ORIGINAL ARTICLE

Comparison of thrice daily 'high' vs. 'medium' premixed insulin aspart with respect to evening and overnight glycaemic control in patients with type 2 diabetes

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Background: The glycaemic control of thrice daily treatment with premixed biphasic insulin aspart (BIAsp) without other antidiabetic therapy was tested in type 2 diabetic patients, in order to compare the glucose control of a 'high' mixture (BIAsp 70) or a 'medium' mixture (BIAsp 50) (70 or 50% soluble IAsp and 30 or 50% protamine-crystallized IAsp, respectively) administered just before dinner.
Aim: To compare these regimens to conventional 30 : 70 premixture on a twice a day basis.
Methods: This randomized, double-blind, two-period crossover study included 16 patients with type 2 diabetes. Twenty four-hour serum glucose and insulin profiles were obtained thrice: (1) after a one-week run-in period with biphasic human insulin (BHI) 30/70 twice daily (run-in), (2) after 4 weeks of treatment with thrice daily BIAsp 70 before breakfast, lunch and dinner (Dinner70 regimen) and (3) after 4 weeks of BIAsp 70 before breakfast and lunch and BIAsp 50 before dinner (Dinner50).
Results: Daytime average serum glucose was lower with Dinner70 compared to run-in (9.6 ± 0.39 mmol/l vs. 11.2 ± 0.61 mmol/l, $p < 0.05$). Postprandial glucose excursions after breakfast and lunch were lower, but fasting morning glucose was higher during the treatment periods than in the run-in period. Twenty four-hour C-peptide AUC was considerably lower during both treatment periods than in the run-in period (run-in/Dinner50 ratio 1.29 [1.08; 1.54] $p < 0.01$; run-in/Dinner70 ratio 1.31 [1.08;1.58], $p < 0.01$).
Conclusions: Switching the dinner dose to BIAsp 50 did not alter overall glucose control significantly from that provided with BIAsp 70. Exploratory analyses between the two active treatment regimens and run-in/BHI indicate that thrice daily BIAsp 70 administration: (1) for optimization of the night-time control, the dinner dose needs adjustment or replacement by a premixed insulin with a larger proportion of basal insulin than BIAsp 50 and (2) none of the premixtures adequately provide for both the evening meal and overnight requirements.

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Introduction

A number of landmark studies, most prominently the Diabetes Control and Complications Trial (DDCT) and the UK Prospective Diabetes Study (UKPDS), have documented that tight glycaemic control reduces the incidence and delays the progression of late diabetic complications associated with type 1 and type 2 diabetes [1–4]. While many people with type 2 diabetes generally

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do not require insulin treatment for survival, an increasing proportion (20–30%) use insulin injections to correct persistent hyperglycaemia [5].

Premixed insulin formulations are often prescribed for insulin-requiring type 2 diabetes because of remnant insulin secretion and ease of administration [6]. Experience has shown that twice daily treatment with the most widely used mixture of 30% soluble human insulin (HI) and 70% protamine-crystallized human insulin (NPH) (BHI 30) partially may compensate for hyperglycaemia after breakfast and dinner, as well as provide sufficient basal insulin requirements until the next injection [6].

Insulin aspart (IAsp) is a rapid-acting insulin analogue that may more closely match the physiological need in the postprandial period than HI by means of faster absorption [7–11]. These advantageous properties vs. HI have been shown to be retained in a range of IAsp-premixed formulations [12–15]. The range includes a 'low' mix (biphasic insulin aspart 30, BIAsp 30), a 'medium' mix (BIAsp 50) and a 'high' mix (BIAsp 70) consisting of 30%, 50% or 70% soluble IAsp combined with 70%, 50% or 30% protamine-crystallized IAsp, respectively.

Premixed HI is almost exclusively administered twice daily. However, with such regimen, postprandial control in general and lunchtime control in particular are not optimally controlled [6, 16–18]. As initially assessed in computer-modelling experiments, the comparatively more dynamic time-action profiles of biphasic insulin analogues provide the possibility to dose thrice daily [19]. This would potentially improve mealtime glucose control without increasing the risk of late postmeal hypoglycaemia [20]. However, before embarking on larger scale clinical studies, it was important to assess the night-time glucose control with use of the 'medium' or the 'high' IAsp mix. Therefore, the objectives of this study were to test the concept of administering BIAsp 70 thrice daily as combined bolus and basal insulin and to test whether switching to BIAsp 50 before dinner would be necessary in order to fully control overnight serum glucose levels.

