Contribution of Postprandial Versus Interprandial Blood Glucose to HbA_{1c} in Type 1 Diabetes on Physiologic Intensive Therapy With Lispro Insulin at Mealtime

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OBJECTIVE — To quantitate the contribution of postprandial blood glucose, which improves with the short-acting insulin analog lispro [Lys(B28),Pro(B29)] in type 1 diabetes, to the overall 24-h blood glucose concentration and the long-term HbA_{1c} concentration under conditions of different postabsorptive blood glucose.

RESEARCH DESIGN AND METHODS — A total of 24 type 1 diabetic patients on long-term intensive therapy with premeal human regular insulin (Hum-R) and bedtime NPH were randomly assigned to a continuation of Hum-R (group 1, $n = 8$), lispro (group 2, $n = 8$), or lispro + NPH (in variable proportions) administered at mealtime (group 3, $n = 8$) for 3 months. NPH administered at bedtime was continued in all three groups. Data from home blood glucose monitoring were collected, and a 24-h plasma glucose and insulin profile was obtained during a 2-day hospital visit to calculate areas under the postprandial glucose curve (3.5 h after breakfast, 3.5 h after lunch, and 3.0 h after dinner for a total of 10.0 h) and the postabsorptive blood glucose curve (the remaining 14.0 h out of 24.0 h) (AUC). Eight nondiabetic subjects were also studied.

RESULTS — The substitution of Hum-R with lispro (group 2) resulted in lower postprandial blood glucose, but greater postabsorptive blood glucose ($P < 0.05$ vs. group 1). The postprandial blood glucose AUC was lower (161 ± 19 vs. 167 ± 20 mg · 100 ml^{-1} · h^{-1}), but the postabsorptive blood glucose AUC was greater (155 ± 22 vs. 142 ± 19 mg · 100 ml^{-1} · h^{-1}) ($P < 0.05$). Therefore, the 24-h blood glucose AUC was no different (NS). Consequently, HbA_{1c} was no different (NS). This occurred because in group 2, mealtime lispro resulted in normal prandial plasma insulin, but also resulted in lower interprandial concentration ($P < 0.05$ vs. group 1). When NPH was added to lispro (30% at breakfast, 40% at lunch, 10% at dinner) in group 3, postabsorptive plasma insulin was similar to group 1 (NS). In group 3, the postprandial blood glucose AUC (153 ± 17 mg · 100 ml^{-1} · h^{-1}) was lower and the postabsorptive blood glucose AUC was no different, as compared with group 1 (NS). Therefore, the 24-h blood glucose AUC was lower (147 ± 17 vs. 155 ± 21 and 158 ± 20 mg · 100 ml^{-1} · h^{-1}), and HbA_{1c} was lower (6.41 ± 0.12 vs. 6.84 ± 0.2 and 6.96 ± 0.2% (groups 3, 1, and 2 respectively, $P < 0.05$). Frequency of hypoglycemia was greater in group 2 ($P < 0.05$), but not in group 3 (NS) vs. group 1.

CONCLUSIONS — Lispro administered at mealtime, which improves postprandial blood glucose, should be associated with optimized replacement of basal insulin to prevent deterioration of postabsorptive blood glucose. NPH administered four times daily normalizes interprandial daily plasma insulin concentration and decreases mean daily blood glucose and HbA_{1c}, with no increase in hypoglycemia.

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The short-acting insulin analog lispro [Lys(B28),Pro(B29)] (Eli Lilly, Indianapolis, IN) (1) is the optimal insulin preparation for subcutaneous injection at mealtime in the physiological intensive treatment of type 1 diabetes (2). Compared with human regular insulin (Hum-R) injected 30 min before meals, lispro administered at mealtime improves the 2-h postmeal blood glucose and reduces the frequency of severe hypoglycemia (3–5).

However, contrary to initial expectations, long-term substitution of Hum-R with lispro in type 1 diabetes has not resulted in improved glycemic control, as indicated by the percentage of HbA_{1c} (3,4,6–10). A similar observation was reported with the short-acting analog B10Asp (11). This observation occurs because in totally C-peptide–negative type 1 diabetes, the shorter duration of lispro action (1) unmasks the greater need for basal insulin (12). When the latter is replaced appropriately, by either continuous subcutaneous insulin infusion (CSII) (13,14) or multiple daily administrations of NPH (15–19), the long-term administration of lispro at mealtime reduces HbA_{1c} (16–19). Therefore, adding a few units of NPH to lispro at each meal, combined with bedtime NPH, is presently being recommended in the physiological intensive therapy of type 1 diabetes (16,18). Among several other advantages, this regimen may protect against unawareness of, and impaired glucose counterregulation to, hypoglycemia (18).

