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ORIGINAL

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Improved postprandial glycaemic control with insulin Aspart in type 2 diabetic patients treated with insulin

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Abstract The effect on postprandial blood glucose control of an immediately pre-meal injection of the rapid acting insulin analogue Aspart (IAsp) was compared with that of human insulin Actrapid injected immediately or 30 minutes before a test meal in insulin-treated type 2 diabetic patients with residual β-cell function. In a double-blind, double dummy crossover design, patients attended three study days where the following insulin injections in combination with placebo were given in a random order: IAsp (0.15 IU/kg body weight) immediately before the meal, or insulin Actrapid (0.15 IU/kg) immediately (Act_0) or 30 minutes before (Act_{-30}) a test meal. We studied 25 insulin-requiring type 2 diabetic patients, including 14 males and 11 females, with a mean age of 59.7 years (range, 43-71), body mass index 28.3 kg/m^2 (range, 21.9-35.0), HbA_{1c} 8.5% (range, 6.8-10.0), glucagon-stimulated C-peptide 1.0 nmol/l (range, 0.3-2.5) and diabetes duration 12.5 years (range, 3.0-26.0). Twenty-two patients completed the study. A significantly improved postprandial glucose control was demonstrated with IAsp as compared to Act_0, based on a significantly smaller postprandial blood glucose excursion (IAsp, 899 ± 609 (SD) mmol/l • min versus Act_0, 1102 ± 497 mmol/l min, $p < 0.01$) and supported by a significantly lower maximum serum glucose concentration (C_{max}) up to 360 min after dosing (IAsp, 10.8 ± 2.2 mmol/l vs. Act_0, 12.0 ± 2.4 mmol/l, $p < 0.02$). No difference was demonstrated in glucose endpoints between IAsp, administered with a meal and Actrapid injected 30 minutes before the meal ($AUC_{glucose}$ IAsp, 899 ± 609 mmol/l min vs. Act_{-30}, 868 ± 374 mmol/l min; C_{max} IAsp, 10.8 ± 2.2 mmol/l vs. Act_{-30}, 11.1 ± 1.8 mmol/l).

No concerns about the safety of IAsp were raised. Immediate pre-meal administration of the rapid-acting insulin analogue Aspart in patients with type 2 diabetes resulted in an improved postprandial glucose control compared to Actrapid injected immediately before the meal, but showed similar control compared to Actrapid injected 30 minutes before the meal. These results indicate that the improved glucose control previously demonstrated with insulin Aspart compared to human insulin in healthy subjects and type 1 diabetic patients also applies to insulin-treated type 2 diabetic patients.

Key words Human insulin analogue • Meal-related insulin • Postprandial glucose excursions • Type 2 diabetes • Treatment

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Introduction

The Diabetes Control and Complications Trial (DCCT) confirmed that intensified treatment reduces the incidence and progression of macrovascular complications in type 1 diabetes [1]. Furthermore, the UK Prospective Diabetes Study (UKPDS) recently demonstrated that tight blood glucose control by insulin or oral hypoglycaemic agents decreases

the risk of micro- and macrovascular complications in patients with type 2 diabetes [2].

In intensified insulin regimens in both type 1 and type 2 diabetic patients, multiple-injection therapy is used to mimic physiological insulin secretion using intermediate-acting insulin at bedtime to meet basal insulin needs, and pre-meal injections of short-acting insulin to meet meal-related insulin requirements.

The absorption, and thereby duration of action, of short-acting human insulin (HI) is delayed due to the propensity of insulin to self-associate into dimers and hexamers, which have to dissociate into the monomeric or dimeric form before being absorbed into the capillaries at the injection site. As a result, injection of HI immediately before or after a meal leads to postprandial hyperglycaemia and risk of hypoglycaemia before the next meal. These disadvantages can be partially helped by injecting HI 30-60 min before the meal and eating snacks [3-6]. However, this practice is inconvenient for the patient and despite being advised differently, a considerable number of patients inject immediately before their meals [7].

