Diabetologia (1995) 38: 592–598

Diabetologia

Long-term comparison of human insulin analogue B10Asp and soluble human insulin in IDDM patients on a basal/bolus insulin regimen

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Summary Recombinant DNA technology allows the production of insulin analogues with faster absorption rates from subcutaneous tissue as compared to soluble human insulin. The human insulin analogue B10Asp (mono/dimeric) is absorbed twice as fast as soluble human insulin (hexameric). A double blind, randomised crossover study with a 1-month run-in period and two 2-month treatment periods was performed in 21 male insulin-dependent diabetic (IDDM) patients aged 18–40 years in order to compare the metabolic control obtained with equimolar doses of the analogue B10Asp vs soluble human insulin (Actrapid) given as mealtime insulin and intermediate acting isophane insulin (Protaphane) at bedtime. At the end of each 2-month study period, the patients were admitted to the metabolic ward. We found significantly higher plasma insulin/analogue levels after breakfast, lunch and dinner with B10Asp as compared to Actrapid ($p < 0.05$). The plasma insulin/analogue levels were significantly lower before lunch and dinner with B10Asp as compared to Actrapid ($p < 0.05$). Also, the plasma insulin/analogue level tended to be lower at bedtime when comparing B10Asp to Actrapid. The 24-h blood glucose profiles showed identical fasting blood glucose, significantly lower blood glucose after breakfast with the analogue ($p < 0.05$), no differences in blood glucose after lunch and dinner but a significantly higher blood glucose at midnight using the analogue ($p < 0.05$). The overall 24-h mean blood glucose concentrations, the daily insulin dose, HbA_{1c}, diet, home blood glucose monitoring and frequency of hypoglycaemia were almost identical in the two treatment periods. In conclusion, the overall glycaemic control remained unchanged and quite good when Actrapid was exchanged dose for dose with the insulin analogue B10Asp in IDDM patients treated with a basal bolus regime. [Diabetologia (1995) 38: 592–598]

Key words IDDM, insulin analogues, metabolic control.

Several randomised studies dealing with small numbers of insulin-dependent diabetic (IDDM) patients have unanimously suggested that the initiation and progression of the early stages of diabetic retinopathy and nephropathy can be delayed or even prevented by strict metabolic control [1–10]. The DCCT trial has confirmed and extended these studies by demonstrating that intense insulin therapy effectively delayed the onset and slowed the progression of diabetic retinopathy, nephropathy and neuropathy in 1441 IDDM patients [11]. Intensive therapy was conducted using an external insulin pump or by three or more daily insulin injections (basal/bolus regime) and guided by frequent blood glucose monitoring. Several clinic-based studies have demonstrated that the basal/bolus treatment regime is appreciated by IDDM patients in general [12–14].

In non-diabetic subjects, meal ingestion is followed rapidly by a rise in plasma insulin concentration, which reaches a peak within 30–60 min and returns to baseline within 4–5 h [15, 16]. In contrast, subcuta-

Received: 7 June 1994 and in revised form: 18 October 1994

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; RIA, radioimmunoassay; IGF-I, insulin-like growth factor-I.

neous injections of soluble insulin, in both non-diabetic [17] and diabetic subjects [18], results in a slower rise in plasma insulin concentration and a lower peak at 90–120 min; also the level tends to be inappropriately high 3–5 h after injection [19]. In diabetic subjects, the initial low plasma insulin level contributes to an excessive postprandial glucose excursion, and a subsequent long period of high insulin levels predisposes to late hypoglycaemia.

Soluble human insulin exists mainly in the hexameric form in pharmaceutical concentrations [20], and the initial delay in absorption has been attributed to the rate of dissociation of hexameric units into dimeric and monomeric molecules at the subcutaneous injection site [21, 22]. Recombinant DNA technology allows the production of insulin analogues with faster absorption rates from subcutaneous tissue as compared to soluble human insulin [23]. The human insulin analogue B10Asp (mono/dimeric) in which B10 histidine is substituted by aspartate is absorbed twice as fast as soluble human insulin from the subcutaneous injection site in both non-diabetic subjects [24] and in IDDM patients [25]. Clamp studies in healthy men have demonstrated that the analogue B10Asp has the same bioavailability but a faster onset of action as compared to soluble human insulin [26].

The aim of our double-blind, randomized crossover study was to evaluate the long-term (2-month) effect on metabolic control of the fast acting insulin analogue B10Asp as compared to soluble human insulin (Actrapid) in IDDM patients on a basal bolus regimen. The fast-acting insulins were given less than 5 min before the three main meals.