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Abbreviations: AUC, area under the curve; CSII, continuous subcutaneous insulin infusion; DiMISEM, Department of Internal Medicine and Endocrine and Metabolic Sciences; lispro, Lys(B28),Pro(B29); Hum-R, human regular insulin.

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

The observation that improved postprandial blood glucose control, per se, after lispro is administered at mealtime (1) does not necessarily result in improved 24-h control and long-term HbA_{1c} control, raises the question of the role of postprandial blood glucose, as compared with postabsorptive blood glucose, as the determinant of mean daily blood glucose concentration in type 1 diabetes treated with lispro at mealtime. One approach to answer the question would be to quantitate the prandial and postabsorptive blood glucose concentrations under different insulin regimens, which optimize the former with or without affecting the latter.

The present study was undertaken to quantitate the contribution of postprandial blood glucose concentration, which improves with lispro in type 1 diabetes, to the overall 24-h blood glucose concentration and long-term HbA_{1c} concentration, under conditions of different interprandial (postabsorptive) blood glucose concentrations.

RESEARCH DESIGN AND METHODS

Subjects

Approval from the institutional review board was obtained for this study. A total of 24 C-peptide–negative (plasma C-peptide <0.15 nmol/l 6 min after 1 mg glucagon i.v.) type 1 diabetic patients (17 men, 7 women; age 33 ± 4 years; BMI 23.5 ± 1.1 kg/h^2, diabetes duration 13 ± 2.1 years, HbA_{1c} 6.84 ± 0.20%), free of detectable microangiopathic complications, were recruited among patients attending the outpatient Diabetes Clinic of the Department of Internal Medicine and Endocrine and Metabolic Sciences (DiMISEM), University of Perugia, Italy. These patients are being treated by our team with intensive insulin therapy (regular insulin at each main meal and NPH insulin at bedtime), as described in detail elsewhere (20). Eight normal age-, sex-, and BMI-matched nondiabetic subjects were also studied.

Design

During the 15-day run-in period, patients continued their previously described model of insulin therapy (20) (i.e., Hum-R at breakfast, lunch, and dinner and NPH insulin at bedtime). After the run-in period, the patients were randomly assigned to three different treatments (n = 8 patients each) and studied for 3 months in three open and parallel groups. One group (group 1) continued Hum-R as meal insulin. The second group (group 2) used lispro as meal insulin. The third group (group 3) injected lispro + NPH at each meal. Bedtime NPH was continued in all three groups. All three groups of patients used pen devices to inject insulin (in group 3, they had separate injections of lispro and NPH at each meal). Lispro was always injected immediately before meals (0–5 min), whereas Hum-R was injected 10–40 min before meals, depending on premeal blood glucose concentration. Patients were instructed to aim for the 90-min postmeal blood glucose to be between 9 and 10 mmol/l and for fasting and premeal blood glucose to be between 7 and 8 mmol/l. In all three groups, the 90-min postprandial blood glucose was used to titrate the dose of lispro or Hum-R, whereas the dose of daily and nocturnal NPH was based on fasting and premeal blood glucose values. In group 3, patients added NPH at breakfast, lunch, and dinner. The percentage of NPH initially added to lispro was ~20–30% of the total insulin dose at breakfast, ~40% of the dose at lunch, and ~10–20% of the dose at dinner. These estimates were made based on previous experience with lispro + NPH mixtures as well as the time interval between meals (16,18).

During the run-in and treatment periods, patients were seen at 1- to 2-week intervals and were in frequent (even daily) telephone contact with us. The percentage of HbA_{1c} was measured at the end of the run-in period, and subsequently after 3 months of treatment. The diet of the run-in period was not changed during the treatment periods. Patients usually had three meals per day with no snacks. In the run-in and treatment periods, patients continued daily blood glucose monitoring before meals, 90 min after meals, and at 0300 each day (the latter at least three times/week). In all study periods, all patients measured capillary blood glucose using chemistrips (Accutrend Glucose teststrips read by means of Accutrend Alpha reflectometer, Boehringer-Mannheim, Mannheim, Germany).

To quantitate the frequency of hypoglycemia (blood glucose ≤3.9 mmol/l), episodes were divided into severe (i.e., coma or neuroglycopenia requiring assistance from a third party, with or without the need for intramuscular glucagon or intravenous glucose, or emergency hospitalization) and mild (defined as any self-treated episode with no need for third party assistance) category.