In the insulin analogue Aspart (IAsp), designed for mealtime therapy, proline is substituted with aspartic acid at the B28 position in order to reduce the above described tendency of self-association seen with human insulin. As a result, IAsp is more rapidly absorbed following subcutaneous injection than HI and can thereby be injected immediately at mealtimes and mimic normal insulin kinetics [8]. This has been demonstrated in various pharmacokinetic studies, and long-term studies in type 1 patients have shown a significant improvement in postprandial glucose dynamics [9-12]. Furthermore, no safety concerns were raised in these trials.

The aim of the present study was to compare the effect on postprandial glycaemic excursions of IAsp (Novo Nordisk, Bagsvœrd, Denmark) given immediately before a test meal, with HI (Actrapid, Novo Nordisk, Bagsvœrd, Denmark) given 30 min or immediately before a test meal in insulin-treated type 2 diabetic patients.

Subjects and methods

Subjects

49 patients aged 40-75 years with type 2 diabetes for at least 15 months and insulin treatment for at least 3 months, but not within the first year of diagnosis, were screened for participation in the study. The inclusion criteria were: no severe late diabetic complications, glucagon stimulated C-peptide levels ≥ 0.32 nmol/l, body mass index (BMI) ≤ 35 kg/m^2, and HbA_{1c} ≤ 10%. 25 patients (14 males and 11 females) fulfilled the inclusion criteria and were randomised. Twenty-two patients completed the study, two patients withdrew consent and one patient failed to eat the full test meals. The patients' characteristics are shown in Table 1.

Table 1 Patient characteristics

	Mean	SD	Range
Age (years)	59.7	7.3	43.0-71.0
Body mass index (kg/m^2)	28.3	4.3	21.9-35.0
HbA_{1c} (%)	8.5	1.0	6.8-10.0
Diabetes duration (years)	12.5	5.2	3.0-26.0
Glucagon-stimulated C-peptide (nmol/l)	1.0	0.6	0.3-2.5

Study design

The trial had a randomised, two-centre, double-blind, double dummy, three-period crossover design. Patients attended one pre-study visit, three study visits (separated by 1-3 weeks) and one post-study, follow-up visit.

Study day (meal test)

The patients were admitted to the metabolic ward at 22.00 the evening before the meal test. Throughout the night, blood glucose (BG) was maintained at 4-7 mmol/l by adjusting a continuous intravenous (IV) infusion of short-acting insulin Actrapid according to BG values measured one to two times per hour using a Beckman Glucose Analyzer (Fullerton, USA) and aiming at a BG between 5 and 8 mmol/l in the morning.

On the three study days each patient received two subcutaneous injections: one injection 30 min before and one injection immediately before a test meal, using the double dummy technique. The following combinations of injections were given in a random order:

– IAsp: placebo 30 min before and 0.15 IU/kg body weight (BW) at mealtime,
– Act_0: placebo 30 min before and 0.15 IU/kg BW at mealtime,
– Act_{-30}: 0.15 IU/kg BW 30 min before and placebo at mealtime,

30 min before the meal the first subcutaneous injection was given. At meal time (time 0) the insulin infusion was stopped, the second injection was given and the standardised test meal containing in total 2000 KJ as 85 g carbohydrates, 16 g fat and 22 g protein was served. Throughout the next 6 hours, 22 blood samples were drawn for measurement of blood glucose, insulin and C-peptide.

The trial was conducted in accordance with the Helsinki Declaration and Good Clinical Practice guidelines.

Analysis

Serum insulin concentrations were assayed using a commercial RIA-kit (Pharmacia, Uppsala, Sweden). Serum glucose was analysed by a standard enzymatic GOD assay [13]. For post hoc analysis, Aspart was analysed with a specific constructed enzyme-linked two-sided immunoassay employing two specific monoclonal mouse anti-insulin and anti-Aspart antibodies (Novo Nordisk, Bagsværd, Denmark). Drugs-of-abuse screen and safety

biochemical and haematological clinical profiles were performed before and after the study using standard methods.

