Diabetes, Obesity and Metabolism 12: 414–420, 2010.

ORIGINAL ARTICLE

Obesity does not influence the unique pharmacological properties of different biphasic insulin aspart preparations in patients with type 2 diabetes

T. Parkner[1,2], S. E. Dyrskog[3], T. Laursen[4], J. W. Chen[1], U. Mouritzen[5], L. Brondsted[5], K. Hermansen[3], T. Lauritzen[6] & J. S. Christiansen[1]

[1]*Department of Endocrinology and Diabetes M, Aarhus University Hospital, Aarhus, Denmark*
[2]*Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark*
[3]*Department of Endocrinology and Metabolism C, Aarhus University Hospital, Aarhus, Denmark*
[4]*Department of Pharmacology, University of Aarhus, Aarhus, Denmark*
[5]*Novo Nordisk A/S, Bagsvaerd, Denmark*
[6]*Department of General Medical Practice, University of Aarhus, Aarhus, Denmark*

Aim: To investigate the influence of obesity in type 2 diabetic patients upon pharmacological properties of different biphasic preparations of insulin aspart.

Methods: A total of 75 type 2 diabetic patients were stratified according to their body mass index (BMI) into 40 non-obese (BMI 23–28 kg/m^2) and 35 obese (BMI 30–35 kg/m^2) subjects. The trial was a double-blinded crossover study. In two periods of 4 weeks each the patients received subcutaneous injections of biphasic insulin aspart 50 (BIAsp 50) or biphasic insulin aspart 70 (BIAsp 70) thrice daily in random order. Insulin doses were titrated individually. At the end of each period 24-h serum profiles of insulin aspart, C-peptide and glucose were recorded. The primary endpoint was the area under the curve of serum glucose concentration during 24 h ($AUC_{Glu(0-24\ h)}$).

Results: The insulin concentration profiles of BIAsp 50 and 70 were as expected according to the content of protamine-bound insulin aspart (50 and 30% respectively). $AUC_{Glu(0-24\ h)}$ BIAsp 50/BIAsp 70 ratios were 0.97 (95% CI: 0.90–1.05, $p = 0.49$) for non-obese and 0.98 (95% CI: 0.92–1.05, $p = 0.55$) for obese. Fasting serum glucose (FSG) BIAsp 50/BIAsp 70 ratios were 0.90 (95% CI: 0.84–0.96, $p = 0.002$) for non-obese and 0.90 (95% CI: 0.84–0.97, $p = 0.006$) for obese. During both treatment regimens the frequency of minor hypoglycaemic episodes was highest for the non-obese group.

Conclusions: The pharmacokinetic and pharmacodynamic characteristics of the two preparations of biphasic insulin aspart were different; however, they were not influenced by the degree of obesity in type 2 diabetic patients.

Keywords: diabetes, insulin, insulin analogue, obesity

Date submitted 31 August 2009; date of first decision 23 October 2009; date of final acceptance 1 November 2009

Introduction

In type 2 diabetes, optimised glycaemic control prevents or delays development of especially microvascular complications in non-obese and obese subjects [1–3]. Progressive pancreatic B-cell failure in type 2 diabetes ultimately renders lifestyle changes and oral glucose-lowering agents insufficient to maintain tight glycaemic control and insulin treatment will frequently be required [4–6]. The first choice is often either an injection of intermediate-/long-acting basal insulin or injection with premix biphasic insulin preparations as a supplement to oral glucose-lowering agents [6–9]. There is no consensus as to which insulin preparation should be the firs choice [10–13]. However, the increased emphasis upon controlling postprandial glucose excursion has prompted the use of biphasic insulin preparations [14,15]. Thus, premixed insulin represents about 40% of the world market in human insulin [16].

The development of biphasic insulin aspart 70 (BIAsp 70: 70% rapid-acting insulin aspart and 30% intermediate-acting protamine-bound insulin aspart) and biphasic insulin aspart 50 (BIAsp 50: 50% insulin aspart and 50% protamine-bound insulin aspart) was aimed at providing premixed insulin analogues that mimic the endogenous serum insulin profile of healthy subjects when given at meals [17]. BIAsp 70 thrice daily have been compared to similar BIAsp 50 regimens, but without testing for differences between subgroups of type 2 diabetic patients [18]. However, as all injected insulin formulations initially are stored in subcutaneous (s.c.) tissue, there are inherent problems with absorption as regards onset of action, peak action, effective duration and variability; although improvements have been made for the different insulin analogues [19,20]. One of the main contributors to

Correspondence to: Dr Tina Parkner, Department of Clinical Biochemistry, Aarhus University Hospital, Norrebrogade 44, 8000 Aarhus C, Denmark.
E-mail: tparkner@dadlnet.dk

insulin properties, beside the insulin preparations themselves, is the vascularisation of the s.c. layer, which can be very different in thickness among patients [21]. A positive correlation has been found between s.c. blood flow and the rate of s.c. insulin absorption [22].

