Use of a Novel Double-Antibody Technique to Describe the Pharmacokinetics of Rapid-Acting Insulin Analogs

TORBJÖRN LINDSTRÖM, MD[1]
CHRISTINA A. HEDMAN, MD[1]
HANS J. ARNQVIST, MD[1,2]

OBJECTIVE — To measure the contribution of bedtime intermediate-acting human insulin on the morning plasma insulin profiles after injection of the rapid-acting insulin analogs lispro and aspart in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS — A total of 14 patients with type 1 diabetes, aged 35 ± 13 years (mean ± SD), participated in this single-blind, randomized crossover study. After taking their usual injection of human intermediate-acting insulin the night before, they were given insulin aspart or insulin lispro (10 units) before a standardized breakfast. The contribution of continuing absorption of the human insulin was measured using a monoclonal antibody not cross-reacting with insulin aspart or lispro, whereas the contribution of the analogs was estimated by subtraction after measurement of all plasma free insulin using an antibody cross-reacting equally with human insulin and both analogs.

RESULTS — The correlation coefficient of the fasting free insulin concentrations measured with both insulin methods was 0.95. Fasting free insulin was 95 ± 25 pmol/l before administration of insulin aspart, when determined with enzyme-linked immunosorbent assay detecting only human insulin, and 71 ± 20 pmol/l before administration of insulin lispro (NS). Both insulin analogs gave marked peaks of free insulin concentrations, lispro at 40 ± 3 min and aspart at 55 ± 6 min after injection ($P = 0.01$). The later part of the profiles, from 4.5 to 5.5 h after injection, were similar and showed almost no contribution of the insulin analogs.

CONCLUSIONS — The combination of insulin assays that detect human insulin only or both human insulin and analogs provides a new tool for studying insulin pharmacokinetics. Using this technique, we showed that 4.5 h after administration of the rapid-acting insulin analogs lispro and aspart, the free insulin levels are almost only attributable to the intermediate-acting insulin given at bedtime.

Diabetes Care 25:1049–1054, 2002

Two rapid-acting insulin analogs are now available for treatment of patients with diabetes. Insulin lispro has an inversion of the amino acids proline and lysine at positions 28 and 29 in the B-chain of the insulin molecule (1), and insulin aspart has a single amino acid substitution in position 28 of the B-chain (2). Lispro has a slightly increased affinity for the IGF-I receptor, but regarding metabolic and mitogenic effects, lispro and aspart are similar to human insulin (3). Both analogs form less stable hexamers than human regular insulin and, owing to this, have a faster absorption, more rapid action, and shorter duration after subcutaneous injection (1,3–13). The short duration of these insulin analogs affects the need for basal insulin substitution.

We have recently reported a comparison of the morning insulin profiles of insulin aspart and lispro after subcutaneous injection in patients with type 1 diabetes treated with preprandial insulin injections during the day and human intermediate-acting insulin at bedtime (14). The insulin profiles of these analogs resembled each other, but there were small differences indicating a slightly faster uptake of insulin lispro. Owing to the contribution of basal intermediate-acting insulin, it was not possible to determine the end of the duration of absorption of the insulin analogs. When using insulin regimens with preprandial injections of insulin analogs, a need for injection of basal intermediate-acting insulin together with the insulin analogs has been advocated (15). By using an insulin assay with monoclonal antibodies, which do not detect the insulin analogs, it is now possible to determine the contribution of intermediate-acting human insulin to the free insulin profile during the day.

The aim of our study was to determine the contribution of the bedtime intermediate-acting insulin to the morning insulin profile after injection of insulin analogs at breakfast in patients with type 1 diabetes. A further aim was to determine at what time after injection the contribution of the insulin analogs to the plasma insulin levels had disappeared. Blood glucose profiles were also measured.

RESEARCH DESIGN AND METHODS

Subjects

A total of 14 patients with type 1 diabetes attending our diabetes unit were recruited for the study: 6 men and 8 women, 35.4 ± 12.5 years of age (mean ± SD;

From the [1]Division of Internal Medicine, Department of Medicine and Care and the [2]Division of Cellbiology, Department of Biomedicine and Surgery, Faculty of Health Sciences, Linköping University, Linköping, Sweden.

Address correspondence and reprint requests to Torbjörn Lindström, MD, Division of Internal Medicine, Department of Medicine and Care, University Hospital, SE-581 85 Linköping, Sweden. E-mail: torbjorn.lindstrom@lio.se.

Received for publication 2 August 2001 and accepted in revised form 25 February 2002.

Abbreviations: AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay.