To determine the 24-h plasma insulin and glucose profiles during the different insulin regimens, the type 1 diabetic patients were studied at the end of the 3rd month of treatment. Type 1 diabetic patients and nondiabetic subjects were admitted to the Clinical Research Center of the DiMISEM between 0700 and 0730 and studied for 24 h. A superficial vein of one forearm was cannulated with an 18-gauge cathether-needle for intermittent blood sampling. During the 24 h, patients were free to move around and maintain their meal schedule and insulin regimen as similar as possible to their traditional everyday lifestyle. Normal subjects had the same diet as type 1 diabetic patients.

Analytical methods

Plasma glucose was measured using a Beckman Glucose Analyzer (Beckman Instruments, Palo Alto, CA). Plasma insulin was measured by a previously described assay (21). To remove antibody-bound insulin, plasma was mixed with an equal volume of 30% polyethylene glycol immediately after blood collection (21). HbA_{1c} was determined by high-performance liquid chromatography using an HI-Auto A1c TM HA 8121 apparatus (DIC, Kyoto Daiichi, Kogaku, Japan) (range 3.8–5.5% in nondiabetic subjects). The intra-assay coefficient of variation in the 5.0–8.0% range in our laboratory was 1.2%.

Statistical analysis

Data (means ± SEM) were analyzed by two-way analysis of variance corrected for repeated measures (22). Glycemic AUCs were calculated according to the rule of trapezoidal area and expressed as milligrams per 100 milliliters per hour. The postprandial period was assumed as the sum of 3.5 h after breakfast, 3.5 h after lunch, and 3.0 h after dinner (for a total of 10.0 h/day). The postabsorptive state was assumed as the remaining part of day and night (14 h/day).

RESULTS

Insulin doses and frequency of hypoglycemia

The total daily insulin requirements were greater in group 2 than in group 1 because of greater requirements of both lispro and NPH insulin (Table 1). In group 3, the total daily insulin requirements were slightly, but not significantly, greater than those of group 1 (P = 0.07). The ratio between short-acting

Table 1—*Insulin requirements in group 1 (Hum-R at each meal), group 2 (lispro at each meal), and group 3 (lispro + NPH at each meal)*

	Group 1	Group 2	Group 3
Daily insulin dose (U)			
Total	34.2 ± 2	41.1 ± 0.3*	37 ± 2.5†
Short-acting	20.9 ± 1.4	23.1 ± 1.8*	18.9 ± 1.5*†
NPH	13.3 ± 0.7	18 ± 2.1*	18.1 ± 0.7*
Insulin units at injection times (lispro/NPH)			
Breakfast	4.9 ± 0.6 (0)	5.4 ± 0.6 (0)*	4.1 ± 0.5 (1.7 ± 0.3)*†
Lunch	8.2 ± 0.9 (0)	9.1 ± 0.8 (0)*	7.5 ± 0.6 (3.5 ± 0.3)*†
Dinner	7.8 ± 0.1 (0)	8.6 ± 0.7 (0)*	7.3 ± 0.4 (0.9 ± 0.2)*†
Bedtime	0 (13.3 ± 0.7)	0 (18 ± 2.1)*	0 (18.1 ± 0.7)*

Data are means ± SD. Units of NPH are given in parentheses. In group 3, ~70/30, ~50/45, and ~90/10 are the percentage proportions between requirement units of lispro and NPH insulin for breakfast, lunch, and dinner, respectively. In all groups, the bedtime NPH was maintained. *$P < 0.05$ vs. group 1; †$P < 0.05$ vs. group 2.

and NPH insulin doses was different in group 3, as compared with group 1 (~10% less short-acting and ~35% more NPH insulin was needed in group 3, as compared with group 1, $P < 0.05$) (Table 1).

Severe hypoglycemia did not occur in any of the three groups. Mild hypoglycemia was more frequent in group 2 (8.1 ± 0.8 episodes/patient-month) than in group 1 (4 ± 0.5 episodes/patient-month) ($P < 0.05$). In group 3 (5.2 ± 1.2 episodes/patient-month), mild hypoglycemia was no different from group 1 (NS).

Blood glucose control

Data from home blood glucose monitoring. At randomization, mean daily premeal and 90-min postmeal blood glucose of the run-in period were not different in the three groups (Table 2). Mean blood glucose during the 3-month treatment in group 1 and group 2 was no different. In fact, despite the 90 min after a meal, blood glucose in group 2 was lower than in group 1 and fasting and premeal blood glucose was greater than in group 1. In contrast, in group 3, mean blood glucose was lower than in the three other groups because of lower postprandial blood glucose, as compared with group 1, and lower fasting and preprandial blood glucose, as compared with group 2. At randomization, HbA_{1c} was not different in the three groups. At the end of the 3-month treatment, HbA_{1c} in group 2 was no different, as compared with group 1, but it was lower in group 3, as compared with the two other groups.