Our hypothesis was that whether a subject was obese or not could influence the absorption of the injected premixed insulin and the individual need [23]. If better glycaemic control was obtained with either BIAsp 70 or 50 thrice daily in subject subgroups based on body mass index (BMI), it might be possible to give recommendations on which premixed ratio should be used, based on the BMI of the subject.

Thus, the purpose of this study was to compare the effects of thrice-daily injections of BIAsp 70 and BIAsp 50 on the 24-h pharmacokinetic and pharmacodynamic profiles in non-obese and obese subjects with type 2 diabetes.

Methods

Patients

Seventy-five patients stratified as 40 non-obese (all Caucasians except one of Asian origin, eight female and 32 men) and 35 obese subjects (all Caucasians, 14 female and 21 men) participated in the study. Their baseline characteristics (the exposed subjects at screening 1 week before the first treatment period) are shown in table 1.

Inclusion criteria were age 30–75 years, BMI of 23–28 kg/m^2 (non-obese) *or* 30–35 kg/m^2 (obese), diagnosed with type 2 diabetes according to World Health Organisation classification (since 1999) for at least 6 months and glycated haemoglobin (HbA1c)$\leq$9.0%. The patients should have stable glycaemic control on any insulin injection regimen and the doses of s.c. injected insulin should remain unchanged for at least 1 month before the study and be in the range of 0.3–1.8 U/kg per day.

Exclusion criteria included patients with BMI $>$28 kg/m^2 *and* $<$30 kg/m^2, any systemic concomitant medication influencing glycaemic control, chronic kidney disease (serum creatinine $\geq$150 μmol/l), abnormal liver function tests (alanine aminotransferase or alkaline phosphatase $\geq$2 times the upper reference limit), severe cardiac insufficiency (New York Heart Association III or IV) or unstable angina/myocardial infarction within the last 12 months, uncontrolled hypertension (systolic blood pressure $\geq$180 and/or diastolic blood pressure $\geq$110 mmHg), planned or existing pregnancy, and any other clinically significant concomitant disorder.

Table 1. Mean ± s.d. characteristics (summary) for the 75 exposed patients with type 2 diabetes at baseline.

	Non-obese	Obese
Patients exposed (number)	40	35
Age (year)	59.5 ± 8.8	61.3 ± 6.9
Weight (kg)	79.4 ± 8.5	94.9 ± 10.7
BMI (kg/m^2)	26.3 ± 1.4	32.1 ± 1.6
Duration of type 2 diabetes (years)	11.1 ± 5.9	12.2 ± 5.5
HbA1c (%)	7.4 ± 0.9	7.6 ± 0.9
Total daily dose of insulin (U/kg)	0.30 ± 0.20	0.36 ± 0.27

Study Design

The trial was a stratified, randomised, double-blinded, two-period crossover study performed at the Department of Endocrinology and Diabetes and the Department of Endocrinology and Metabolism, Aarhus University Hospital, Aarhus, Denmark. The local Ethical Committee and the Danish Medicines Agency approved the trial protocol. The study was conducted in accordance with the Declaration of Helsinki 2000 and by the principles of Good Clinical Practice. Written informed consent from all the patients was obtained before enrolment in the study.

Patients were stratified according to their BMI prior to randomisation; 23–28 kg/m^2 in the non-obese group and 30–35 kg/m^2 in the obese group. The trial comprised a run-in period of 1 week and two treatment periods of 4 weeks. Between the treatment periods there was no washout period.

In the first treatment period the patients were randomised to either thrice-daily s.c. injections of BIAsp 50 or BIAsp 70 (both Penfill® 3 ml, 100 IU/ml, Novo Nordisk A/S, Bagsvaerd, Denmark), whereas they were treated with the opposite treatment regime in the second treatment period. The total daily dose when starting each treatment period was equal to the dose the subject received when entering the trial (the day prior to the first treatment period), thus the same individual starting dose was used in both treatment regimens for each subject. The total dose was divided into three doses of trial insulin injected just before breakfast (approximately 30%), lunch (approximately 30%) and dinner (approximately 40%). The percentage given in the parentheses indicate the recommended starting ratio [23].