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

range 22–59), BMI 24.7 ± 3.9 kg/m^2, duration of diabetes 22.9 ± 9.6 years. Except for two patients, who had low C-peptide levels (0.04 and 0.11 nmol/l), all patients were C-peptide negative. The breakfast insulin dose was 11.1 ± 0.7 units (mean ± SE; a usual morning short-acting insulin dose between 6 and 14 units was required), whereas the lunch and dinner doses were 10.2 ± 0.8 units and 11.4 ± 0.7 units, respectively. Seven of the patients were treated with human regular insulin (Actrapid U-100; Novo-Nordisk, Bagsvaerd, Denmark), and five patients were treated with insulin lispro (Humalog U-100; Lilly, Indianapolis, IN). One patient used Humalog Mix 50 (U-100; Lilly, Indianapolis, IN), and one patient injected both Humalog and Actrapid. Human intermediate-acting insulin was used at bedtime (21.7 ± 2.4 units). Ten patients used Insulatard (U-100; Novo-Nordisk, Bagsvaerd, Denmark), three patients used Monotard (U-100; Novo-Nordisk, Bagsvaerd, Denmark), and one patient used Humulin NPH (U-100; Lilly, Indianapolis, IN). The 10 patients treated with NPH insulin took 22.1 ± 2.4 units, and the 3 patients treated with lente insulin took 20.3 ± 1.5 units. None of the participating patients were treated with intermediate-acting insulin in the morning. The mean total insulin dose was 54 ± 4 units.

HbA_{1c} at baseline was 7.3 ± 0.3% (reference range 3.2–5.4%). All patients had normal serum creatinine levels, one patient had microalbuminuria; none of the patients had manifest nephropathy. The study protocol was approved by the local ethical committee and conducted according to the declaration of Helsinki. All patients gave informed consent.

Study design

The study was designed as a single-blind, randomized crossover study. Randomization with prefilled envelopes was used. Seven of the patients were randomized to insulin lispro (Humalog U-100; Lilly, Indianapolis, IN) on the first occasion, and seven patients were randomized to insulin aspart (NovoRapid U-100; Novo-Nordisk, Bagsvaerd, Denmark). On the second study day, 5–21 days later, the patients received the alternative insulin analog. Between the study days, the patients continued their usual insulin treatment.

All patients arrived fasting at 0645 to the clinic and had taken their ordinary mealtime insulin and intermediate-acting insulin the previous day and evening. They had taken their bedtime intermediate-acting insulin at median 2220 (interquartile range 0.5 h). After initial blood samples were collected, all patients were given 10 units of one of the insulins subcutaneously in the abdominal wall. The study nurse gave the injection at 0730, immediately before breakfast. The energy content of the standardized breakfast was 418 kcal, and the nutrient content was 21 g protein (20 E%, percentage of the total energy intake of the breakfast), 11 g fat (23 E%), and 59 g carbohydrate (57 E%). The breakfast was finished in 15 min. No snack was given, and the patients were not served lunch until 1300, when the study was finished. Plasma free insulin (Iso-Insulin ELISA [enzyme-linked immunosorbent assay]; Mercodia AB, Uppsala, Sweden) and blood glucose concentrations were measured from samples collected at 0729 and then every 5 min from 0735 to 0750, every 10 min from 0750 to 0810, every 5 min from 0810 to 0820, every 10 min from 0810 to 0910, and every 30 min after 0930. In the EDTA samples collected at 0729, 0810, 0910, 1100, and 1300, free insulin concentrations were also determined with Insulin ELISA, which detects human insulin but not the insulin analogs. The concentrations of basal insulin lying between these times were calculated by linear interpolation. If blood glucose was ≤3.5 mmol/l, 20 ml of glucose 30% was injected.

Biochemical analysis

Free insulin was determined after immediate polyethylene glycol precipitation, as previously described (16). Insulin was measured by two different methods. Iso-Insulin ELISA (Mercodia AB, Uppsala, Sweden) is a two-site ELISA containing two monoclonal antibodies against insulin. Human insulin was used for the standard curve. When equimolar concentrations of human insulin, insulin lispro, and insulin aspart were tested, identical results were obtained, indicating 100% cross-reactivity between lispro, aspart, and human insulin in this assay (Fig. 1*A*). Free insulin concentrations were also determined with Insulin ELISA (Mercodia AB, Uppsala, Sweden), which detects human insulin but do not cross-react with insulin lispro or insulin aspart (Fig. 1*B*). Even at concentrations up to 60,000 pmol/l, there was no cross-reactivity with insulin aspart or lispro (data not shown). Fasting insulin concentrations were measured with both insulin methods, and the results were correlated with each other. The adjusted R^2 value was 0.89. The Insulin ELISA method gave higher values than the Iso-Insulin ELISA method. The values measured with the Insulin ELISA assay, detecting only human insulin, were adjusted to the same level as those of the Iso-Insulin ELISA assay, detecting both human insulin and the insulin analogs by using the equation Iso-Insulin ELISA = (1.39 × Insulin ELISA) + 22.6, obtained by linear regression of the fasting insulin values. The SE of the slope was 0.10 and the SE of the intercept was 7.2. The interassay coefficient of variation for a control serum at the level of 80 pmol/l insulin was <9% for both methods. C-peptide was measured with an ELISA from Dako Diagnostics (Cambridgeshire, U.K.) based on two monoclonal antibodies against C-peptide. Intra-assay and interassay coefficients were <6%.