The 24-h plasma insulin and glucose profiles. With lispro at meals (groups 2 and 3), plasma insulin concentration increased earlier to greater peaks 60 to 90 min after each meal, as compared with Hum-R ($P < 0.05$), and was no longer different from nondiabetic control subjects (NS) (Figs. 1–3). However, in group 2, plasma insulin after 150–180 min after meals was lower and remained lower until the next insulin injection, as compared with group 1 (14.4 ± 1 vs. 19 ± 0.6 μU/l, $P < 0.05$) (Fig. 1). In group 2, plasma glucose was lower between 1 and 3 h after meals ($P < 0.05$), but greater over a 2- to 3-h period before lunch and dinner, and overnight, as compared with group 1 ($P < 0.03$) (Fig. 2). Consequently, despite the lower postprandial glycemic AUC (161 ± 19 vs. 167 ± 20 mg · 100 · $ml^{-1} \cdot h^{-1}$), the postabsorptive glycemic AUC was greater in group 2 than in group 1 (155 ± 22 vs. 142 ± 19 mg · 100 $ml^{-1} \cdot h^{-1}$). Therefore, the 24-h plasma glucose AUCs were no different (158 ± 20 vs. 155 ± 21 mg · 100 $ml^{-1} \cdot h^{-1}$, group 2 and group 1, respectively, NS) (Fig. 3).

When NPH was added to each lispro injection in group 3, plasma insulin peaked postprandially as in group 2, but it was greater for at least 2 h before the insulin injection at lunch, dinner, and bedtime, as compared with group 2 (17.1 ± 1.3 vs. 13.6 ± 1.0 μU/ml, $P < 0.05$) (Fig. 1). Postprandial plasma glucose was lower, as compared with group 1 ($P < 0.002$), and preprandial plasma glucose was lower, as compared with group 2 ($P < 0.02$) (Fig. 2). Consequently, the postprandial glycemic AUC was lower, as compared with group 1 (153 ± 17 vs. 167 ± 20 mg · 100 $ml^{-1} \cdot h^{-1}$, $P < 0.05$) and the postabsorptive glycemic AUC was lower, as compared with group 2 (141 ± 16 vs. 155 ± 22 mg · 100 $ml^{-1} \cdot h^{-1}$, $P < 0.05$). Therefore, the area under the 24-h plasma glucose (146 ± 17 mg · 100 $ml^{-1} \cdot h^{-1}$) was lower than in the two other groups ($P < 0.05$) (Fig. 3).

CONCLUSIONS — In this study, simple substitution of Hum-R with lispro (group 2) improved the postprandial, but not the interprandial, including postabsorptive nocturnal blood glucose because

Table 2—*Blood glucose control at randomization and during 3-month treatment in the three groups examined*

	Group 1	Group 2	Group 3
Premeal blood glucose (mmol/l)			
Randomization	8.4 ± 0.3	8.5 ± 0.4	8.4 ± 0.4
Treatment	8.3 ± 0.3	8.9 ± 0.2*	8.2 ± 0.2†
Postmeal blood glucose (mmol/l)			
Randomization	9.2 ± 0.3	9.3 ± 0.3	9.1 ± 0.4
Treatment	9.3 ± 0.3	8.7 ± 0.2*	8.5 ± 0.3*
Mean daily blood glucose (mmol/l)			
Randomization	9.0 ± 0.4	8.8 ± 0.3	8.9 ± 0.3
Treatment	8.9 ± 0.3	9.0 ± 0.2	8.1 ± 0.2†
HbA_{1c}			
Randomization	6.79 ± 0.17	6.89 ± 0.16	6.83 ± 0.18
Treatment	6.84 ± 0.2	6.96 ± 0.2	6.41 ± 0.12†

Blood glucose numbers are derived from data of home blood glucose monitoring. *$P < 0.05$ vs. group 1; †$P < 0.05$ vs. groups 2 and 3.