In both treatment periods the daily insulin doses and the ratios of daily dose between meals were individually titrated (visits and regular phone contacts between the investigational site and the subjects) to achieve optimal doses according to the glycaemic response. The patients were instructed to inject s.c. with a 8-mm needle (NovoFine® 30 G, Novo Nordisk A/S, Bagsvaerd, Denmark) just before their three main meals each day, using the same region (abdominal wall preferably or thigh) for BIAsp 50 and BIAsp 70. The target for self-monitored (standard plasma calibrated GlucoMeter) preprandial plasma glucose levels was 5.0–7.2 mmol/l and for 1–2 h postprandial peak plasma glucose levels $<$10 mmol/l [24].

At the end of each 4-week treatment period the patients were admitted to the Department of Endocrinology and Diabetes for 24-h profile days. Subjects were told not to have significant changes in daily eating habits within the last week before these profile days. Within the last 24 h before each profile day subjects were not allowed intake of more than two alcohol units or strenuous exercise, and were withdrawal from the profile day if they had any acute conditions judged to be relevant by the investigator. Subjects fasted from 23:00 hours the night before each profile day. On profile days the subject administrated the trial insulin within 5 min before eating individually standardised meals at approximately 8:05, 13:05 and 18:05 hours. Subjects were allowed to choose between three types of standard meals, which were most like what they would have had at home. The meals had the exact same amount and content of fat, carbohydrate and protein on both profile days.

Also, activities were standardised individually in the clinic. During the 24-h profile days, serum insulin aspart, C-peptide and glucose were recorded with regular intervals, monitoring especially around meals (figure 1). For safety reasons, bedside glucose monitoring was performed (standard plasma calibrated GlucoMeter) before and 90 min after each meal, at 22:00 and 2:00 hours, and in the event of hypoglycaemic symptoms.

Hypoglycaemic episodes were defined as (i) *symptomatic only* if plasma glucose $\geq$3.1 mmol/l or the episode was not confirmed by plasma glucose measurement; (ii) *minor* if plasma glucose <3.1 mmol/l without hypoglycaemic symptoms or if the patient was able to treat the episode without assistance; and (iii) *major* if plasma glucose <3.1 mmol/l *and* the patient required intravenous glucose infusion.

Laboratory Assessments

Serum insulin aspart was measured by means of an immune assay specific for insulin aspart: Serum insulin aspart enzyme-linked immunosorbent assay (ELISA) (Capio Diagnostik A/S, Copenhagen, Denmark). Serum C-peptide was also measured by a validated ELISA method (Capio Diagnostik A/S). Serum glucose were analysed using a standardised enzymatic glucose oxidase method (Capio Diagnostik A/S).

Statistical Analyses

Twenty-eight subjects were required in each of the two BMI groups to detect a difference >15% between the area under the curve of serum glucose concentration during 24 h ($AUC_{Glu(0-24\ h)}$) with s.d. = 0.25 between the two insulin treatment regimens with a power of 80% and a significance level of 5% (two-sided alpha). Calculating with a dropout frequency of 15%, and expected non-evaluable profiles of three subjects, a total of 36 subjects where needed in each BMI group. This required a total of 72 subjects for the study.

Two analyses populations were defined; the efficacy and the safety population. The efficacy population included all randomised and exposed patients who had at least one evaluable 24-h serum glucose profile. This included 39 patients (one withdrawal) in the non-obese group and 33 (two withdrawals) in the obese group. The safety population included all 75 randomised and exposed patients.

No run-in trial treatment or washout period was included before or between the two treatment periods, as the half-life of insulin is very short (for the longest acting insulin formulations effective duration of approximately 1 day) compared to the duration of each treatment period (4 weeks). Thus, any carry-over effect from previous treatment period was not considered to affect the resulting profiles recorded on the last day of each treatment period. Further, the doses when starting each treatment period needed to be equal to the doses each subject entered the trial with in order to minimize a possible period effect.