Patients and methods

Subjects

Sixteen type 2 diabetic mellitus patients were recruited from the diabetic outpatients clinic of the department of endocrinology at Aarhus Kommune hospital. The demographic and baseline characteristics are summarized in table 1. Eligible patients were treated with twice daily BHI 20 or 30 for more than 3 months and had reasonably stable glucose control with glycosylated haemoglobin (HbA_{1c}) < 11%. Excluded were those grossly overweight (body mass index, BMI > 35) and those with impaired renal function (serum creatinine > 150 μmol/l), proliferative retinopathy or any evidence of rapidly progressing complications. All 16 subjects completed the study. One subject was excluded from the analysis of efficacy endpoints due to a protocol violation (incorrect mixture of insulin during run-in period). The study was approved by the Ethics Committee in Aarhus County and by the Danish Medicines Agency and was performed in accordance with the 'Declaration of Helsinki' [21] and in accordance with Good Clinical Practice (GCP). Written consent was obtained from all patients.

Table 1 Subject characteristics. Mean (range) or count

Number of subjects	16
Age (year)	59.3 (46–75)
Gender (male/female)	8/8
Race (% Europid)	100
BMI (kg/m^2)	27.7 (21–32)
Smoker	5/16
Duration of diabetes (year)	12 (4–25)
Baseline HbA1c (%)	8.63 (6.2–10.6)
Baseline serum fructosamine (umol/l)	329 (174–467)
Baseline insulin dose (IU/kg)	
Breakfast	0.40 (0.15–0.85)
Dinner	0.24 (0.06–0.37)

Study design

The study was a single-centre, randomized, double-blind, two-period crossover study in type 2 diabetic subjects. All patients were on a stable twice daily BHI 30 therapy during the 1-week run-in period (referred to as 'run-in') and were subsequently randomized to receive one of two treatment regimens for 26–30 days in a crossover fashion: in one period, BIAsp 70 was administered immediately before breakfast, lunch and dinner ('Dinner70'); in the other period, BIAsp 70 was administered immediately before breakfast and lunch and BIAsp 50 before dinner ('Dinner50').

The subjects attended formal visits at screening, at randomization to period 1, after 2 and 4 weeks (first period), after 6 and 8 weeks (i.e. 2 and 4 weeks into second period) and at 9 weeks (poststudy visit).

Treatment

During the run-in period, the subjects were treated with BHI twice daily before breakfast and dinner, dosed as before entering the study (run-in; BHI 30). Subjects were

then randomly assigned to a study-product sequence: Dinner70 or Dinner50 and were instructed to take their biphasic insulin within 5 min of starting a meal and to continue to do so throughout the period, to administer their biphasic insulin by subcutaneous injection, maintain the same injection region throughout the study and change location within each site after each injection. The initial total daily dose of BIAsp for both treatment periods was to be the same as at the end of the run-in; the twice daily dose during the run-in period was then divided into three equal doses of study product for breakfast, lunch and dinner administration. The initial dose level was fixed at each treatment period to provide as similar conditions as possible between treatment periods. The dose was then frequently adjusted aiming for the targets of glycaemic control (see below).

24-h profiles

Twenty four-hour serum sampling was performed at the end of the run-in period and at the end of the treatment periods. Subjects attended the hospital in the afternoon on the day before these visits and stayed at the hospital overnight. They were instructed to avoid strenuous exercise and alcohol within 24 h. Standard meals with the same content on all three occasions of fat (25–37%), calorie (1500, 2000 or 2500 kcal) and protein (10–20%) were administered at fixed time points during the 24-h profiles. Blood was collected for separation of serum samples starting at 17:00 hours hourly for 24 h and additionally every 15 min for 2 h after meals and at 2.5 h after meals (in total 46 sample points/profile).

Subjects were provided with new calibrated blood glucose-monitoring devices (One Touch® Profile™, Lifescan, Milpitas, CA, USA), instructed in conducting self-monitoring of blood glucose (FBG daily and 8-point profiles weekly) and were informed about targets for glycaemic control; prandial (4–7 mmol/l), postprandial (<10 mmol/l) and 02:00 hours (4–7 mmol/l). The results of these measurements were used to help the subjects and the investigators optimize insulin dosing. The subjects were supplied with a diary to record hypoglycaemic episodes between visits.