Subjects and methods

Subjects. Twenty-four IDDM patients fulfilling the following criteria were consecutively enrolled from the outpatient clinic at Steno Diabetes Center. Inclusion criteria were: age 18 to 40 years, male, duration of diabetes more than 1 year, treated with a multiple injection regimen for longer than 6 months, haemoglobin A_{1c} (HbA_{1c}) less than 10 %, body mass index (BMI) less than 27 kg/m^2 and a stable metabolic control (HbA_{1c} varying less than 1 % for the previous 6 months). Exclusion criteria were: history of hypoglycaemic unawareness, local lipodystrophy, urinary albumin excretion greater than 300 mg/24 h, proliferative retinopathy, other medication or any concurrent disease. All patients had a stimulated C-peptide value lower than 0.60 pmol/ml [27]. The glucagon/C-peptide test was carried out after an overnight fast. Blood samples for plasma C-peptide determination were obtained before and 6 min after an i. v. bolus injection of 1 mg glucagon (Novo Nordisk, Bagsværd, Denmark) as described previously [27]. The study was completed by 21 patients; 3 patients were excluded, one patient because of a gastroscopic verified ulcus duodenus at the first visit, a second because his HbA_{1c} rose more than 3 % during the run-in period; the reason for this increase was unknown. The third patient had an infected toenail at the first hospitalisation and required antibiotic and surgical treatment. The results from the three excluded patients were not used in the calculations. All the subjects included in the study were Caucasian, and all gave informed consent to participate in the study. The study was approved by the local ethical committee.

Methods. We performed a double-blind randomised, crossover study with an open 4-week run-in period during which the patients were treated with soluble human insulin (Actrapid HM; 600 nmol/ml (100 IU/ml), Novo Nordisk, Bagsværd, Denmark) less than 5 min before breakfast, lunch and dinner and with intermediate acting isophane insulin (Protaphane HM, 600 nmol/ml; (100 IU/ml), Novo Nordisk) at bedtime. All mealtime insulin was injected subcutaneously in a skinfold at a 45° angle with a Novo Pen using a 12.5 mm-long cannula in the abdominal wall. Bedtime insulin was injected in a similar way in the thigh.

After the run-in period the patients were randomised to either Actrapid or insulin analogue B10Asp (600 nmol/ml (100 IU/ml), Novo Nordisk) as premeal insulin for 8 weeks; thereafter the patients were taken to the metabolic ward where 24-h profiles of blood glucose and plasma free-insulin/analogue were obtained, and HbA_{1c} were measured. The subjects were then changed to the other type of premeal insulin for another 8 weeks and finally taken to the metabolic ward for 24-h blood glucose and plasma free-insulin/analogue profiles and HbA_{1c} measurement. During hospital admission the patients received a diet according to the information they had given to the dietician. The individual diets were isocaloric at the two admissions. During the 2 days prior to admission to the metabolic ward we collected seven capillary blood samples on each day from each patient (blood glucose was measured, preprandially, 90 min postprandially and before bedtime). The samples were sent to our laboratory for analysis.

During the run-in period the patients were seen in the outpatient clinic at day 0, week 2 and for randomisation at week 4. During the treatment periods, the patients were seen in the outpatient clinic at weeks 1 and 2. A telephone consultation was performed at weeks 3 and 6. The day before each visit to the outpatient clinic, the patients performed a 7-point blood glucose profile with a Reflolux II M, Boehringer-Mannheim GmbH, (Mannheim, Germany). The insulin doses were recorded and adjusted according to the following goals: fasting blood glucose 4 to 7 mmol/l and postprandial blood glucose level less than 11 mmol/l. Hypoglycaemic events for the previous week were recorded and graded as mild if the patients could treat themselves with carbohydrate supplements, and graded as serious if third-party assistance was required. Mild hypoglycaemic events were recorded during each contact with the patients. Serious hypoglycaemic events were recorded for the complete study. At each clinic visit injection sites were inspected.

The patients were instructed to continue their usual diet (three main meals and two to three snacks) and their everyday activities. To ensure that no quantitative or qualitative changes had occurred in their diet the patients were interviewed by a dietician and their body weight recorded at entry, crossover and at the end of the study.