Mean values were summarised. The $AUC_{Glu(0-24\ h)}$ was calculated by the trapezoid method, log-transformed, and analysed using an analysis of variance (ANOVA) model with an overall mean, a fixed treatment effect, a fixed effect of period, a random subject effect, and a measurement error. The effect of treatment was tested using a two-sided test and a significance level of 5%. The analysis was performed for non-obese and obese subjects, separately. The other efficacy endpoints were analysed in the same manner, except that the effect of treatment was tested with a significance level of 1%. The safety endpoints were only summarised.

All statistical analyses were produced using SAS® software, version 8.2.

Results

Insulin Doses

Total daily doses and mean doses of BIAsp 50 vs. BIAsp 70 at breakfast, lunch and dinner respectively, were not significantly different in the non-obese group (table 2). Likewise, in the obese group, the corresponding doses were not significantly different. Moreover, table 2 indicates more than twice as high total mean doses of both premixed insulin analogues in both BMI groups compare to the baseline values (table 1).

Twenty-Four-Hour Insulin Aspart and C-Peptide Concentrations

Figure 1 upper panel illustrates 24-h mean serum insulin aspart profiles after thrice-daily injections with BIAsp 50 or 70 in the two BMI groups. Serum insulin aspart levels were higher in the 4 h after meals (area under the curve of serum insulin concentration, $AUC_{Ins(0-4\ h)}$) for BIAsp 70 compared with BIAsp 50 in both BMI group, but only for the obese this was significant at all three meals (for all $p < 0.01$). The opposite was true during the night (22:00–8:00), where the insulin aspart levels ($AUC_{Ins(22-8\ h)}$) were highest for BIAsp 50 in both BMI group, but only significant for the non-obese ($p = 0.003$).

Figure 1, middle panel shows the corresponding mean serum C-peptide profiles.

Twenty-Four-Hour Glucose Concentrations

Figure 1 lower panel demonstrates the resulting overall 24-h mean serum glycaemic control for treatment with BIAsp 50 vs. BIAsp 70 in the two BMI groups. The $AUC_{Glu(0-24\ h)}$ BIAsp 50/BIAsp 70 ratio was 0.97 (95% CI: 0.90–1.05) for the non-obese group ($p = 0.49$). The $AUC_{Glu(0-24\ h)}$ BIAsp 50/BIAsp 70 ratio was 0.98 (95% CI: 0.92–1.05) in the obese group ($p = 0.55$).

During the day (8:00–22:00), both insulin regimens resulted in similar glucose control measured as $AUC_{Glu(8-22\ h)}$ BIAsp 50/BIAsp 70 ratio in the non-obese ($p = 0.77$) and the obese group ($p = 0.44$). During the night (22:00–8:00), BIAsp 70 provided higher mean glucose values measured as $AUC_{Glu(22-8\ h)}$ BIAsp 50/BIAsp 70 in the non-obese group ($p = 0.02$), but not in the obese group ($p = 0.78$).

Fasting Serum Glucose

Figure 1 and table 3 shows that the mean fasting serum glucose (FSG) values at the end of the 24-h profiles were high for both insulin treatment regimes in both BMI groups. The FSG BIAsp 50/BIAsp 70 ratio was 0.90 (95% CI: 0.84–0.96) for