Pharmacokinetic and pharmacodynamic assessments

Serum fructosamine and pharmacodynamic and pharmacokinetic measurements of serum glucose, insulin and C-peptide were obtained from the 24-h profiles at run-in and after each of the two treatment periods. The AUC of glucose, insulin and C-peptide were calculated by the trapezoidal method and compared over 24 h. Excursions (EXC) were measured as the baseline-corrected AUC over the specified time periods. Additional pharmacodynamic endpoints were weighted average glucose, maximum postmeal glucose ($C_{\max(glu)}$) and insulin ($C_{\max(ins)}$) and the time taken to reach these values ($t_{\max(glu)}$) and ($t_{\max(ins)}$).

Analyses

Concentrations of IAsp, HI and C-peptide were measured, each using a specific two-sided ELISA [22, 23]. Serum glucose was measured by the glucose oxidase method [24]. Total serum concentrations of insulin were measured using the sum of the concentrations of IAsp plus HI.

Statistical method

The power calculation based on a previous study showed that 12 subjects needed to complete the study in order to have 80% power to detect a 17% treatment difference in mean fasting serum glucose. All endpoints were analysed by ANOVA and, with the exception of $t_{\max}$, log-transformed before analysis. For the fasting serum glucose, a significance level of 5% was used. For all other endpoints, a significance level of 1% was used. For $t_{\max}$, the comparison between regimens was done using Wilcoxon sign rank test. Comparisons between the two treatment regiments and run-in should be cautiously interpreted, as they are not part of a randomized comparison.

Results

Overall Glycaemic Control

Serum fructosamine levels did not differ between the Dinner50 and the Dinner70 regimens after 4 weeks of treatment (316 ± 53 μmol/l vs. 318 ± 60 μmol/L; NS; table 2).

The mean 24-h serum glucose, insulin and C-peptide profiles during the run-in period and the two active treatment periods are presented in fig. 1.

24-h Glycaemic Control

There were no significant differences in mean serum glucose level between treatments. However, $AUC_{24\,h(glu)}$ tended to be lower with the Dinner70 regimen compared to the Dinner50 regimen (NS, table 2).

Daytime and Nighttime Glucose Control

Daytime average serum glucose levels did not differ between the two treatment periods but were lower for the Dinner70 treatment than in the run-in period (9.6 ± 0.39 mmol/l vs. 11.2 ± 0.61 mmol/l, $p < 0.05$; table 2). In general, daytime serum glucose levels were higher than during the night.

Night-time serum glucose did not differ between the treatment periods or between the treatment and run-in periods.

Preprandial Glucose Control

There were no treatment differences in preprandial glucose levels. However, fasting serum glucose was significantly higher during the treatments than during the run-in period (10.2–10.7 mmol/l in the treatment periods vs. 7.8 mmol/l during run-in, table 2).

Postprandial Glucose Control

Postprandial 4-h serum glucose excursions were lower after breakfast and lunch during both treatment periods compared to the run-in period, and $t_{max(glu)}$ was higher and appeared later at the end of the run-in period compared to either of the treatment periods.

Postprandial 4-h serum glucose excursions after dinner tended to be larger with the Dinner50 regimen than with the Dinner70 regimen ($p = 0.085$, table 2). The results of the comparisons for C_{max} and t_{max} were consistent with those for $EXC_{0\text{-}4(glu)}$.

