patient analysis. With an overall repeated measures ANOVA (RMANOVA), the outcomes of the overall 8-point glucose profiles were analyzed. The pre-dinner, 2-h post-dinner, and bedtime glucose values were evaluated separately with a paired *t* test. Data that could not be considered to follow a normal distribution (i.e., hypoglycemic event) were analyzed with a Wilcoxon's signed-rank test; because this test calculates significance levels using only the cases in which a within-patient difference exists (values ≠ 0), only those cases are shown in Table 2. Statistical analysis was undertaken with SPSS software (Version 10.0).

RESULTS — In all, 138 patients entered the run-in period. The 17 nonrandomized patients included 9 who did not meet the inclusion criteria, 6 who chose not to enter the study, 1 who was withdrawn at the investigator's discretion, and 1 who did not tolerate once daily NPH insulin. Of the 121 randomized patients, 104 completed both study periods; 3 patients withdrew because of increased hypoglycemia during the 2 × NPH protocol, 1 did not tolerate the 1 × NPH protocol, 6 did not endure the study load, 5 were noncompliant, 1 moved abroad, and 1 became pregnant.

Baseline characteristics, given in Table 1, did not differ significantly between groups, except for a slight gender difference ($P = 0.04$). The distribution of the two sequence groups differed regarding the number of patients who had used 2 × NPH before the study: nine subjects were in the 1 × NPH−2 × NPH sequence, and two subjects were in the 2 × NPH−1 × NPH sequence (Fisher's exact test, $P = 0.05$). At baseline, the HbA_{1c} level was not significantly different between groups (Table 1).

HbA_{1c} and blood glucose profiles

The results of HbA_{1c} levels are presented in Table 2. HbA_{1c} analysis was performed with a sample size of 102 patients, as 2 patients were missing values. The mean HbA_{1c} level was 7.2 ± 0.92% during the 1 × NPH protocol and 7.1 ± 0.95% during the 2 × NPH protocol. HbA_{1c} levels were similar for both therapy regimens.

Glucose levels are presented in Fig. 1. Within subjects, the overall 8-point glucose profiles revealed a trend toward an overall lower glucose level (8-point profile) during the 2 × NPH protocol (RMANOVA, $P = 0.085$). Pre-dinner glucose values were 0.76 mmol/l lower during the 2 × NPH than during the 1 × NPH protocol (*t* test, $P = 0.004$; CI 0.25–1.27). The 2-h post-dinner glucose values were 0.66 mmol/l lower during the 2 × NPH protocol (*t* test, $P = 0.023$; CI 0.09–1.22). In addition, the post-dinner glucose values showed a period effect ($P = 0.018$, independent samples *t* test): in the 1 × NPH−2 × NPH sequence group, the 2-h post-dinner glucose value was 0.022 ± 2.8 mmol/l lower, and in the 2 × NPH−1 × NPH sequence group, the 2-h post-dinner glucose value was 1.4 ± 2.7 mmol/l lower during treatment with 2 × NPH.

Hypoglycemia

Differences in frequencies of mild, severe, and asymptomatic hypoglycemia are presented in Table 2. The frequency of mild hypoglycemia in the evening hours and nighttime increased during the 2 × NPH protocol, with a median difference of 0.56 events/30 days (range −3.4 to 5.5; $P = 0.001$) and 0.48 events/30 days (range −1.5 to 3.4; $P = 0.053$), respectively.