HbA_{1c} was analyzed with our hospital routine method (reference range 3.2–5.4%). A comparison with other laboratories, including the Diabetes Control and Complications Trial (DCCT), has been reported by our group (17).

Blood glucose was analyzed with the Hemocue method (Hemocue, Mission Viejo, CA).

Statistical analysis

Statistical analysis was made using StatView software (version 4.5; SAS Institute, Cary, NC). Results are given as means ± SE if not otherwise stated. Differences between groups were tested with Wilcoxon's signed-rank test. A P value <0.05 was considered statistically significant. Areas under the curve (AUCs) were calculated with the trapezoidal method. Predefined end points according to the protocol were the time for 50% increase, time for the individual peaks, and time for 50% decrease of free insulin from the peak and free insulin concentrations at the end of the insulin profile.

RESULTS — All 14 patients completed the study. One patient was excluded from the analysis because he mistakenly took a large extra dose of insulin the night before the study.

With the Iso-Insulin ELISA method, detecting both human insulin and the insulin analogs, fasting free insulin concentration was 56 ± 16 pmol/l before administration of insulin aspart and 30 ±

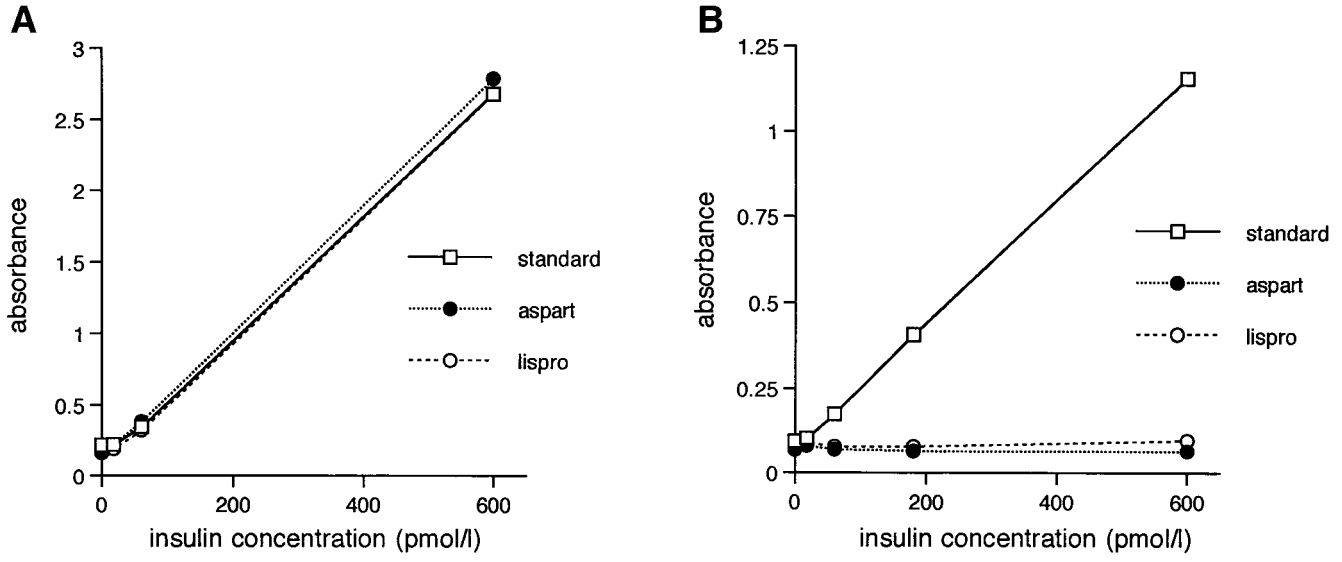

Figure 1—A: *Detection of insulin aspart and insulin lispro by the Iso-insulin ELISA method. Insulin aspart and lispro from commercial insulin vials were diluted in phosphate buffer containing 0.1% albumin to 18, 60, and 600 pmol/l. As human insulin, the human standard of the kit was used. Data shown are representative of three experiments.* B: *Detection of insulin aspart and insulin lispro by the Insulin ELISA method. Insulin aspart and lispro from commercial insulin vials were diluted in phosphate buffer containing 0.1% albumin to 18, 60, 180, and 600 pmol/l. As human insulin, the human standard of the kit was used. Data shown are representative of three experiments.*