Efficacy and safety criteria

The primary efficacy endpoint was the incremental area under the serum glucose curve ($AUC_{glucose}$). Secondary efficacy endpoints were maximum serum glucose concentration during the 360 min after dosing (C_{max}), time to maximum serum concentration during the 360 min (t_{max}), minimum serum glucose concentration in the interval from t_{max} to 360 min ($t_{360\ min}$) after dosing (C_{min}), time to maximum insulin and C-peptide concentrations and incremental area under the serum insulin ($AUC_{insulin}$) and C-peptide ($AUC_{C\text{-}peptide}$) curves. Safety evaluation included a physical examination, haematology, biochemistry and urine screening, reactions at the injection site and adverse event reports including hypoglycaemic episodes.

Statistical analyses

Previous trials have shown an intrasubject standard deviation of serum glucose AUC of approximately 40 mmol/l·min. Using a significance level of 5% and ensuring statistical power of 80% to detect a difference of 40 mmol/l min, it was estimated that 18 individuals would be required. All endpoints (with the exception of t_{max}) were log transformed before analysis of variance (ANOVA), with the subject as a random effect and treatment as a fixed effect. Treatment comparisons were represented by an estimated mean, a *p* value and a 95% confidence interval (CI). A comparison of treatments with respect to t_{max} was done using the non-parametric Friedmann test, followed by a paired comparison of the treatments using the Wilcoxon signed rank test. A non-parametric 95% CI was constructed for the median for each treatment comparison. All tests were performed within individuals at the 5% significance level. All statistical programming was conducted in SAS version 6.11 on a UNIX platform.

Linear models were analysed using PROC MIXED.

Results

Blood glucose

Fasting blood glucose (t = 0) was 6.2 ± 0.8 mmol/l (IAsp), 6.4 ± 0.8 mol/l (Act_0) and 6.3 ± 0.7 mmol/l (Act_{-30}) on the three study days with no significant difference between days (Fig. 1). The postprandial glucose excursion, estimated as absolute incremental area over baseline ($AUC_{glucose}$), was significantly smaller for IAsp (899 ± 609 mmol/l min) compared with Act_0 (1102 ± 497 mmol/l min), $p < 0.01$. There was no difference comparing Act_{-30} (868 ± 374 mmol/l·min) and IAsp (899 ± 609 mmol/l min), $p = 0.44$.

The maximum blood glucose concentration (C_{max}) was significantly lower for IAsp (10.8 ± 2.2 mmol/l) compared to Act_0 (12.0 ± 2.4 mmol/l) ($p < 0.02$), but not different compared to Actrapid injected 30 minutes before the meal (Act_{-30}, 11.1 ± 1.8 mmol/l), $p = 0.97$. No significant differences were observed between times to maximum glucose concentrations (IAsp, 113.0 ± 91.0 min; Act_0, 93.0 ± 33.7 min; Act_{-30}, 110.2 ± 61.1 min) or minimum glucose con-