A between-sequence group difference of the frequency of mild hypoglycemia in the evening resulted in a significant period effect: the treatment sequences 1 × NPH−2 × NPH and 2 × NPH−1 × NPH had a median within-patient difference of 0.27 (range −3.4 to 4.6) events/30 days, and 0.86 (range −2.1 to 5.5) events/30 days, respectively ($P = 0.004$, Mann Whitney *U* test), with the hypoglycemia

Table 2—*Mean difference of HbA_{1c} and median differences of hypoglycemic frequencies during the study periods (1 × NPH minus 2 × NPH)*

	n	Difference	*P*
HbA_{1c}	102	0.061 ± 0.068	NS
Mild hypoglycemia (events/30 days)	96	−0.93 (−13.7 to 15.4)	0.002*
00.00–05.59 h	62	−0.48 (−3.4 to 1.5)	0.053
06.00–11.59 h	83	−0.25 (−5.1 to 7.11)	NS
12.00–17.59 h	78	−0.40 (−6.2 to 3.8)	NS
18.00–23.59 h	70	−0.56 (−5.5 to 3.4)	0.001*
Severe hypoglycemia (events/patient years)	28	−5.8 (−95.6 to 21.5)	0.083
00.00–05.59 h	9	−5.8 (−9.1 to 17.4)	NS
06.00–11.59 h	11	−5.8 (−43.4 to 6.6)	NS
12.00–17.59 h	12	−5.5 (−17.4 to 7.3)	NS
18.00–23.59 h	11	−6.9 (−26.0 to 5.8)	0.007
Asymptomatic hypoglycemia (events/30 days)	71	0.06 (−12.8 to 5.2)	NS

Data are means ± SE or median (range), unless otherwise noted. Mild, severe, and asymptomatic hypoglycemia are presented of selected patients with unequal (≠0) frequencies during 1 × NPH and 2 × NPH. Patients with equal hypoglycemic frequencies during 1 × NPH and 2 × NPH were not included in the results. Frequencies of hypoglycemia within the remaining patient subsets were used to determine differences between treatments. HbA_{1c} significance determined by *t* test, all other levels of significance determined using Wilcoxon's signed-rank test. *Additional period effect (Mann-Whitney *U* test).

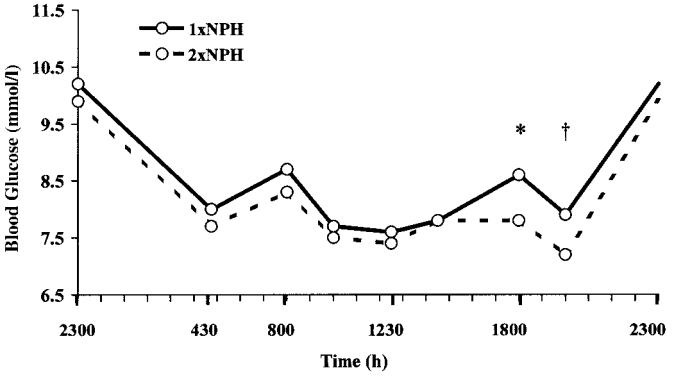

Figure 1—*The 8-point glucose profiles. *paired* t *test, mean difference = 0.76 mmol/l,* P *= 0.004, CI 0.25–1.27; †paired* t *test, mean difference = 0.64 mmol/l,* P *= 0.027, CI 0.1–1.2, period effect.*

rate being higher during treatment with 2 × NPH in both cases.

The overall (both study periods) frequency of severe hypoglycemic events was 1.8 events per patient year. The frequency of severe hypoglycemia in the evening hours increased during the 2 × NPH protocol, with a median difference of 6.9 events/patient year (range 5.8–26; $P = 0.007$). Three patients were hospitalized in the 2 × NPH protocol because of severe hypoglycemia leading to coma with an epileptic insult ($n = 2$) or a car accident ($n = 1$).

Body weight and insulin dosage

Body weight and the total daily insulin dosages were similar in both treatment regimens. During the 2 × NPH protocol, the total daily dosage of insulin lispro was 3.7 IU lower ($P < 0.001$; CI 2.3–5.1), whereas the total daily dosage of NPH insulin was 3.3 IU higher ($P < 0.001$; CI 2.5–4.0). The mean lunch NPH dosage was 7.2 IU (median 6 IU, range 3–18), which was on average 35% (median 30%, range 15–80%) of the bedtime dosage.