15 pmol/l before administration of insulin lispro (NS). When determined with the Insulin ELISA method, detecting only human insulin, fasting free insulin was 95 ± 25 pmol/l before administration of insulin aspart and 71 ± 20 pmol/l before administration of insulin lispro (NS) (Fig. 2). There was a slight nonsignificant hump at 40 and 100 min after injection but then a slow decrease in the human insulin concentrations attributed to the intermediate-acting insulin during the morning profile (Fig. 2). The concentration of human free insulin was 58 ± 15 pmol/l after administration of insulin aspart and 49 ± 8 pmol/l after insulin lispro (NS) at 5.5 h (Fig. 2.), i.e., 61 and 69% of the fasting concentrations, respectively (both NS).

The fasting insulin concentrations and insulin profiles of the patients treated with NPH insulin and the three patients treated with lente insulin were similar, and their data are not separated.

All concentrations measured with the Insulin ELISA method, detecting only human insulin, were adjusted to the same level as those of the Iso-Insulin ELISA method, detecting both human insulin and the insulin analogs. These adjusted concentrations are shown as intermediate-acting insulin in Fig. 3. This figure also depicts the insulin peaks attributable to the insulin analogs.

Both insulin analogs gave marked peaks of free insulin concentrations, lispro at 40 ± 3 min and aspart at 55 ± 6 min after injection ($P = 0.01$; the latter slightly later than the time observed when not adjusting for basal insulin). Maximum insulin concentration after subtraction of adjusted basal insulin concentration was 295 ± 26 pmol/l on insulin lispro and 274 ± 29 pmol/l on insulin aspart (NS), and both occurred at 40 min after the injection, in contrast to the mean of the individual peaks, which differed in time as given above. From fasting insulin levels, lispro reached 50% of peak concentration at 21 ± 1 min and aspart reached 50% of peak concentration at

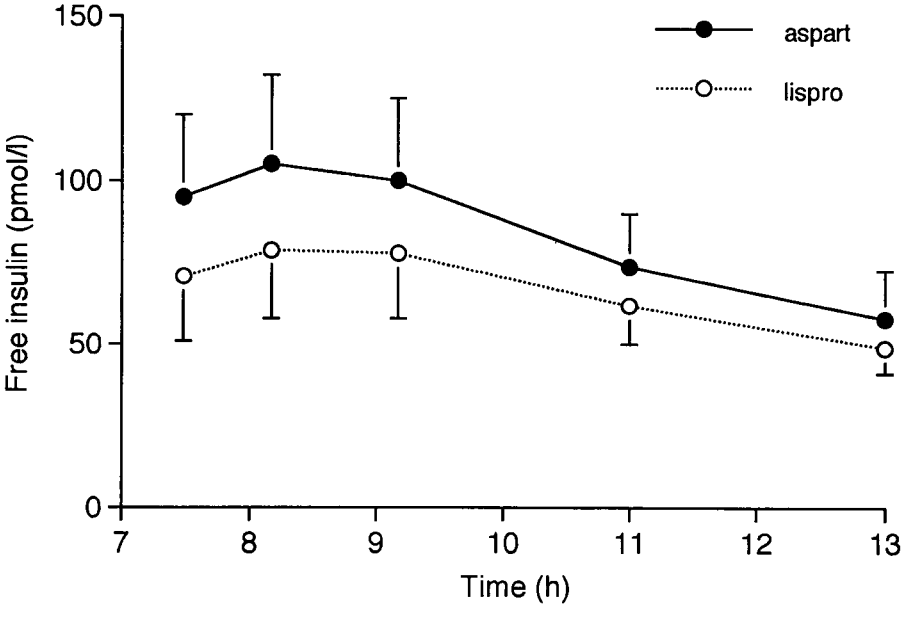

Figure 2— *Morning plasma concentrations of free insulin attributable to basal intermediate-acting insulin administered at bedtime (~ 2200) in 13 patients with type 1 diabetes after a 10-unit single subcutaneous injection of insulin lispro (○) and insulin aspart (●) at 0730, immediately before breakfast. The concentrations are means ± SE.*