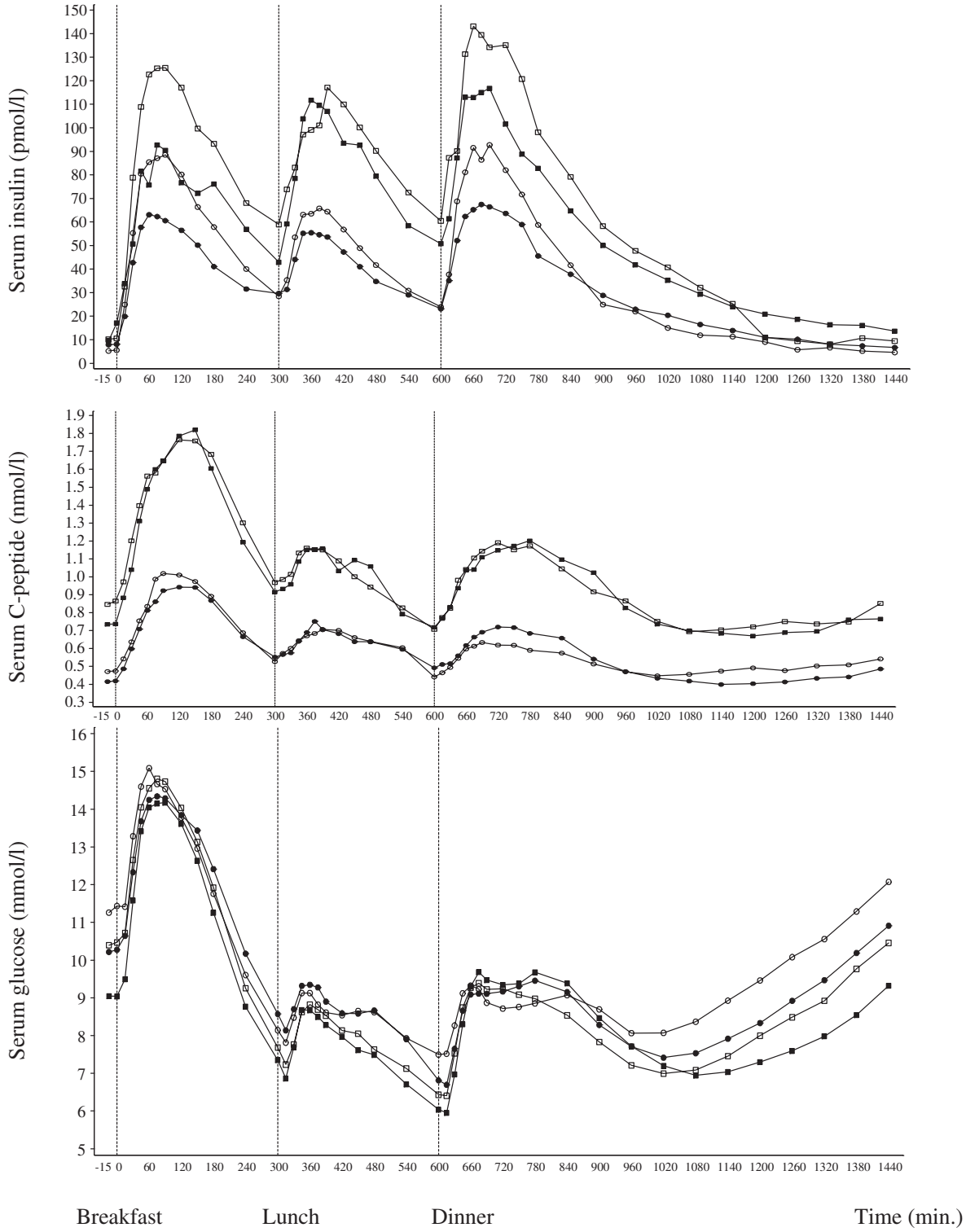

Figure 1. Mean 24-h serum insulin aspart, C-peptide and glucose profiles by treatment (BIAsp 50 and 70 thrice daily) and strata (non-obese and obese) in the end of each treatment period. (●) Non-obese treated with BIAsp 50; (○) Non-obese treated with BIAsp 70; (■) Obese treated with BIAsp 50; (□) Obese treated with BIAsp 70.

Table 2. Mean ± s.d. insulin doses by meal (summary) and p values of the BIAsp 50/BIAsp 70 ratios (ANOVA) at profile days in the end of each treatment period.

	Non-obese			Obese		
	BIAsp 50	BIAsp 70	BIAsp 50/70 p value	BIAsp 50	BIAsp 70	BIAsp 50/70 p value
Breakfast (U/kg)	0.28 ± 0.10	0.30 ± 0.12	0.09	0.31 ± 0.11	0.32 ± 0.12	0.25
Lunch (U/kg)	0.17 ± 0.08	0.17 ± 0.08	0.67	0.25 ± 0.10	0.24 ± 0.11	0.21
Dinner (U/kg)	0.23 ± 0.10	0.24 ± 0.10	0.70	0.29 ± 0.11	0.30 ± 0.12	0.33
Total daily (U/kg)	0.68 ± 0.24	0.71 ± 0.23	0.07	0.84 ± 0.31	0.87 ± 0.33	0.55

Table 3. Mean FSG ± s.d. (summary) and p values of the BIAsp 50/BIAsp 70 ratios (ANOVA) at the end of the 24-h profiles in the end of each treatment period.