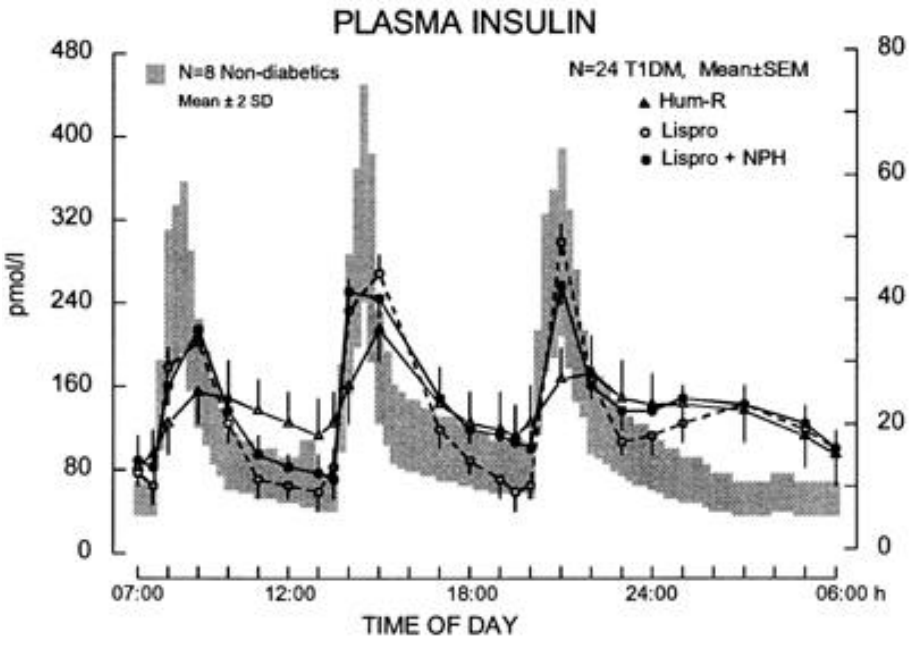

Figure 1—*Plasma insulin concentrations in the three groups of type 1 diabetes patients. Group 1 received Hum-R at each meal, group 2 received lispro, group 3 received lispro + NPH in variable proportions (Table 1). Bedtime NPH was maintained in all three groups. Values from eight nondiabetic subjects are shown in the shaded area (means ± 2 SD).*

of lower interprandial and early night plasma insulin concentration (Fig. 1). This occurred despite near-normalization of prandial insulin, which was no longer different from normal nondiabetic subjects. Because the improvement in the postprandial glycemic area equalled the deterioration in the interprandial glycemic area (Fig. 3), mean blood glucose and HbA_{1c} did not change after 3 months (Table 2). This explains why a number of previous studies have not demonstrated a decrease in the percentage of HbA_{1c} despite lower 1- and 2-h postmeal blood glucose in type 1 diabetic subjects given lispro (or B10Asp) at mealtime (3,4,6–11).

In contrast, when NPH was added to lispro at each meal in group 3, the postprandial improvement in blood glucose was similar to that of group 2; in addition, however, interprandial plasma insulin was greater than that in group 2 (Fig. 1). Because there was no deterioration of postabsorptive blood glucose, the improved postprandial blood glucose resulted in lower mean daily blood glucose and lower HbA_{1c} (Table 2).

In this study, the postprandial period was defined as a 3.5-h period after breakfast, 3.5-h period after lunch, and 3.0-h period after dinner. While this assumption may well be correct in the Mediterranean region for breakfast and dinner, it is probably underestimated for lunch. However, had we calculated a longer period as the postprandial period for lunch, the effect of mealtime lispro in the absence of daily addition of NPH (group 2) would have been even worse than obtained in this study.

One important finding in this study is that plasma insulin concentration in group 3, who were treated with the unconventional strategy of NPH injected four times daily, is in the physiological range both in the postprandial as well as between meals during the daytime. Thus, the strategy of multiple NPH daily insulin injections did not result in hyperinsulinemia between meals; probably, this is the reason of no increase (15–17,19, this study) or decrease (18) in the frequency of hypoglycemia despite better glycemic control.

Two comments are worth discussing. First, whenever lispro is used in place of Hum-R as meal insulin in C-peptide–negative type 1 diabetic patients, care should be taken to physiologically replace basal insulin. If this is not done (group 2 of this study), blood glucose control does not improve. Rather, it may deteriorate because of the risk for postprandial hypoglycemia that may increase because of the tendency to increase lispro doses owing to greater preprandial hyperglycemia (Table 1). Previous observations in experimental controlled settings have pointed out the problem in the afternoon hours with lispro administered at lunch (12) and in the fast-