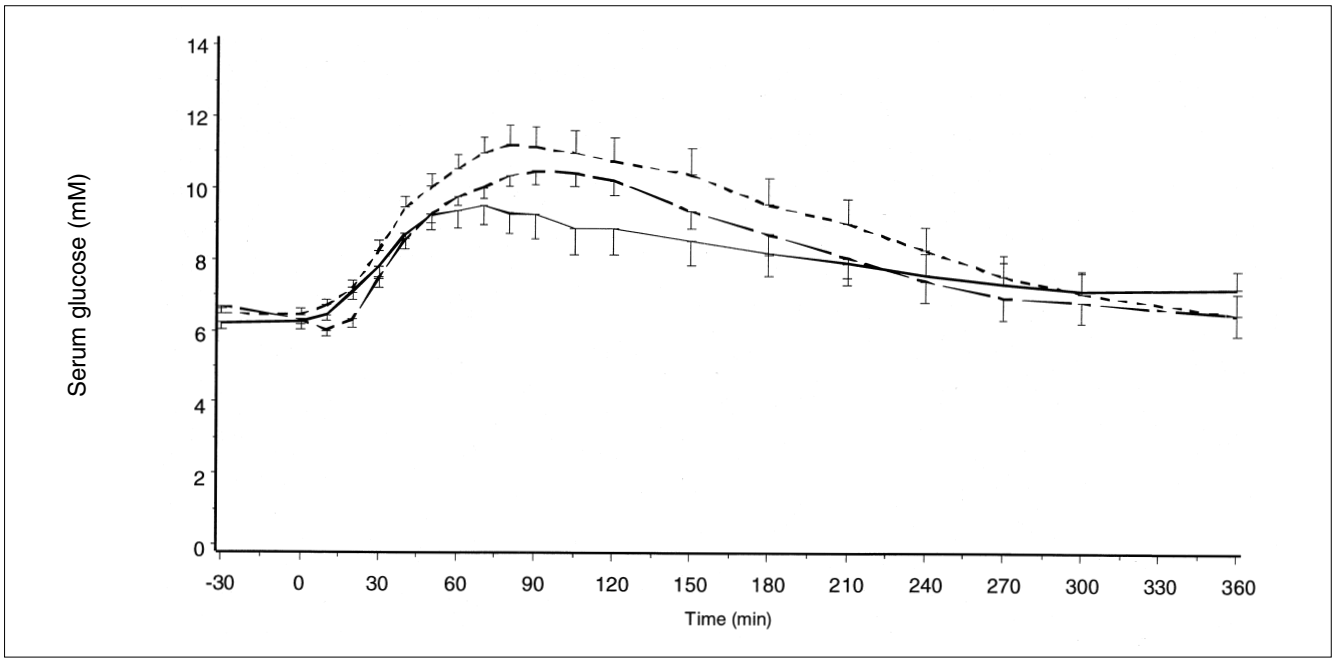

Fig. 1 Serum glucose profiles. Treatment with insulin Aspart (IAsp) (—), human insulin Actrapid injected immediately before meal (Act_0) (- - -), and insulin Actrapid 30 minutes before the meal (Act_{-30}) (— - — -). Values are mean ± SEM

centrations (IAsp, 6.6 ± 2.8 mmol/l; Act_0, 6.2 ± 2.8 mmol/l; Act_{-30}, 6.3 ± 2.7 mmol/l).

Insulin

Immediately pre-meal injection of IAsp resulted in an early and steep increase in serum insulin concentration (Fig. 2). There also was a significantly higher maximum insulin concentration (C_{max}, 74.8 ± 43.2 mU/l) as compared to both Act_0 (56.6 ± 29.2 mU/l, $p < 0.001$) and Act_{-30} (56.2 ± 37.2 mU/l, $p < 0.0004$). Maximum insulin concentration was reached significantly earlier with IAsp (t_{max}, 61.7 ± 27.8 min) as compared to human insulin (Act_0, 90.2 ± 39.8 min, $p < 0.002$; Act_{-30}, 82.5 ± 51.4 min, $p < 0.05$) (Fig. 2). For the incremental area under the curve ($AUC_{insulin}$) no statistical difference between IAsp (242.0 ± 69.8 mU/l h) and Actrapid (Act_0, 223.2 ± 152.2 mU/l h; Act_{-30}, 229.3 ± 162.2 mU/l h) was observed.

C-peptide

C-peptide profiles are shown in Fig. 3. The analysis of $AUC_{Cpeptide}$ showed no significant difference between IAsp (3.5 ± 3.0 nmol/l h) and Actrapid (Act_0, 4.0 ± 2.2 nmol/l h; Act_{-30}, 3.2 ± 1.9 nmol/l). The maximum C-peptide concentration (C_{max}) was significantly lower for IAsp (0.9 ± 0.7 nmol/l), $p < 0.008$), whereas no significant difference was observed between IAsp and Act_{-30} (0.9 ± 0.5 nmol/l).