Laboratory analysis. During the hospital admissions blood glucose and plasma insulin/analogue were measured each hour during the daytime (07.00 to 24.00 hours) and each 2 h during the nighttime (00.00 to 07.00 hours). Blood glucose concentrations were determined by the hexokinase method [28]. HbA_{1C} was determined by high performance liquid chromatography (DIAMAT Analyzer; Bio-Rad, Richmond, Calif., USA) [29] where the normal range of HbA_{1C} in our laboratory is 4.1–6.1 %.

Blood samples for plasma insulin/analogue concentration measurement were centrifuged for 10 min at 4 °C and anti-

Table 1. Clinical characteristics of 21 male IDDM patients completing 8 weeks treatment with Actrapid and insulin analogue B10Asp

Age (years)	28 (23–33)
Duration of diabetes (years)	11 (2–28)
Duration of basal/bolus treatment (years)	3.5 (1–5)
HbA_{1c} (%)[a]	8.0 ± 1.2
BMI (kg/m^2)[a]	23.6 ± 1.8
Retinopathy *(n)* (nil/simplex/proliferative)	0/21/0
Urinary albumin excretion (mg/24 h)	17 (10–25)

Median (range), except [a] mean ± SD

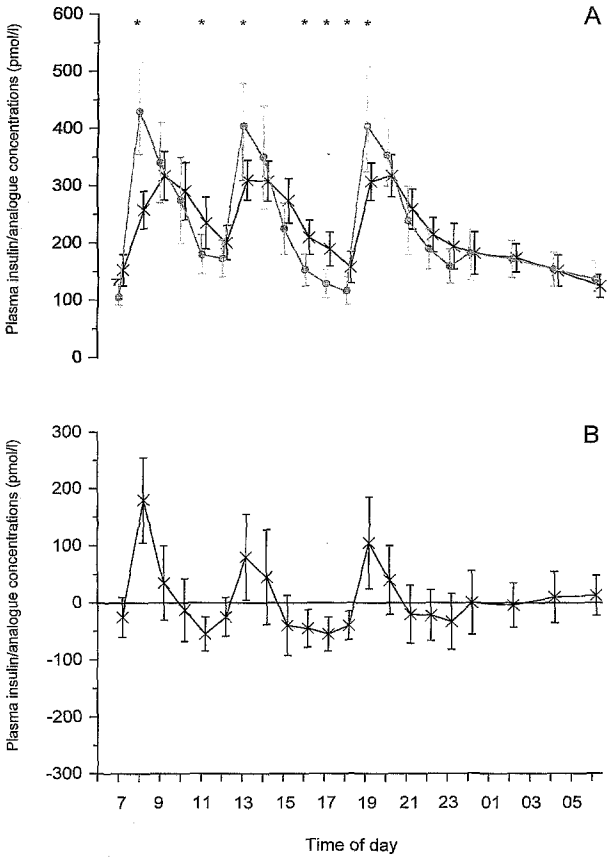

Fig. 1. A Twenty-four hour plasma free insulin/analogue concentrations (pmol/l) in 21 IDDM patients treated with soluble human insulin (Actrapid) -×××- or insulin analogue B10Asp -●●●- for 8 weeks. Values given as mean ± 2 SEM. * $p < 0.05$ comparing the two insulins. **B** B10Asp – Actrapid. Values given as mean difference (95 % confidence interval). Values obtained during hospital admission

bodies bound to insulin were precipitated by addition of a 300 g/l polyethylene glycol solution to the plasma. These samples were stored at – 80 °C for less than 1 month and then analysed for insulin levels. Plasma free insulin concentrations were measured by a radioimmunoassay (RIA) technique with coefficients of variation between 6 and 14 % in the physiological range [30]. Plasma analogue concentrations were determined by standard RIA using M8309 antibody, 125-I-monoiodinated-insulin and analogue-calibrators, essentially as described by Heding [30]. M-value was calculated as described by Schlichtkrull et al. [31] using the 24-h blood glucose values from the hospital admissions. The following safety parameters were measured using standard methodology at entry, crossover and at the end of the study; yeast antibodies IgG, lactate dehydrogenase, alkaline phosphatase, aspartate aminotransferase, creatinine, leucocytes and differential count, haemoglobin, haematocrit, cholesterol, high-density (HDL)-cholesterol, low-density (LDL)-cholesterol was calculated by the Friedewald equation [32], triglycerides, prothrombin, activated prothrombin, fibrinogen and thrombocytes. At the same time insulin antibodies IgG, analogue B10Asp antibodies IgG were determined by anti-insulin (Novo Nordisk, Immunochemical Department, Cat. No.: 7351542) using mono-125I-(Tyr-A14)-Human Insulin or mono-125I-(Tyr-A14)-B10Asp.