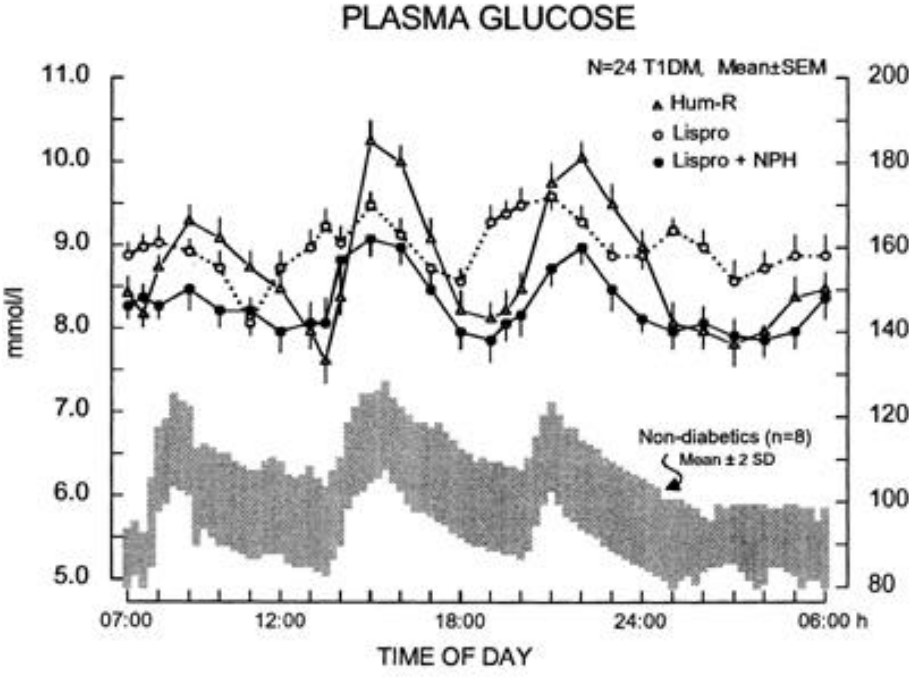

Figure 2—*Plasma glucose concentrations in the three groups of type 1 diabetes patients. See legend to Fig. 1.*

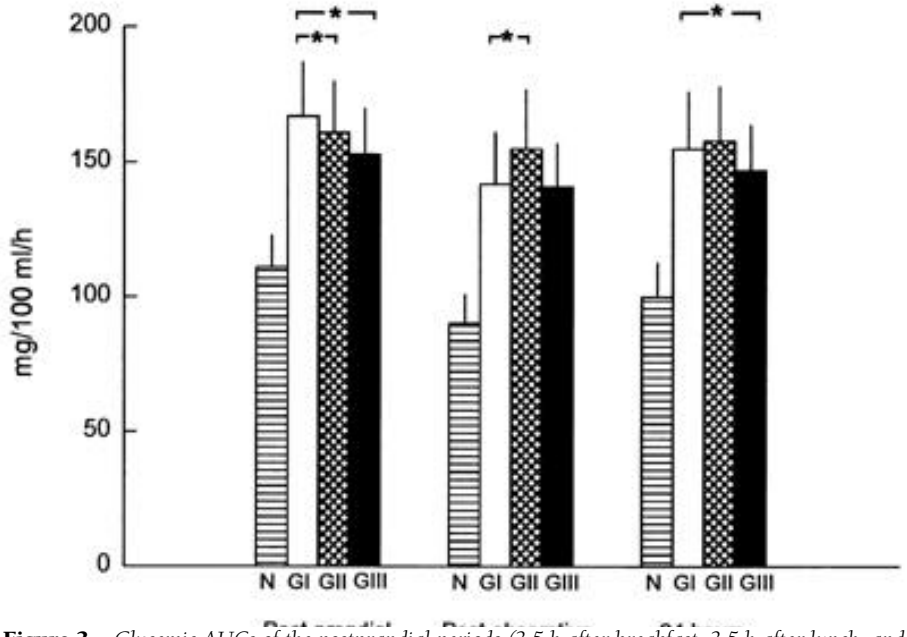

Figure 3—*Glycemic AUCs of the postprandial periods (3.5 h after breakfast, 3.5 h after lunch, and 3.0 h after dinner for a total of 10.0 h), postabsorptive state (remaining 14.0 h), and total (24.0 h) in the three groups studied. Data expressed as milligram per 100 ml per hour. Group 1 (G1) (Hum-R at meals); group 2 (G11) (lispro at meals); group 3 (G111) (lispro + NPH at meals). *P < 0.05. Data from eight normal, nondiabetic subjects are shown for comparison.*

ing state with lispro administered at dinner (23). In this regard, this study does confirm these observations and expand its consequences to long-term blood glucose control and percentage of HbA_{1c}. On the other hand, this study offers a strategy to overcome the problem.