Safety

There were no clinically significant changes in safety parameters. No serious adverse events were reported and no patients withdrew from the trial due to adverse events. Overall, 24 patients experienced a total of 18 mild or moderate adverse events. Five cases of mild hypoglycaemia were recorded during the study days. Two cases did not demand any treatment, one episode occurred during the night and was treated with IV glucose as this was easy during the clamp procedure, and two cases occurred in relation to the last blood sample and were treated with soft drinks. One episode occurred after injection of IAsp, one after injection of Act_0 and two after injection of Act_{-30}. In all cases patients continued the study. There were no clinically significant changes in any of the laboratory parameters.

Discussion

The present data confirm that an immediately pre-meal injection of the fast-acting insulin analogue Aspart (IAsp) improves the postprandial serum glucose profile as compared to short-acting human insulin Actrapid administered immediately before the meal (Act_0) in insulin-treated type 2 diabetic patients. Injection of IAsp provided a significantly smaller incremental $AUC_{glucose}$ and C_{max}. We were not able to demonstrate any significant treatment effects comparing IAsp and HI injected 30 minutes before (Act_{-30}) the meal, while AUC glu-

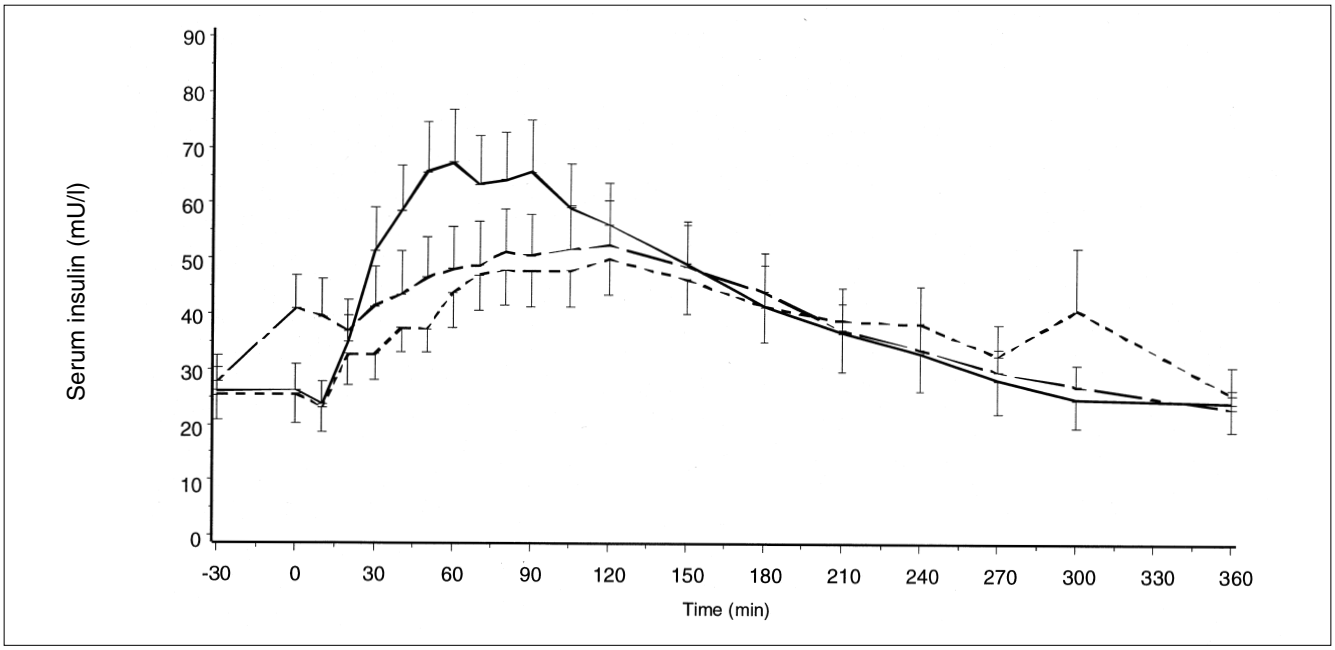

Fig. 2 Serum insulin profiles. Treatment with insulin Aspart (IAsp) (—), human insulin Actrapid injected immediately before meal (Act_0) (- - -) and insulin Actrapid 30 minutes before the meal (Act_{-30}) (— - — -). Values are mean ± SEM

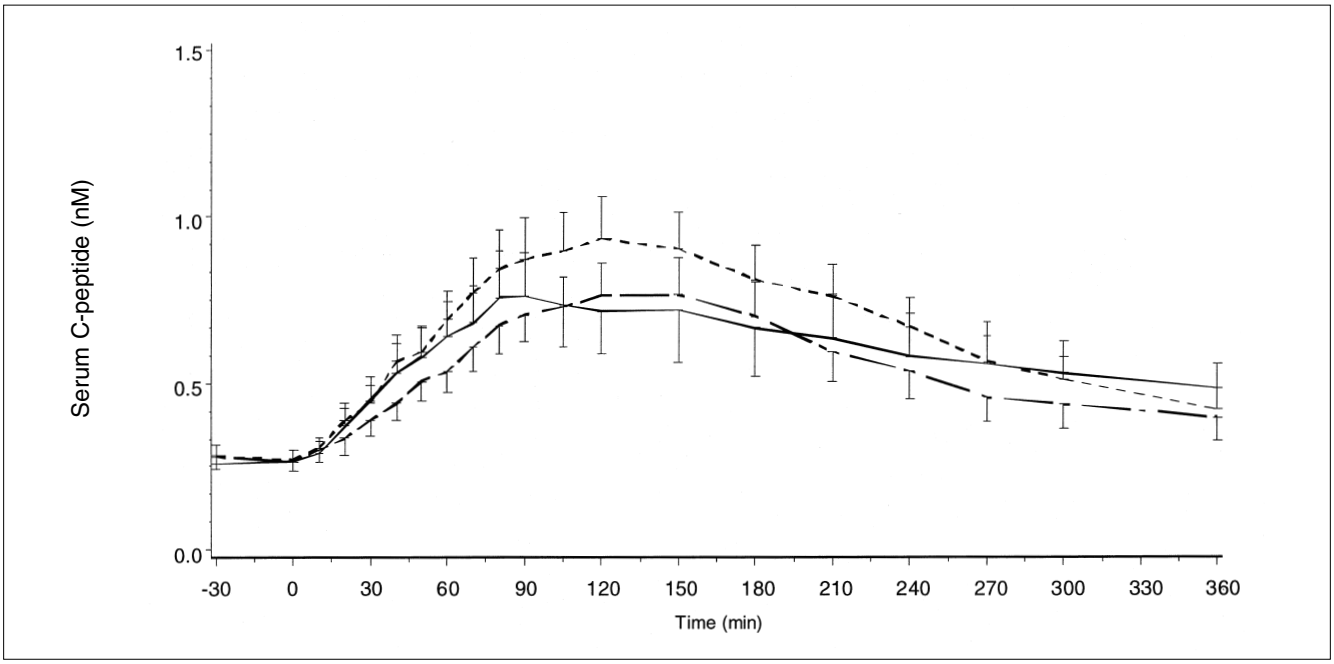

Fig. 3 Serum C-peptide profiles. Treatment with insulin Aspart (IAsp) (—), human insulin Actrapid injected immediately before meal (Act_0) (- - -) and insulin Actrapid 30 minutes before the meal (Act_{-30}) (— - — -). Values are mean ± SEM