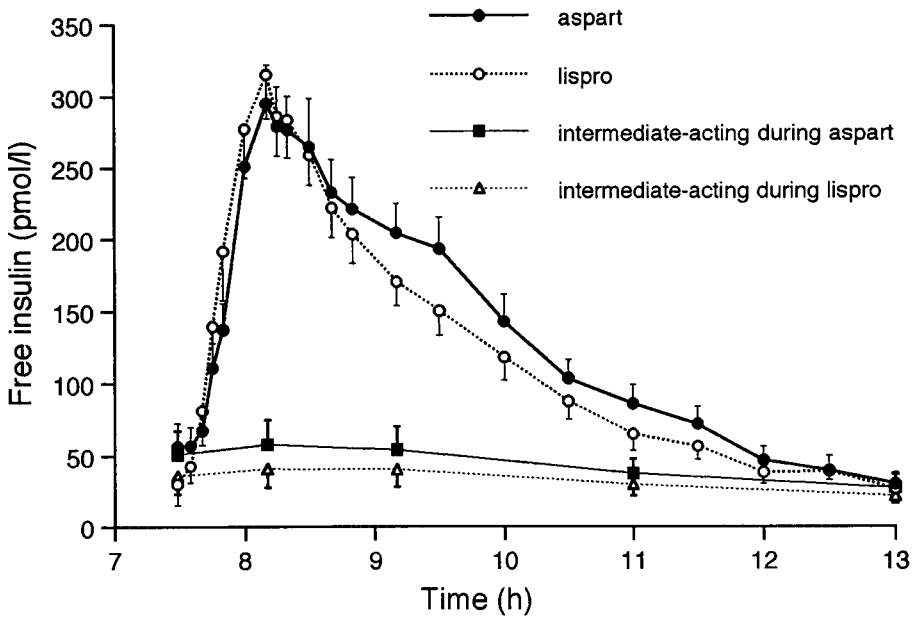

Figure 3— *Morning plasma concentrations of free insulin in 13 patients with type 1 diabetes after a 10-unit single subcutaneous injection of insulin lispro (○) and insulin aspart (●) at 0730, immediately before breakfast. This figure also depicts the free insulin concentrations attributable to the basal intermediate-acting insulin adjusted to the same level and attributable to the intermediate-acting insulin administered at bedtime the night before (~2200). The concentrations are means ± SE.*

26 ± 2 min ($P = 0.03$). The AUC of free insulin for the first 40 min (from 0729 to 0810), after subtracting the fasting insulin concentration, was 5,985 ± 651 pmol/l × min during insulin lispro and 4,008 ± 543 pmol/l × min during insulin aspart ($P < 0.03$). The decrease of free insulin concentration from peak concentration to 50% of the maximum concentration was found at 97 ± 9 min during insulin lispro and 125 ± 15 min during insulin aspart ($P < 0.04$), when adjusting for the effect of the human intermediate-acting insulin. The later parts of the profiles, from 4.5 to 5.5 h after injection, were very similar during both insulins (Fig. 3), and adjustment for the insulin concentrations caused by the intermediate-acting insulin showed almost no contribution of the insulin analogs at this time.

Blood glucose concentrations are shown in Fig. 4. The fasting blood glucose concentration was 11.2 ± 1.0 mmol/l before injection of insulin aspart and 13.7 ± 1.4 mmol/l before injection of insulin lispro (NS). The course of the blood glucose profiles was similar, with peak concentrations 40 min after beginning of the standardized breakfast and no clear increase at the end of the profile. The total AUC was 2,970 ± 321 mmol/l × min during insulin aspart and 3,662 ± 497 mmol/l × min during insulin lispro (NS).

CONCLUSIONS — The present study shows that by using an insulin assay with monoclonal antibodies that do not detect the insulin analogs lispro and aspart, both analogs modified in the B-chain at the same amino acid position (1,2), it is possible to differentiate between the contribution of human insulin and insulin analogs to the insulin profile. Even at very high concentrations (60,000 pmol/l), there was no cross-reactivity of insulin lispro or insulin aspart with Insulin ELISA. The difference in cross-reactivity with insulin lispro and aspart between different insulin assays provides a unique tool to study insulin pharmacokinetics. To our knowledge, this is the first report applying this method in a clinical investigation.