The second question is the efficacy, safety, and feasibility of the proposed regimen of NPH four times daily to optimize replacement of basal insulin when lispro is used at mealtime in place of Hum-R. The results of this study do speak in favor of this regimen. Plasma insulin was in the physiological range during the daily hours, both after meals and during the interprandial state. To our surprise, type 1 diabetic subjects well accepted the need for doubling the number of insulin injection with pens at mealtime and continued on such a regimen also after the end of the 3-month study. One problem, however, was nighttime.

Between midnight and 0700, plasma insulin in type 1 diabetes of group 3 (as well as other two groups) was greater than in normal nondiabetic control subjects. This is due to the greater dose of NPH at bedtime, as compared with the small NPH doses used at meals in combination with lispro. At the dose of ~0.2 U/kg used in this study, NPH has a peak of action between 4 and 6 h after injection with a subsequent decrease in its effect (24). This pattern, confirmed in the present study (Fig. 2), increases the risk for nocturnal hypoglycemia and is responsible for fasting hyperglycemia (24). Thus, while the daytime regimen of multiple small doses of NPH at each meal mimics satisfactorily the physiology of interprandial plasma insulin, the problem remains with the nighttime dose of NPH. At present, a better approach to nocturnal replacement of basal insulin is the technique of CSII (13,14,24). An alternative possibility for the near future might be use of a new soluble long-acting insulin analog that seems to have a peakless, prolonged action profile (25).

In this study, the amount of NPH added to each lispro injection was the direct function of the time interval between insulin injections, with the largest dose used at lunch to provide basal insulin over an extended period of hours that elapse between lunch and dinner. This reflects typically the Mediterranean lifestyle of diabetic patients of this present study. Of course, the figures of daily NPH doses given in Table 1 would be somehow different at different latitudes of different countries.

What is good about the short-acting insulin analog lispro is that its advantages in the postprandial state have somehow forced us to rediscuss the strategy of replacement of basal insulin. Pumps have been rediscovered (13,14); however, for the (many) nonpump user type 1 diabetic patients, it is important to optimize the use of NPH. It is intriguing to conclude from the data of this study that such an old preparation, first introduced in 1946 by Hagedorn, works so well at least during the daytime in conjunction with the new short-acting insulin analog lispro invented nearly 50 years later.

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References

1. Howey DC, Bowsher RR, Brunelle RL, Woodworth JR: [Lys(B28), Pro(B29)] human insulin: a rapidly absorbed analogue of human insulin. *Diabetes* 43:396–402, 1994
2. Bolli GB: Rapid-acting insulin analogs. *Curr Opin Endocrinol Diabetes* 4:277–281, 1997
3. Anderson JH, Brunelle R, Koivisto VA, Pfuetzner A, Trautman M, Vignati L, Di Marchi R, the Multicenter Insulin Lispro Study Group: Insulin analogue treatment reduces postprandial hyperglycemia and frequency of hypoglycemia in IDDM patients. *Diabetes* 46:265–270, 1997
4. Holleman F, Schmitt H, Rottiers R, Rees A, Symanowski S, Anderson J, the Benelux-U.K. Insulin Lispro Study Group: Reduced frequency of severe hypoglycemia and coma in well-controlled IDDM patients treated with insulin lispro. *Diabetes Care* 20:1827–1832, 1997
5. Brunelle RL, Llewelyn J, Anderson VA, Gale EAM, Koivisto VA: Meta-analysis of the effect of insulin lispro on severe hypoglycemia in patients with type 1 diabetes. *Diabetes Care* 21:1726–1731, 1998
6. Garg SK, Carmain JA, Braddy KC, Anderson JH, Vignati L, Jennings MK, Chase HP: Pre-meal insulin analogue insulin lispro versus Humulin R insulin treatment in young subjects with type 1 diabetes. *Diabet Med* 13:47–52, 1996
7. Pfuetzner A, Kuestner E, Forst T, Schulze-Schleppinghoff B, Tratuman M, Haslbeck M, Schatz H, Beyer J, the German Insulin Lispro/IDDM Study Group: Intensive insulin therapy with insulin lispro in patients with type 1 diabetes reduces the frequency of hypoglycemic episodes. *Exp Clin Endocrinol* 104:25–30, 1996