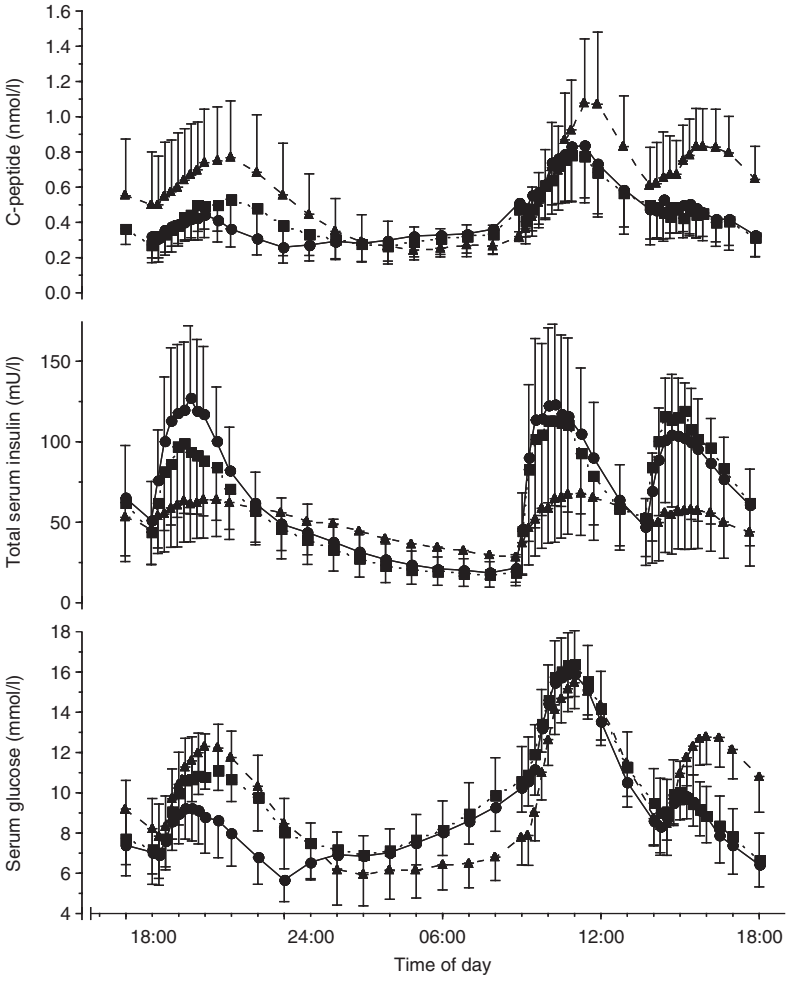

Fig. 1 Mean serum glucose, serum total insulin and C-peptide profiles at run-in (dashed, triangles) and during thrice daily BIAsp therapy (BIAsp 70; solid line, circles; BIAsp 50, dashed, squares).

Table 2 Measures of glucose control as obtained from 24-h serum glucose profiles

		Treatment periods		ANOVA between treatment periods (Dinner50/Dinner70 or Dinner50 – Dinner70)		
Endpoint	**Run-in mean ± SD**	**Dinner50 mean ± SD**	**Dinner70 mean ± SD**	**Ratio/diff**	**95% CI**	**p value**
Overall control						
Fructosamine (umol/l)	320 ± 63	316 ± 53	318 ± 60	−1.93	[−95.5; 15.6]	NS
24-h serum glucose						
$AUC_{24\,h\ (glu)}$ (mmol/l*min)	13567 ± 3469	13763 ± 3405	12500 ± 1968	1.08	[0.96; 1.22]	0.19
Daytime serum glucose						
$AVE_{8-22\,h\ (glu)}$ (mmol/l)	11.2 ± 2.4‡	10.6 ± 2.7	9.6 ± 1.5	0.95	[−0.36; 2.25]	0.14
Night-time serum glucose						
$AVE_{23-7\,h\ (glu)}$ (mmol/l)	7.0 ± 2.9	8.2 ± 2.3	7.4 ± 1.7	0.78	[−0.12; 1.67]	0.083
Preprandial serum glucose						
$Pre\text{-}meal_{(glu),\ breakfast}$ (mmol/l)	7.8 ± 2.7†‡	10.7 ± 3.5	10.2 ± 2.5	1.03	[0.90; 1.17]	NS
$Pre\text{-}meal_{(glu),\ lunch}$ (mmol/l)	8.6 ± 2.7	9.5 ± 3.6	8.5 ± 2.4	1.08	[0.94; 1.26]	NS
$Pre\text{-}meal_{(glu),\ dinner}$ (mmol/l)	7.7 ± 4.1	7.3 ± 5.2	6.7 ± 3.0	1.00	[0.72; 1.38]	NS
Postprandial serum glucose						
Breakfast						
EXC0–4 h (glu),breakfast (mmol/l*min)	1248 ± 504†‡	954 ± 492	845 ± 372	1.06	[0.88; 1.28]	NS
$C_{max\ (glu),\ breakfast}$ (mmol/l)	16.2 ± 2.8	17.2 ± 3.4	16.3 ± 2.3	1.05	[0.96; 1.14]	NS
$t_{max\ (glu),\ breakfast}$ (min)	119 ± 38	115 ± 28	107 ± 21	8.3	[−3.2; 19.9]	0.14
Lunch						
$EXC_{0-4\,h\ (glu),lunch}$ (mmol/l*min)	653 ± 298†‡	468 ± 309	442 ± 299	1.08	[0.64; 1.81]	NS
$C_{max\ (glu),\ lunch}$ (mmol/l)	13.3 ± 2.8†‡	10.9 ± 3.0	10.5 ± 2.1	1.02	[0.87; 1.20]	NS
$t_{max(glu),\ lunch}$ (min)	126 ± 31†‡	51 ± 41	55 ± 37	−3.9	[−24.9; 17.1]	NS
Dinner						
$EXC_{0-4\,h\ (glu),\ dinner}$ (mmol/l*min)	783 ± 525	749 ± 482	489 ± 347	1.63	[0.93; 2.86]	0.085
$C_{max\ (glu),\ dinner}$ (mmol/l)	12.5 ± 3.9	11.9 ± 4.1	9.6 ± 2.9	1.22	[1.00; 1.50]	0.052
$t_{max\ (glu),\ dinner}$ (min)	118 ± 61	132 ± 48	81 ± 44	50.8	[14.8; 86.8]	<0.01