At least three 24-h urine samples were collected. The urinary albumin concentration was determined by RIA with a single antibody [33]. Body mass index (BMI) was calculated as kg body weight/height m^2. Retinopathy was assessed by fundus photography after pupillary dilation.

Statistical analysis

All data are given as mean ± SD except 24-h profiles of blood glucose and plasma insulin/analogue concentrations which are given as mean ± 2 SEM. Comparison of normally distributed parameters was done using standard analysis of variance (ANOVA) for two crossover periods, hence evaluation of treatment differences were carried out as within patient comparisons. Area under the curve (AUC) was calculated by means of the trapezoid rule. Comparison of frequencies was done using Wilcoxon's signed rank test. A *p*-value (two-tailed) less than 0.05 was considered significant. The study was designed to detect a difference of 0.5 % in HbA_{1c} with a power of 90 % (alpha = 0.05) provided 21 patients completed the study. All calculations were made with a commercially available program (Statistical Analysis System, SAS Institute, Raleigh, N. C., USA).

Results

The clinical characteristics of the 21 male IDDM patients completing the study are shown in Table 1. The 24-h profiles of plasma free insulin/analogue levels for B10Asp and Actrapid are shown in Figure 1. We found significantly higher plasma insulin/analogue levels after breakfast, lunch and dinner with B10Asp as compared to Actrapid ($p < 0.05$). The plasma insulin/analogue levels were significantly lower before lunch and dinner ($p < 0.05$) and tended to be lower at bedtime with B10Asp as compared to Actrapid. There was no difference in the calculated area under the curve (AUC) comparing the two insulins ($p = 0.70$) during 24 h. Furthermore, no differences were found comparing the two insulins between the meals and during the night.

The corresponding 24-h blood glucose profiles are shown in Figure 2. The fasting blood glucose was identical using the two fast-acting insulins. Blood glucose was significantly lower after breakfast comparing B10Asp to Actrapid ($p < 0.05$). There was no significant difference in blood glucose from lunch up to 21.00 hours using the two insulins. From 21.00 until

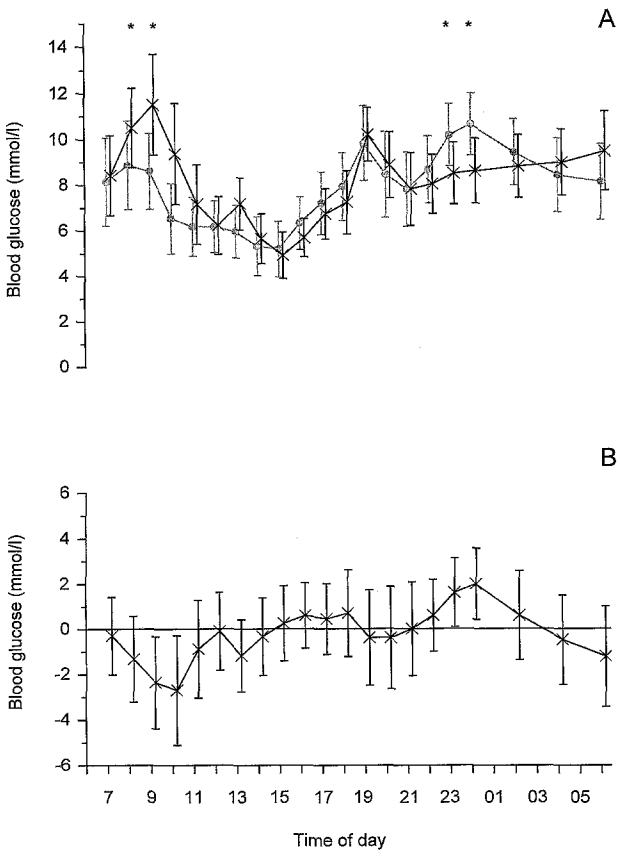

Fig. 2. A Twenty-four hour blood glucose concentrations (mmol/l) in 21 IDDM patients treated with soluble human insulin (Actrapid) -×××- or insulin analogue B10Asp -●●●- for 8 weeks. Values given as mean ± 2 SEM. * $p < 0.05$ comparing the two insulins. **B** B10Asp – Actrapid. Values given as mean difference (95 % confidence interval). Values obtained during hospital admission