8. Rowe R, Anderson JH, Gale E: A double-blind comparison of insulin lispro and regular insulin in patients on a multiple injection regimen (Abstract). *Diabetes* 45 (Suppl. 2):71A, 1996
9. Jacobs MAJM, Keulen ETP, Kanc K, Casteleijn S, Scheffer P, Deville W, Heine RJ: Metabolic efficacy of preprandial administration of Lys(B28),Pro(B29) human insulin analog in IDDM patients. *Diabetes Care* 20:1279–1286, 1997
10. Heller SR, Amiel SA, Macdonald IA, Tattersall RB: Does insulin lispro preserve the physiological defenses to hypoglycaemia during intensive insulin therapy? (Abstract). *Diabetologia* 41 (Suppl. 1):A241, 1998
11. Nielsen FS, Jorgensen LN, Ipsen M, Voldsgaard AI, Parving HH: Long-term comparison of human insulin analogue B10Asp and soluble human insulin in IDDM patients on a basal/bolus insulin regimen. *Diabetologia* 38:592–598, 1995
12. Torlone E, Pampanelli S, Lalli C, Del Sindaco P, Di Vincenzo A, Rambotti AM, Modarelli F, Epifano L, Kassi G, Perriello G, Brunetti P, Bolli GB: Effect of the short-acting insulin analog [LYS(B28),PRO(B29)] on postprandial blood glucose control in IDDM. *Diabetes Care* 19:945–952, 1996
13. Zinman B, Tildesley H, Chiasson JL, Tsui E, Strack TR: Insulin lispro in CSII: results of a double-blind crossover study. *Diabetes* 46:440–443, 1997
14. Melki V, Renard E, Lassman-Vague V, Boivin S, Guerci B, Hanaire-Broutin H, Bringer J, Belicar P, Jeandidier N, Meyer L, Blin P, Augendre-Ferrante B, Tauber J-P: Improvement of HbA_{1c} and blood glucose stability in IDDM patients treated with lispro insulin analog in external pumps. *Diabetes Care* 21:977–982, 1998
15. Ebeling P, Jansson P-A, Smith U, Lalli C, Bolli GB, Koivisto VA: Strategies toward improved control during insulin lispro therapy: importance of basal insulin. *Diabetes Care* 20:1287–1289, 1997
16. Del Sindaco P, Ciofetta M, Lalli C, Perriello G, Pampanelli S, Torlone E, Brunetti P, Bolli GB: Use of short-acting insulin analogue lispro in intensive treatment of type 1 diabetes mellitus: importance of appropriate replacement of basal insulin and time-interval injection-meal. *Diabet Med* 15:592–600, 1998
17. Jansson P-A, Ebeling P, Smith U, Conget I, Coves MJ, Gomis R, Lalli C, Del Sindaco P, Bolli GB, Koivisto VA: Improved glycemic control can be better maintained with insulin lispro than with human regular insulin. *Diabetes Nutr Metab* 11:194–199, 1998
18. Lalli C, Ciofetta M, Del Sindaco P, Torlone E, Pampanelli S, Compagnucci P, Cartechini MG, Bartocci L, Brunetti P, Bolli GB: Long-term intensive treatment of type 1 diabetes with the short-acting insulin analog lispro in variable combination with NPH insulin at mealtime. *Diabetes Care* 22:468–477, 1999
19. Colombel A, Murat A, Anton B, Krempf M, Charbonnel B: A multiple daily injection regimen using lispro and multiple NPH insulins compared to regular insulin: a randomized crossover study. *Diabet Med.* (In press)
20. Pampanelli S, Fanelli C, Lalli C, Ciofetta M, Del Sindaco P, Lepore M, Modarelli F, Rambotti AM, Epifano L, Di Vincenzo A, Bartocci L, Annibale B, Brunetti P, Bolli GB: Long-term intensive insulin therapy: effects of HbA_{1c}, risk for severe and mild hypoglycaemia, status of counterregulation and unawareness of hypoglycaemia. *Diabetologia* 39: 677–686, 1996
21. Perriello G., Torlone E, Di Santo S, Fanelli C, De Feo P, Santeusanio F, Brunetti P, Bolli GB: Effects of storage temperature of insulin on pharmacokinetics and pharmacodynamics of insulin mixtures injected subcutaneously in subjects with IDDM. *Diabetologia* 31:811–815, 1988
22. Zar J: *Biostatistical analysis*. Englewood Cliffs, Prentice Hall, 1984
23. Ahmed ABE, Home PD: The effect of the insulin analog lispro on nighttime blood glucose control in type 1 diabetic patients. *Diabetes Care* 21:32–37, 1998
24. Bolli GB, Perriello G, Fanelli C, De Feo P: Nocturnal blood glucose control in type 1 diabetes mellitus. *Diabetes Care* 16 (Suppl. 3):71–89, 1993
25. Pieber TR, Eugène-Jolchine I, Derobert E: Efficacy and safety of HOE 901 in patients with type 1 diabetes: a four week randomised, NPH insulin-controlled trial (Abstract). *Diabetologia* 41 (Suppl. 1):A49, 1998