*Ratios are presented for EXC, AUC, C_{max} and premeal and differences are presented for fructosamine, AVE, and t_{max}. The estimated ratio/diff and confidence interval (CI) are based on an ANOVA with adjustment treatment.
†$p < 0.05$ between run-in and Dinner50.
‡$p < 0.05$ between run-in and Dinner70.

24-h Serum Insulin Profiles

A repeated measures analysis of individual IAsp concentration-time profiles, at the end of both treatment periods, indicated that the shapes of the Dinner50 and the Dinner70 profiles differed significantly between treatments ($p < 0.01$). There was a trend for larger IAsp AUC with Dinner70 than with Dinner50 (table 3). As visually depicted, treatment with BIAsp thrice daily provided higher total insulin levels after all three meals and somewhat lower levels during the night compared to twice daily treatment with to BHI during the run-in period (fig. 1). Although the total insulin profile for the run-in period has been included in fig. 1, a comparison between this and profiles for the two treatment regimens should be interpreted with some caution since this comparison was not included as part of the trial design.

As expected the maximum serum insulin concentrations after dinner were lower with the Dinner50 regimen than with the Dinner70 regimen (table 3).

C-peptide profiles

The C-peptide profiles did not differ between treatments but each treatment had a lower $AUC_{24\,h}$ compared to the run-in period (fig. 1). With Dinner70 compared to baseline the ratio was 1.29 [1.08;1.54,$p < 0.01$]. With Dinner50 the ratio to baseline was 1.31 [1.08;1.58,$p < 0.01$]

Discussion

In current clinical practice, the next treatment step after insufficient treatment with twice daily BHI treatment is

Table 3 Total insulin and insulin aspart pharmacokinetics as obtained from 24-h insulin profiles

Endpoint	Run-in mean ± SD	Dinner50 mean ± SD	Dinner70 mean ± SD	ANOVA comparison mean ratio/difference*	p value
24-h total serum Insulin					
$AUC_{24\,h\ (ins)}$ (mmol/l*min)	72800 ± 62100†‡	85500 ± 69800	91200 ± 66500		
24-h IAsp					
$AUC_{24\,h\ (ins)}$ (mmol/l*min)	N/A	64695 ± 60878	68712 ± 54407	0.87 [0.76; 1.00]	0.057
Postprandial IAsp					
Breakfast					
$C_{max\ (ins),\ breakfast}$ (mU/l)		83 ± 86	90 ± 80	0.84 [0.66; 1.07]	0.15
$t_{max\ (ins),\ breakfast}$ (min)		77 ± 32	81 ± 31	−4.4 [−29.0; 20.5]	NS
Lunch					
$C_{max\ (ins),\ lunch}$ (mU/l)		110 ± 130	88 ± 72	1.04 [0.86; 1.25]	NS
$t_{max\ (ins),\ lunch}$ (min)		77 ± 33	75 ± 38	1.6 [−26.3; 29.5]	NS
Dinner					
$C_{max\ (ins),\ dinner}$ (mU/l)		87 ± 82	109 ± 84	0.72 [0.57; 0.91]	<0.01
$t_{max\ (ins),\ dinner}$ (min)		99 ± 40	74 ± 33	24.8 [2.95; 46.6]	<0.05
24-h C-peptide					
$AUC_{24\,h\ (ins)}$ (nmol/l*min)	828 (149)†‡	611 ± 24	593 ± 23	1.01 [0.88; 1.16]	NS