	Non-obese			Obese		
	BIAsp 50	BIAsp 70	BIAsp 50/70 p value	BIAsp 50	BIAsp 70	BIAsp 50/70 p value
FSG (mmol/l)	10.9 ± 4.3	12.1 ± 5.0	0.002	9.3 ± 2.6	10.5 ± 3.6	0.006

Table 4. Numbers (frequency) of hypoglycaemic episodes by classification at profile days in the end of each treatment period.

	Non-obese		Obese	
	BIAsp 50	BIAsp 70	BIAsp 50	BIAsp 70
Symptoms only (number)	105 (33%)	95 (33%)	86 (57%)	85 (47%)
Minor (number)	217 (67%)	191 (67%)	65 (43%)	94 (53%)
Major (number)	0	1	0	0
All (number)	322 (100%)	287 (100%)	151 (100%)	179 (100%)

the non-obese group ($p = 0.002$). Similar, the FSG BIAsp 50/BIAsp 70 ratio was 0.90 (95% CI: 0.84–0.97) in the obese group ($p = 0.006$) (table 3).

Safety. Table 4 summarises the numbers and frequency of hypoglycaemic episodes by classification at the profile days. Subjects experiencing at least one episode of hypoglycaemia, ranged from 79% of subjects during treatment with BIAsp 50 in the obese group to 90% of subjects whilst on treatment with BIAsp 70 in the non-obese group. For both insulin treatments and in both BMI groups, the vast majority of hypoglycaemic episodes occurred during daytime. No safety concerns were raised, as assessed by the incidence of hypoglycaemic episodes and adverse events.

Discussion

Overall the obese group of patients with type 2 diabetes had higher 24-h mean serum insulin aspart levels than the non-obese group with type 2 diabetes (figure 1), reflecting the higher daily insulin doses used in the obese subjects (table 2). Moreover, even though we try to mimic everyday life in the more heterogeneous population of patients with type 2 diabetes, the pharmacokinetic profiles of BIAsp 50 and 70 were as expected according to a previous pharmacokinetic trial in patients with type 1 diabetes [25]. In both non-obese and obese subjects, treatment with BIAsp 70 thrice daily resulted in higher postprandial mean serum insulin aspart concentrations than BIAsp 50 thrice daily. The opposite was seen during the night, and BIAsp 50 provided the highest mean serum insulin aspart levels during the night hours (after 3:00) in the obese group (figure 1). These findings can be explained by the higher content of the rapid-acting insulin aspart in BIAsp 70 and of the intermediate-acting protamine-bound insulin aspart in BIAsp 50, as insulin parts in mixed preparations are preserved [25–27].

There was no difference in overall 24-h daily mean glycaemic control after thrice-daily BIAsp 50 vs. thrice-daily BIAsp 70 in non-obese and obese subjects with type 2 diabetes respectively. To our knowledge this is the first time to be shown in a clinical set-up. It was a reflection of the similar mean glucose profiles during daytime, whereas, treatment with both insulin regimens resulted in higher mean serum glucose levels during night hours (after 2:00) in the non-obese group compared with the obese group (figure 1). Again, this reflected the higher insulin doses used during the day in the obese subjects, especially at lunch and dinner (table 2). However during night-time, there was only a significant difference between the insulin regimens in the non-obese group with BIAsp 70 providing higher mean glucose values than BIAsp 50. This may be because of more preserved insulin sensitivity in non-obese than in obese subjects [28,29].

In general, FSG was high with both treatments and in both BMI groups (figure 1) [24]. Treatment with BIAsp 70 resulted in significantly higher FSG levels compared with BIAsp 50 in both BMI strata as expected from the different proportion of intermediate insulin in the mixtures (table 3). Overall, the suboptimal insulin delivery during the last part of the night, indicating the need for a higher ratio of prolonged insulin in the evening, such as, for example, BIAsp 30, for night-time glycaemic control [18,23,30].

The selected BMI criteria for the two strata were chosen in an attempt to develop guidelines for use of BIAsp 50 and BIAsp 70 in non-obese and obese patients with type

2 diabetes. The 4-week treatment periods allowed for near optimal titration of the two insulin regimes when balanced by hypoglycaemic episodes. The latter probably explains why the targets for glycaemic control in this study were not met during the treatment periods [24], especially during mornings (figure 1). However, the majority of patients did experience at least one episode of hypoglycaemia during the supervised profile days. For both treatment regimens the frequency of minor hypoglycaemic episodes was highest in the non-obese group (table 4). The presence of some preserved endogenous insulin, verified by C-peptide levels, possible acting as an insulin buffer, may have contributed to the overall safety profile without any concerns [31–34]. With both treatment regimens the highest concentrations of C-peptide were found in the obese group (figure 1).