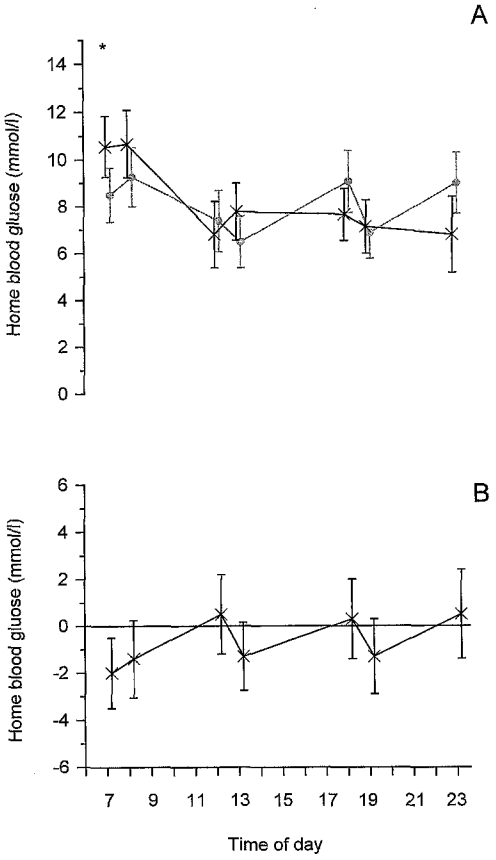

Fig. 3. A Home blood glucose concentrations (mmol/l) in 21 IDDM patients treated with soluble human insulin (Actrapid) -×××- or insulin analogue B10Asp -●●●-. Values given as mean ± SEM. * $p < 0.05$ comparing the two insulins. **B** B10Asp – Actrapid. Values given as mean difference (95 % confidence interval). Values obtained at home

Table 2. Metabolic control in 21 male IDDM patients at the end of each 8-week treatment period

Treatment period	B10Asp	Actrapid	*p*-value
HbA_{1c} (%)	7.7 ± 0.9	7.8 ± 0.6	NS
Mean blood glucose (mmol/l)	7.8 ± 2.3	8.1 ± 1.5	NS
M-value	24 ± 19	26 ± 14	NS
Triglycerides (mmol/l)	1.0 ± 0.6	0.9 ± 0.6	NS
Total cholesterol (mmol/l)	4.2 ± 0.8	4.2 ± 0.7	NS
HDL-cholesterol (mmol/l)	1.4 ± 0.3	1.3 ± 0.3	NS
LDL-cholesterol (mmol/l)	2.4 ± 0.7	2.4 ± 0.7	NS

Mean ± SD

03.00 hours there was a rise in blood glucose levels during B10Asp as compared to Actrapid treatment. The difference reached statistical significance at 23.00 and 24.00 hours ($p < 0.05$). AUC was similar comparing the two insulins ($p = 0.63$) during 24 h. Furthermore, no differences were found comparing the two insulins between the meals and during the night. Twenty-four h mean blood glucose and M-value were not different, comparing the two insulin treatments (Table 2).

The home blood glucose profiles, based on the seven samples per day sent to the hospital for analysis, (Fig. 3) showed statistically significant lower fasting blood glucose comparing B10Asp to Actrapid ($p < 0.05$). Also, the blood glucose level was lower after breakfast, lunch and dinner comparing B10Asp to Actrapid but the differences did not reach statistical significance.

We found no statistically significant differences in HbA_{1c} and lipids comparing the two insulin regimens (Table 2). Insulin doses, BMI, hypoglycaemic events, total energy intake and snack consumption were not different comparing the two insulin (Table 3).

Insulin antibodies were higher comparing B10Asp to Actrapid using human insulin as tracer 19 ± 14 vs 17 ± 12 % ($p < 0.05$), but not different using B10Asp as tracer 10 ± 8 vs 9 ± 5 %. There was no difference in any of the safety parameters comparing the two insulins. No local reactions were observed at the injection sites.

Table 3. Insulin dose, total energy intake and BMI in 21 male IDDM patients at the end of each treatment period

Treatment period	B10Asp	Actrapid	*p*-value
Insulin dose (IU)			
morning	10 ± 4	10 ± 4	NS
lunch	9 ± 3	9 ± 3	NS
dinner	12 ± 4	12 ± 4	NS
bedtime	21 ± 4	21 ± 4	NS
Total energy intake (kJ)	8444 ± 2223	8440 ± 2036	NS
Number of snacks	2.0 ± 1.0	2.5 ± 1.0	NS
Hypoglycaemic events (no.)			
Mild (number/ week/patient)	1.4	1.3	NS
Severe (number whole study)	0	3	NS
BMI (kg/m^2)	23.2 ± 1.7	23.2 ± 1.8	NS