The results obtained with Insulin ELISA, detecting only human insulin, were higher than the results obtained by Iso-Insulin ELISA and had an adjusted R^2 value of 0.89. It is well known that different insulin assays may give different results, and in a recent report of an insulin assay not cross-reacting with insulin lispro, such a difference was also found (18). Although the method determining only human basal insulin gave higher values than the Iso-Insulin ELISA method, this could be adjusted for, because fasting insulin concentrations were determined with both methods at a time when the insulin analogs had not yet been administered. By using this combination of methods, we could separate the contribution of intermediate-acting basal insulin and of the insulin analogs to the morning insulin profile. Our study shows that during the last part of the profile, 4.5 h after

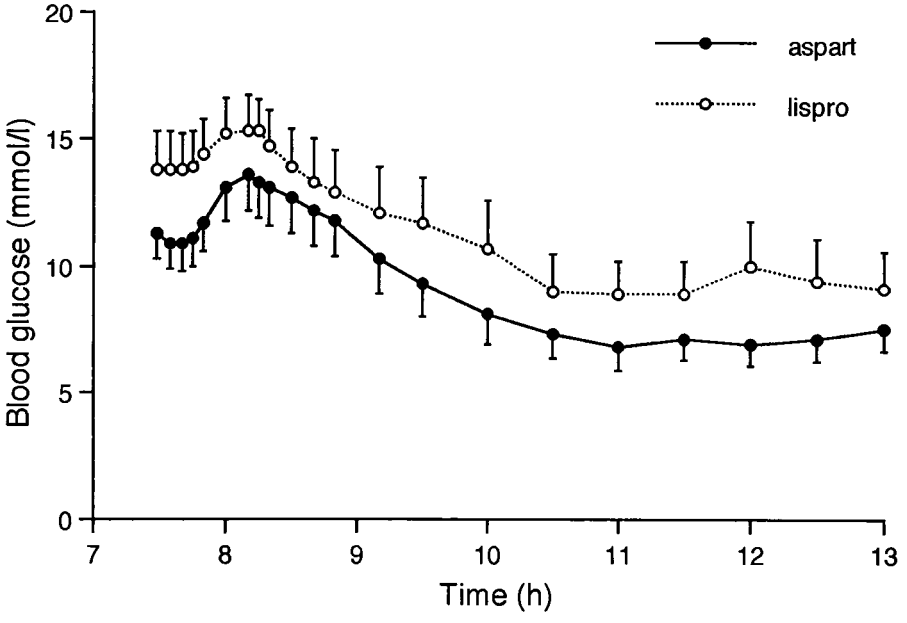

Figure 4— *Blood glucose concentrations in 13 patients with type 1 diabetes after a 10-unit single subcutaneous injection of insulin lispro (○) and insulin aspart (●) at 0730, immediately before breakfast. The concentrations are means ± SE.*

injection of 10 units of the analogs, the concentration of insulin consists almost solely of human intermediate-acting insulin.

The profile of the basal insulin shows a small nonsignificant hump. This suggests some cross-reactivity in the insulin assay from the high levels of insulin analogs at this time, but we found no evidence for such cross-reactivity, even with very high insulin concentrations. Other possibilities include augmented absorption of insulin during the meal or stimulation of endogenous insulin by the meal. Only two patients had low measurable C-peptide levels, but inspection of their insulin profiles suggests that stimulation of small amounts of endogenous insulin might have contributed to some extent.

The use of rapid-acting insulin analogs is rapidly increasing, due to benefits such as improved postprandial glucose control (11,19) and administration immediately before a meal. In certain studies, improved glycemic control without concomitant increase of the number of hypoglycemias have been found (10,19). The insulin profiles of the two now available rapid-acting insulin analogs have been compared with those of human regular insulin in healthy individuals (1,4) and patients with diabetes (11,19). To our knowledge, the only previous report of a direct comparison of the insulin profiles of these analogs is our recently published letter based on the same patient material (14). The main finding of that study is that there are small but significant differences between insulin lispro and insulin aspart in the free insulin profiles obtained after subcutaneous injection in patients with type 1 diabetes. Insulin lispro shows a more rapid onset, reaches the maximum peak concentration earlier, and also shows a more rapid decline than insulin aspart. Adjustment with measurements of the contribution of the intermediate-acting insulin on the insulin concentrations does not change these conclusions. It should be noted that these differences are small in contrast to the previously observed marked differences of these insulin analogs in comparison with human regular insulin (1,4).

In various studies, the time from subcutaneous injection to maximum plasma concentration has been reported to be 42–68 min for lispro (1,5–7) and 32–70 min for aspart (3,8–13). Our results on the time to reach the peak concentration of free insulin after subcutaneous injection of these analogs are, thus, in the same range, as previously reported. The variation in the time from injection to maximal plasma concentration reported in the different studies may be due to different time points chosen for blood sampling (1,3,5–13), different doses of insulin injected (20,21), and different properties of the subcutaneous tissue (22). For insulin lispro, it has been stated that the pharmacokinetics is not influenced by the dose (23), but this may be questioned. Comparing the insulin analogs in a crossover design and using the same dose of insulin as in our study eliminates most of this variation and makes it possible to detect small differences.