Although, pharmacokinetic differences between BIAsp 50 and 70 were confirmed, possible overall pharmacodynamic differences may have been too small to be detected with the present study design. Stratification into BMI groups might not have been the most optimal way to show possible differences between non-obese and obese patients. Another approach for stratification could have been measuring the hip–waist ratio and/or using bioelectrical impedance as an indirect measure of body composition [35].

In summary, this trial did not confirm the hypothesis that treatment with BIAsp 50 and BIAsp 70 thrice daily provides significantly different overall glycaemic control in non-obese and obese subjects with type 2 diabetes.

Acknowledgements

We would like to thank the participating doctors: L. C. Gormsen and M. Krag for their efficacy in dealing with practical issues. Also the participating nurses: K. Lund, U. Sigsgaard and L. Jolin-Laursen for their effort. The trial was supported by an unrestricted grant from Novo Nordisk.

References

1. Stratton IM, Adler AI, Neil HA et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000; **321**: 405–412.
2. Shichiri M, Kishikawa H, Ohkubo Y, Wake N. Long-term results of the Kumamoto Study on optimal diabetes control in type 2 diabetic patients. Diabetes Care 2000; **23**(Suppl. 2): B21–B29.
3. Skyler JS, Bergenstal R, Bonow RO et al. Intensive glycemic control and the prevention of cardiovascular events: implications of the ACCORD, ADVANCE, and VA Diabetes Trials: a position statement of the American Diabetes Association and a Scientific Statement of the American College of Cardiology Foundation and the American Heart Association. J Am Coll Cardiol 2009; **53**: 298–304.
4. Levy J, Atkinson AB, Bell PM, McCance DR, Hadden DR. Beta-cell deterioration determines the onset and rate of progression of secondary dietary failure in type 2 diabetes mellitus: the 10-year follow-up of the Belfast Diet Study. Diabet Med 1998; **15**: 290–296.
5. Wright A, Burden AC, Paisey RB, Cull CA, Holman RR. Sulfonylurea inadequacy: efficacy of addition of insulin over 6 years in patients with type 2 diabetes in the U.K. Prospective Diabetes Study (UKPDS 57). Diabetes Care 2002; **25**: 330–336.
6. Nathan DM, Buse JB, Davidson MB et al. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2009; **32**: 193–203.
7. European Diabetes Policy Group 1999. A desktop guide to Type 2 diabetes mellitus. Diabet Med 1999; **16**: 716–730.
8. Davis T, Edelman SV. Insulin therapy in type 2 diabetes. Med Clin North Am 2004; **88**: 865–895.
9. International Diabetes Federation. Global Guideline for Type 2 Diabetes. Available from: http://www.idf.org (Online), 2009.
10. Raskin PR, Hollander PA, Lewin A, Gabbay RA, Bode B, Garber AJ. Basal insulin or premix analogue therapy in type 2 diabetes patients. Eur J Intern Med 2007; **18**: 56–62.
11. Raskin P, Allen E, Hollander P et al. Initiating insulin therapy in type 2 Diabetes: a comparison of biphasic and basal insulin analogs. Diabetes Care 2005; **28**: 260–265.
12. Kilo C, Mezitis N, Jain R, Mersey J, McGill J, Raskin P. Starting patients with type 2 diabetes on insulin therapy using once-daily injections of biphasic insulin aspart 70/30, biphasic human insulin 70/30, or NPH insulin in combination with metformin. J Diabetes Complications 2003; **17**: 307–313.
13. Janka HU, Plewe G, Busch K. Combination of oral antidiabetic agents with basal insulin versus premixed insulin alone in randomized elderly patients with type 2 diabetes mellitus. J Am Geriatr Soc 2007; **55**: 182–188.
14. American Diabetes Association. Postprandial blood glucose. American Diabetes Association. Diabetes Care 2001; **24**: 775–778.
15. International Diabetes Federation. Guideline for management of postmeal glucose. Available from: http://www.idf.org (Online), 2009.
16. Turner HE, Matthews DR. The use of fixed-mixture insulins in clinical practice. Eur J Clin Pharmacol 2000; **56**: 19–25.
17. Polonsky KS, Given BD, Van Cauter E. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. J Clin Invest 1988; **81**: 442–448.
18. Ejskjaer N, Rasmussen M, Kamp N, Lindholm A, Christiansen JS. Comparison of thrice daily 'high' vs. 'medium' premixed insulin aspart with respect to evening and overnight glycaemic control in patients with type 2 diabetes. Diabetes Obes Metab 2003; **5**: 438–445.
19. Reynolds NA, Wagstaff AJ. Insulin aspart: a review of its use in the management of type 1 or 2 diabetes mellitus. Drugs 2004; **64**: 1957–1974.
20. Parkner T, Laursen T, Vestergaard ET et al. Insulin and glucose profiles during continuous subcutaneous insulin infusion compared with injection of a long-acting insulin in Type 2 diabetes. Diabet Med 2008; **25**: 585–591.
21. Sindelka G, Heinemann L, Berger M, Frenck W, Chantelau E. Effect of insulin concentration, subcutaneous fat thickness and skin temperature on subcutaneous insulin absorption in healthy subjects. Diabetologia 1994; **37**: 377–380.
22. Vora JP, Burch A, Peters JR, Owens DR. Absorption of radiolabelled soluble insulin in type 1 (insulin-dependent) diabetes: influence of subcutaneous blood flow and anthropometry. Diabet Med 1993; **10**: 736–743.
23. Clements MR, Tits J, Kinsley BT, Rastam J, Friberg HH, Ligthelm RJ. Improved glycaemic control of thrice-daily biphasic insulin aspart compared with twice-daily biphasic human insulin; a randomized, open-label trial in patients with type 1 or type 2 diabetes. Diabetes Obes Metab 2008; **10**: 229–237.
24. American Diabetes Association. Standards of medical care in diabetes – 2007. Diabetes Care 2007; **30**(Suppl. 1): S4–S41.
25. Thorisdottir RL, Parkner T, Chen JW, Ejskjaer N, Christiansen JS. A comparison of pharmacokinetics and pharmacodynamics of biphasic insulin aspart 30,