*Ratios are presented for AUC and C_{max}; and differences are presented for and t_{max} The estimated ratio/diff and confidence interval (CI) are based on an ANOVA with adjustment for treatment.

†$p < 0.05$ between run-in and Dinner50.

‡$p < 0.05$ between run-in and Dinner70.

basal-bolus treatment. Basal-bolus treatment may present as a substantial burden for an elderly patient with type 2 diabetes. Thrice daily treatment with a combination of BIAsp 70 and BIAsp 50 may offer a new treatment option for diabetic patients who are suboptimally controlled on twice daily premixed HI, with the potential to provide lower postprandial glucose excursions, thereby preceding the traditional basal-bolus treatment.

The present study was designed to test the concept of administering BIAsp 70 thrice daily in type 2 diabetic subjects and to investigate whether switching to BIAsp 50 at dinner would be necessary to maintain fasting serum glucose. Previous results of pharmacokinetic modelling with various mixtures of IAsp and NPH indicated that thrice daily mealtime injection of BIAsp 70 might offer an opportunity to mimic the endogenous insulin profile of normal healthy individuals [19, 20]. The current study showed excellent match to the computerized predictions and demonstrated an overall adequate glucose control with thrice daily premixed IAsp 'high' mixture injections.

The study included 16 type 2 diabetic subjects who were generally poorly controlled with twice daily premixed HI (mean HbA_{1c} of 8.6%) with differing degrees of residual endogenous insulin release. This heterogeneity is, however, unlikely to be of consequence due to the cross-over design of the study.

Glucose levels during the first 4 h following dinner showed a tendency to be lower after injecting BIAsp 70 than after BIAsp 50. This is likely to be a consequence of the higher soluble component in BIAsp 70. However, administration of BIAsp 50 with dinner in place of BIAsp 70 failed to yield lower fasting serum glucose levels.

The exploratory analyses comparing the profiles at the end of the treatment periods with the profiles obtained at the end of the run-in period should be interpreted with caution because these comparisons were not randomized, and it is not possible to differentiate between treatment effects and study effects. Nevertheless, these analyses were hypothesis-generating for establishing suitable dosing regimens for future studies with thrice daily treatment with BIAsp 70 and BIAsp 50. Results form the exploratory comparisons indicated that thrice daily treatment with BIAsp 70 provides lower daytime glucose excursions and improved daytime control compared to twice daily treatment with premixed HI. However, fasting serum glucose levels were significantly lower with twice daily BHI at the end of run-in. Thus, it is likely that the advantage gained during the daytime with the thrice daily regimen was lost during the night. In order to optimize glucose control during the night, the dinner dose of BIAsp 50 should be replaced by another biphasic IAsp with a greater proportion of protracted BIAsp, a 'low' mixture.

The results indicated, that in the patients tested, the proportion of protracted IAsp in BIAsp 50 was not enough to provide insulin action into the early morning hours. This means that many patients would need at least two different mixtures to adequately cover the diurnal insulin need in a thrice daily regimen. Although this is a drawback, thrice daily insulin administration still is simpler and would require fewer injections than the basal-bolus regimen.

The patients in the present study had long-standing diabetes (mean 12 years). Overnight control may be easily maintained in patients at earlier stages of the disease progression. Thus, even if the patients in the current study required more longer acting insulin in the evening, a 'high' mix for each meal might be sufficient in patients with earlier type 2 diabetes.

The results of the present study have been brought forward, and there are now early indications that the thrice daily treatment concept may provide a lower HbA_{1c} than traditional premixed insulin therapy [25]. Future studies will also have to address the comparison to the traditional basal-bolus regimen.

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Duality of interest

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