CONCLUSIONS

— The most important and unexpected finding observed in this study was that no improvement in HbA_{1c} levels occurred in patients using twice daily basal insulin during insulin lispro intensive therapy. As expected, during the 2 × NPH protocol, the pre-dinner glucose concentrations improved, but bedtime and nighttime glucose levels remained equally as high as with the 1 × NPH protocol. The frequency of severe hypoglycemia increased in the evening hours during the 2 × NPH protocol. Mild hypoglycemia increased mainly in the evening hours as well, although the period effect indicated that the group injecting 2 × NPH in the second treatment period was able to avoid mild hypoglycemia.

Our main result—the absence of an advantageous HbA_{1c} level using 2 × NPH insulin—was not expected. The following considerations validate this negative result. First, a type II error is unlikely, because we overestimated the HbA_{1c} group variance of 1.1 (2) in the power analysis; in reality, the group variance was only 0.76 and the available sample size of 102 patients was abundant. Second, a selection bias might have occurred. Although two dropout subjects did not tolerate 1 × NPH, several patients who already used twice daily NPH did not consent to participate in the study because they did not want to switch back to 1 × NPH. Some factors lighten the gravity of this possible selection bias. In 8 of 15 centers, no patient used twice daily NPH insulin before this study, whereas in 4 of 15 centers no patient refused to abort a twice daily NPH regimen. Moreover, the eight participants who had prior experience with 2 × NPH and completed the study did not show improved HbA_{1c} levels during the 2 × NPH protocol. Finally, it was hypothesized that the 4-month period was sufficient to adapt to the experimental 2 × NPH regimen. After all, the efficacy of insulin regimens in most studies to date have been evaluated with treatment periods of 3 months.

Perhaps a subset of patients could be identified who did benefit from 2 × NPH. After all, the subset of patients with very tight glycemic control might have less room for HbA_{1c} improvement, whereas the subset of patients with moderate glycemic control might have benefited more from 2 × NPH. Also, some authors emphasize that the long mealtime interval between lunch and supper in Mediterranean countries compared with northern European countries (8) such as the Netherlands could be an argument for an additional injection of basal insulin in intensive insulin lispro therapy. We performed an analysis to identify the mentioned subsets of patients benefiting from the additional NPH insulin and found that not baseline HbA_{1c} levels ($r = -0.011$, NS) (Fig. 2), nor the lunch-to-dinner interval at randomization ($r = 0.013$, NS), nor the pre-dinner glucose concentrations at randomization ($r =$

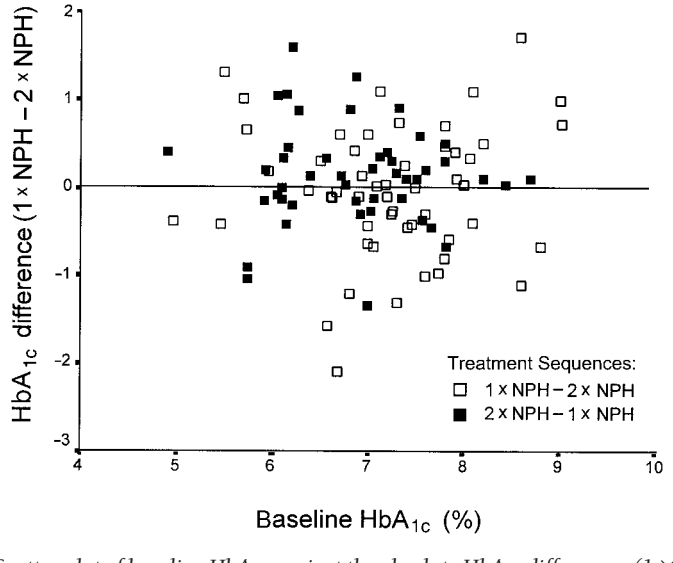

Figure 2—*Scatter plot of baseline HbA_{1c} against the absolute HbA_{1c} differences (1 × NPH minus 2 × NPH) during the study.* r *= 0.011, NS*

0.012, NS) could predict a decreased HbA_{1c} level during the 2 × NPH protocol.