Mean ± SD

Discussion

Our long-term double blind randomised crossover study clearly showed a more physiological plasma insulin/analogue profile with the fast-acting insulin analogue B10Asp, as compared to soluble human insulin (Actrapid) in IDDM patients treated with a basal/bolus regime [15, 16]. Even though there was a trend towards lower postprandial blood glucose values this beneficial effect of B10Asp was offset by higher blood glucose levels during the night resulting in an overall 24-h glycaemic control and HbA_{1c} nearly identical to that obtained during treatment with Actrapid. Both treatments resulted in a fairly well-controlled diabetes (HbA_{1c} 7.8 %).

Absorption of soluble insulin from subcutaneous tissues is too slow to mimic the normal rapid increment of insulin in blood in response to a meal [15–19]. Therefore the development of fast-acting analogues (monomeric/dimeric) was considered of potential major clinical importance. Acute studies in healthy men and male IDDM patients has revealed that B10Asp as compared to human soluble insulin is characterized by half-maximal action obtained within 40 min after subcutaneous injection vs 60 min, higher metabolic clearance and shorter apparent plasma half-life and the same overall bioavailability. The in vitro insulin receptor affinity of B10Asp is 300 % that of human insulin when assessed by binding to human hepatoma cells [34]. Despite the improved plasma insulin/analogue profiles no major impact on overall glycaemic control was observed in our study. This may in part be explained by the existence of a saturable endothelial cell barrier which hampers the passage of insulin from plasma to the interstitial fluid and target organ cells, thus delaying and diminishing the hypoglycaemic effect of an increased plasma insulin/analogue concentration [35].

At our hospital IDDM patients are generally instructed to inject soluble insulin 30 min before the three main meals. Before conducting the present study we performed an anonymous questionnaire in 100 consecutive IDDM patients on basal bolus regimen regarding their actual timing of pre-meal insulin injection [36]. Forty-five percent took insulin 0–10 min before meals, 25 % between 11–20 min before meals and the remaining 30 % of the patients more than 20 min before meals. These findings led us to choose the preprandial insulin injections of less than 5 min before the meals, allowing for the unique opportunity to conduct a double blind randomised study comparing the two fast-acting insulin preparations.

Previous attempts to obtain more physiological plasma insulin concentrations by enhancing the absorption of soluble insulin include local massage [37, 38], the use of jet injector devices [39, 40] or sprinkler needles [41] and injection of insulin with aprotinin [42]. The enhanced absorption occurs mainly due to increased regional blood flow and increased capillary surface area product available for insulin passage.

From acute studies [25, 43] the possibility of increased frequency of postprandial hypoglycaemia using a fast-acting analogue might be suspected. There was no difference in the frequency of mild hypoglycaemic events comparing the two insulins. To insure that hypoglycaemia was not prevented by an increase in energy intake, i.e. more snacks, the patients were interviewed by a dietician three times during the study. No changes occurred in total energy intake or consumption of snacks during the study. The insulin doses were the same using the two insulins. These two facts are consistent with the unchanged BMI during the study.

No differences were found in serum lipids comparing the two insulins. Likewise, no differences were found in the safety parameters. Higher levels of insulin antibodies were found with B10Asp but only when using human insulin as tracer and the ratios between the two treatments were not significantly different.

We observed no side effects using the insulin analogue B10Asp over an 8-week period. Supraphysiological and pharmaceutical concentrations of B10Asp have been shown to induce a dose-dependent carcinogenic effect in the mammary glands in female but not in male rats [44]. All patients (male) receiving B10Asp in our study will be followed-up by physical examination and mammography at the local oncology department for at least the next 10 years. The carcinogen effect of B10Asp is likely to be caused by the increased affinity of the analogue to the insulin-like growth factor-1 (IGF-1) receptors. In rat aortic cells, B10Asp is five times more potent regarding binding to the IGF-1 receptor and also more

potent regarding incorporation of thymidine and stimulating cell growth as compared to soluble human insulin [45]. Therefore, future studies with B10Asp cannot be recommended.

In conclusion, despite differences in plasma insulin/analogue levels and 24-h blood glucose profiles, overall glycaemic control remained unchanged when Actrapid was exchanged dose for dose with the insulin analogue B10Asp. We found no side effects using B10Asp but animal studies have shown a dose-dependent carcinogenic effect of the analogue. The search for a fast-acting insulin analogue with low IGF-1 receptor affinity should be continued.

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