cose was higher after Act_0 compared with IAsp (Fig. 1). These findings support previous results of phase I and II trials [8-11] with IAsp and are in accordance with earlier experience with rapid-acting insulin analogues in type 1 [14, 15] and type 2 [14, 16, 17] diabetic patients. The main reason for the improvement in postprandial control is a faster absorption of IAsp compared with Actrapid. The area under the insulin curve was not different between IAsp and Actrapid, but the peak insulin concentration was about 32% higher and reached earlier with IAsp. The difference between the insulin curves vanished 2 hours after the meal, suggesting that IAsp provides a more physiological approach for the treatment of type 2 diabetes. This finding support the idea that the timing of insulin delivery is of major importance in the regulation of glucose response to a meal [18, 19].

In Type 2 patients, a loss of the first-phase insulin secretion occurs [18]. Bruce and coworkers have demonstrated that restoration of an early but not late rise in prandial plasma insulin concentration was associated with a better prandial glucose profile after the ingestion of a mixed meal, supporting the hypothesis that the initial β-cell response is a major determinant of prandial glucose tolerance [18]. This improvement in glucose tolerance is primarily due to a more pronounced suppression of hepatic glucose production and not improved glucose utilisation [19].

We found a longer time to maximal insulin concentration and the difference between the insulin concentration after IAsp or Act was not so pronounced as previously observed in type 1 patients. Therefore, we performed an exploratory post hoc analysis with a new IAsp-specific monoclonal assay. We found absorption of IAsp, in these C-peptide-positive type 2 diabetic patients, to be slower with a *t* max of 75.5 min compared to 40-50 min seen in type 1 patients [8]. Such a difference in rate of insulin absorption between type 1 and type 2 diabetic patients has been previously described [20]. In accordance with the higher variation in subcutaneous thickness, and thereby more varied absorption in type 2 patients compared to type 1 patients, we have seen a higher intrasubject variation in blood glucose in these patients than estimated. This results in a lower statistical power and thereby a more than 20% risk that the observed lack of difference between Act_{-30} and IAsp is indeed not true (statistical type 2 error).

Another explanation is that the endogenously secreted insulin has a confounding effect on the profile of insulin concentration, which is the sum of absorbed and secreted insulin in type 2 patients. Since the maximal C-peptide concentration was higher at Act_0 compared with IAsp, endogenous insulin secretion contributes more to maximal insulin level in patients where Act were administered at meal. Another reason for the lack of difference between IAsp and Act_{-30} in this study could be the interference of the IV clamp insulin needed to obtain euglycemia before injection of the study drug. However, given the fast $t_{1/2}$ of insulin, we have estimated that even with a maximum infusion rate of 2 IU/h, the circulating intravenous insulin dose at the end of the clamp infusion would only be 2% of the subcutaneous trial drug dose. Accordingly we do not believe the IV infusion to have major influence on peripheral insulin level during the meal.

The 30-minute injection-to-meal interval recommended in the labelling for HI is often shortened in daily clinical life due to convenience. It is known that 60%-70% of diabetes

patients actually use an interval of less than 20 minutes, despite instructions to inject the insulin 30 minutes before the meal [5, 7]. The present study demonstrates that use of rapid-acting insulin analogues allows patients to shorten the injection-to-meal interval. This in accordance with previous studies in type 1 and 2 patients [11, 21, 22].

No safety concerns regarding IAsp were raised in this trial or other trials with insulin analogues. Severe hypoglycaemia is the main concern in insulin treatment [1]. No severe episodes were reported in this trial.

Recently, the UKPDS established that good metabolic control decreases the risk of microvascular complications in type 2 diabetes [2], and concluded that insulin regimens should be used more in type 2 diabetes. The present trial provides evidence that the improved postprandial glucose control demonstrated by IAsp compared with HI in healthy volunteers and type 1 diabetic patients also applies to individuals with insulin-treated type 2 diabetes without induction of hyperinsulinaemia. The clinical use of insulin analogues provides a more physiological approach for the treatment of type 2 diabetes.

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