50, 70 and pure insulin aspart: a randomized, quadruple crossover study. Basic Clin Pharmacol Toxicol 2009; **104**: 216–221.

26. Weyer C, Heise T, Heinemann L. Insulin aspart in a 30/70 premixed formulation. Pharmacodynamic properties of a rapid-acting insulin analog in stable mixture. Diabetes Care 1997; **20**: 1612–1614.
27. Jacobsen LV, Sogaard B, Riis A. Pharmacokinetics and pharmacodynamics of a premixed formulation of soluble and protamine-retarded insulin aspart. Eur J Clin Pharmacol 2000; **56**: 399–403.
28. Bonadonna RC, Groop L, Kraemer N, Ferrannini E, Del Prato S, DeFronzo RA. Obesity and insulin resistance in humans: a dose-response study. Metabolism 1990; **39**: 452–459.
29. Basu R, Chandramouli V, Dicke B, Landau B, Rizza R. Obesity and type 2 diabetes impair insulin-induced suppression of glycogenolysis as well as gluconeogenesis. Diabetes 2005; **54**: 1942–1948.
30. Ligthelm RJ, Mouritzen U, Lynggaard H et al. Biphasic insulin aspart given thrice daily is as efficacious as a basal-bolus insulin regimen with four daily injections: a randomised open-label parallel group four months comparison in patients with type 2 diabetes. Exp Clin Endocrinol Diabetes 2006; **114**: 511–519.
31. Groop LC, Pelkonen R, Koskimies S, Bottazzo GF, Doniach D. Secondary failure to treatment with oral antidiabetic agents in non-insulin-dependent diabetes. Diabetes Care 1986; **9**: 129–133.
32. Zangeneh F, Arora PS, Dyck PJ et al. Effects of duration of type 2 diabetes mellitus on insulin secretion. Endocr Pract 2006; **12**: 388–393.
33. Parkner T, Moller MK, Chen JW et al. Overnight CSII as supplement to oral antidiabetic drugs in Type 2 diabetes. Diabetes Obes Metab 2007; **10**: 556–563.
34. Parkner T, Laursen T, Chen JW et al. Overnight versus 24 hours of continuous subcutaneous insulin infusion as supplement to oral antidiabetic drugs in type 2 diabetes. J Diabetes Sci Technol 2007; **1**: 704–710.
35. Moyad MA Current methods used for defining, measuring, and treating obesity. Semin Urol Oncol 2001; **19**: 247–256.