The insulin analogs lispro and aspart have both been shown to give lower postprandial blood glucose rise than human regular insulin (5–7,11,13). The somewhat more rapid increase of free insulin levels after injection of insulin lispro might therefore be beneficial compared with insulin aspart, but we found no corresponding difference in the increase of blood glucose concentration in the first part of the profile. The overall blood glucose control was poor during the investigation. It should be noted that our investigation was primarily designed to study the plasma insulin profiles. To study the blood glucose–lowering effect under optimal conditions, it would be desirable to standardize the morning blood glucose by an overnight insulin infusion and to assess the effect on glucose metabolism by a glucose clamp (8,11).

The shorter duration of rapid-acting insulin analogs compared with human regular insulin (24) emphasizes the need to provide sufficient basal insulin substitution. In a study by Jacobs et al. (7), investigating treatment with insulin lispro before main meals and NPH insulin given at bedtime, there was a slight increase in glycerol levels before lunch, suggestive of enhanced lipolytic activity and compatible with low insulin levels at this time. This finding, together with our finding that both insulin analogs had almost no contribution to the insulin levels the hour before lunch, supports the use of basal insulin administration in the morning. An argument against this view is that there was a rather slow decrease of the insulin levels ascribed to the human intermediate-acting insulin with 61–69% of fasting concentrations remaining at 1300 and also no apparent increase of blood glucose concentrations during the later morning profile in our study. The slightly slower decrease of free insulin concentrations after the insulin peak with insulin aspart might (although the later insulin profile was similar as after insulin lispro) influence the need for daytime basal insulin. It is also possible that some patients will need basal insulin substitution in the morning and some will not, owing to variation in meal intervals and to the variation of insulin absorption not apparent in the mean plasma concentrations. Previous studies have given some support that intermediate-acting insulin must be given more than once daily for improvement of glycemic control in type 1 diabetes during treatment with insulin lispro (15,25), whereas only an evening dose might be sufficient when using insulin aspart (26). With NPH insulins, more than once-daily administration might be considered, because their effective duration has been shown to be ~13–14 h (27,28). Another alternative is to use a more long-acting basal insulin substitution (28).

In summary, the combination of an insulin assay that measures only human insulin and an assay that also measures insulin analogs provides a new tool when studying insulin pharmacokinetics. The insulin levels in the period 4.5–5.5 h after injection of the rapid-acting insulin analogs lispro and aspart in the morning are almost solely attributable to the intermediate-acting insulin given at bedtime the night before.

Acknowledgments— Financial support was obtained from the Swedish Medical Research Council (04952), the Swedish Diabetes Association, Barndiabetesfonden, and the County of Östergötland, Sweden. No support was obtained from any pharmaceutical company for this study.

We thank research nurses Christina Dahlgren, Kerstin Krogager, and Ewa Petterstedt as well as dieticians Karin Fridell and Ann-Charlotte Orre-Pettersson for their assistance with the study.

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. Brange J, Völund A: Insulin analogs with improved pharmacokinetic profiles. *Adv*