The other outcome measures, glucose profiles and frequencies of hypoglycemia, revealed a few advantages of the 2 × NPH regimen. The trend toward a lower overall glucose level (RMANOVA) during the 2 × NPH protocol can be explained by the significantly decreased pre-dinner glucose values. These lower concentrations were insufficient to lower HbA_{1c} levels, but were accompanied by increased frequencies of severe hypoglycemia in the evening in a small group of patients (n = 11). Less evident was the increased frequency of mild hypoglycemia during the 2 × NPH protocol, because a period effect occurred. This period effect, indicating less increased frequencies of mild hypoglycemia in the evening hours during 2 × NPH in the group with the treatment sequence 1 × NPH−2 × NPH, suggests that the investigators in the first study period became aware of increased hypoglycemia in the evening hours and advised the patients starting with 2 × NPH in the second study period to inject less predinner insulin lispro. This suggestion is supported by the occurrence of a period effect in the post-dinner glucose concentrations, but cannot be confirmed by a period effect of lispro or NPH insulin dosages.

Despite our attempts to lower bedtime, nighttime, and fasting glucose values, these values remained high in both treatment regimens. Both the actual risk of late afternoon/evening hypoglycemia and the fear of nighttime hypoglycemia may have precluded the achievement of our goals. We feel that the prolonged exposure to high glucose values at night might have prohibited the actual improvement in metabolic control. On the other hand, in addition to decreased postprandial glucose excursions and increased flexibility, the important improvement of rapid-acting insulin analogs is less severe nighttime hypoglycemia (1,2), which might evolve from higher glucose levels at night.

Why did expectations of improving basal insulinemia by a second dose of NPH insulin run high? A clamp study of both insulin lispro and NPH insulin administration resulted in improved postprandial glucose profiles (11). Nonrandomized studies for once or more daily NPH have also confirmed the beneficial effects of multiple daily NPH insulin (6,7,12). Finally, randomized parallel studies have shown improved metabolic control on multiple low dosages (3–4 times daily) of NPH insulin with mealtime insulin lispro compared with regular human insulin with once or twice daily NPH insulin (5,8). However, a clamp study is an artificial, laboratory-based situation that cannot be projected directly into the reality of day-to-day experiences of diabetic patients. It is doubtful whether the latter studies are compatible with real-life diabetes management, because the studies were performed in one highly specialized center with a 1–2 week interval of patient visits and frequent (even daily) telephone contacts (5,8). More recently, a regimen with human soluble insulin with once or twice daily NPH was compared with the rapid-acting insulin analog aspart with multiple (3–4) daily injections of NPH; the latter regimen did not result in an improvement of HbA_{1c} or overall glycemic control (13). Thus we feel that sound evidence for benefits of multiple NPH insulin regimens in day-to-day diabetes care is lacking.

Although the possibility cannot be excluded that twice daily NPH insulin on an individual basis can be beneficial in obtaining metabolic control, overall, the equal HbA_{1c} levels and increased frequency of hypoglycemia events in the evening overshadowed the slight improvement in evening glucose profiles during a regimen with twice daily NPH insulin. Therefore, generalized use of a second injection of NPH insulin at lunchtime cannot be recommended to all adult patients with good glycemic control in type 1 diabetes using intensive insulin lispro therapy.

APPENDIX

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Acknowledgments— We acknowledge the contributions of D. van Loon and B. Smaling, in the clinical laboratory of St. Antonius Hospital, Nieuwegein. We also thank C. van Bolhuis (Eli Lilly Nederland BV, Houten, The Netherlands) for advice in executing this trial. Eli Lilly & Company supported this study by contributing a grant.

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