Drug Delivery Rev 35:307–335, 1999
3. Kurtzhals P, Schäffer L, Sörenssen A, Kristensen C, Jonassen I, Schmid C, Trüb T: Correlations of receptor binding and metabolic and mitogenic potencies of insulin analogs designed for clinical use. *Diabetes* 49:999–1005, 2000
4. Heinemann L, Heise T, Jorgensen LN, Starke AAR: Action profile of the rapid-acting insulin analogue B28Asp. *Diabet Med* 10:535–539, 1993
5. Heinemann L, Heise T, Wahl LC, Trautmann ME, Ampudia J, Starke AAR, Berger M: Prandial glycaemia after a carbohydrate-rich meal in type 1 diabetic patients: using the rapid acting insulin analogue [Lys (B28), Pro (B29)] human insulin. *Diabet Med* 13:625–629, 1996
6. Howey DC, Bowsher RR, Brunelle RL, Rowe HM, Santa PF, Downing-Shelton J, Woodworth JR: [Lys (B28), Pro (B29)]-human insulin: effect on injection time on postprandial glycemia. *Clin Pharmacol Ther* 58:459–469, 1995
7. 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
8. Kang S, Creagh F, Peters JR, Brange J, Völund A, Owens DR: Comparison of subcutaneous soluble human insulin and insulin analogues (Asp^{B9}, Glu^{B27}, Asp^{B10}, Asp^{B28}) on meal related plasma glucose excursions in type 1 diabetic subjects. *Diabetes Care* 14:571–577, 1991
9. Wiefels K, Hübinger A, Dannehl K, Gries FA: Insulinkinetic and -dynamic in patients under insulin pump therapy after injection of human insulin or the insulin analogue (B28 Asp). *Horm Metab Res* 27: 421–424, 1995
10. Home PD, Barriocanal L, Lindholm A: Comparative pharmacokinetics and pharmacodynamics of the novel rapid-acting insulin analogue, insulin aspart, in healthy volunteers. *Eur J Clin Pharmacol* 55:199–203, 1999
11. Lindholm A, Mc Ewen J, Riis AP: Improved postprandial glycemic control with insulin aspart. *Diabetes Care* 22:801–805, 1999
12. Mudaliar SR, Lindberg FA, Joyce M, Beerdsen P, Strange P, Linn A, Henry RR: Insulin aspart (B28 Asp-insulin): a fast-acting analog of human insulin. *Diabetes Care* 22:1501–1506, 1999
13. Heinemann L, Heinrichs S, Weyer C, Heise T, Rauhaus M: Variability of the metabolic effect of soluble insulin and the rapid-acting insulin analog insulin aspart. *Diabetes Care* 21:1910–1914, 1998
14. Hedman CA, Lindstrom T, Arnqvist HJ: Direct comparison of insulin lispro and aspart shows small differences in plasma insulin profiles after subcutaneous injection in type 1 diabetes. *Diabetes Care* 24: 1120–1121, 2001
15. Ebeling P, Jansson P-A, Smith U, Lalli C, Bolli GB, Koivisto VA: Strategies toward improved control during insulin lispro therapy in IDDM. *Diabetes Care* 20:1287–1289, 1997
16. Arnqvist H, Olsson PO, von Schenck H: Free and total insulin as determined after precipitation with polyethylene glycol: analytical characteristics and effects of sample handling and storage. *Clin Chem* 33:93–96, 1987
17. Kullberg CE, Bergstrom A, Dinesen B, Larsson L, Little RR, Goldstein DE, Arnqvist HJ: Comparisons of studies on diabetic complications hampered by differences in GHb measurements. *Diabetes Care* 19:726–729, 1996
18. Sapin R, Le Galudec V, Gasser F, Pinget M, Grucker F: Elecsys insulin assay: free insulin determination and the absence of cross-reactivity with insulin lispro. *Clin Chem* 47:602–605, 2001
19. Andersson JH Jr, Brunelle RL, Koivisto VA, Trautmann ME, Vignati L, DiMarchi R, the Multicenter Insulin Lispro Study Group: Improved mealtime treatment of diabetes mellitus using an insulin analogue. *Clin Ther* 19:62–72, 1997
20. Olsson PO, Jorfeldt L, Arnqvist HJ, Gerhardsson P: Pre- and postprandial hyperinsulinemia during insulin pump treatment; role of the subcutaneous bolus and basal infusion. *Diabetes Res* 16:55–61, 1991
21. Kaku K, Matsuda M, Urae A, Irie S: Pharmacokinetics and pharmacodynamics of insulin aspart, a rapid-acting analog of human insulin, in healthy Japanese volunteers. *Diabetes Res Clin Pract* 49:119–126, 2000
22. Young RJ, Hannan WJ, Frier BM, Steel JM, Duncan LJ: Diabetic lipohypertrophy delays insulin absorption. *Diabetes Care* 7:479–480, 1984
23. Woodworth J, Howey D, Bowsher R, Lutz S, Santa P, Brady P: [Lys (B28), Pro (B29)] human insulin(K): dose-ranging vs. humulin R (H) (Abstract). *Diabetes* 42 (Suppl. 1):54A, 1993
24. Ter Braak EW, Woodworth JR, Bianchi R, Cerimele B, Erkelens DW, Thijssen JHH, Kurtz D: Injection site effects on the pharmacokinetics and glucodynamics of insulin lispro and regular insulin. *Diabetes Care* 19:1437–1440, 1996
25. Ahmed ABE, Home PD: Optimal provision of daytime NPH insulin in patients using the insulin analog lispro. *Diabetes Care* 21:1707–1713, 1998
26. Home PD, Lindholm A, Hylleberg B: Improved glycemic control with insulin aspart: a multicenter randomized double-blind crossover trial in type 1 diabetic patients. *Diabetes Care* 21:1904–1909, 1998
27. Woodworth JR, Howey DC, Bowsher RR: Establishment of time-action profiles for regular and NPH insulin using pharmacodynamic modeling. *Diabetes Care* 17:64–69, 1994
28. Lepore M, Pampanelli S, Fanelli C, Porcellati F, Bartocci L, Di Vinzenzo A, Cordoni C, Costa E, Brunetti P, Bolli GB: Pharmacokinetics and pharmacodynamics of subcutaneous injection of long-acting human insulin analog glargine, NPH insulin, and ultralente human insulin and continuous subcutaneous infusion of insulin lispro. *Diabetes* 49:2142–2148, 2000