DIABETES/METABOLISM RESEARCH AND REVIEWS
Diabetes Metab Res Rev 2000; **16**: 262–268.
DOI: 10.1002/1520-7560(2000)9999:9999<::AID-DMRR120>3.0.CO;2-1

RESEARCH ARTICLE

Symptomatic and counterregulatory hormonal responses to acute hypoglycaemia induced by insulin aspart and soluble human insulin in Type 1 diabetes

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Received: 8 February 2000
Revised: 29 March 2000
Accepted: 3 April 2000
Published online: 19 June 2000

Abstract

Background The aim of this study was to assess hypoglycaemia awareness with the insulin analogue, insulin aspart. The counterregulatory hormonal and symptomatic responses to hypoglycaemia induced by insulin aspart were compared with soluble human insulin in a double-blind, randomised, two-period crossover trial in patients with Type 1 diabetes. The primary objective was to compare the blood glucose threshold for autonomic activation during hypoglycaemia induced by insulin aspart and soluble human insulin. Secondary objectives were to compare the counterregulatory, symptomatic and physiological responses to hypoglycaemia.

Methods 20 patients were screened, 17 were randomised and 16 completed the study. Acute hypoglycaemia was induced by intravenous infusion of insulin aspart or soluble human insulin (100 U ml^{-1} at a rate of 2 mU kg^{-1} min^{-1}).

Results No statistical difference between insulin aspart and soluble human insulin was shown for the primary blood glucose endpoint; mean arterialised blood glucose concentrations ($\pm$SD) at the onset of autonomic activation were 1.88 ± 0.39 mmol L^{-1} for insulin aspart and 1.89 ± 0.43 mmol L^{-1} for soluble human insulin (not significant). No statistical differences were observed between the two insulins for the secondary endpoints: counterregulatory hormonal responses, autonomic responses, hypoglycaemia symptom scores, cognitive function and blood glucose responses. No serious adverse events were reported during the study.

Conclusions Insulin aspart and soluble human insulin elicit the same counterregulatory and symptomatic responses to acute hypoglycaemia in patients with Type 1 diabetes. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords insulin aspart; Type 1 diabetes; hypoglycaemia; counterregulatory hormones; autonomic reaction

Introduction

Insulin aspart is a rapid-acting insulin analogue that can be administered immediately before meals in people with Type 1 diabetes. The aim of the treatment is to obtain an insulin action profile closer to the non-diabetic state, consequently preventing postprandial hypoglycaemia. The proline at the B28

position in the insulin molecule is replaced with aspartic acid, which reduces the tendency to self-associate. As a result, insulin aspart has a faster onset and shorter duration of action, can be administered immediately before food, and provides better overall glycaemic control when compared with soluble human insulin [1–8].

The generation of warning symptoms for incipient hypoglycaemia should alert individuals with insulin-treated diabetes to take corrective action. However, warning symptoms may be diminished or absent, so increasing the risk of severe hypoglycaemia. Although unproven, it has been suggested that impaired awareness of hypoglycaemia is associated with human insulin preparations as compared with porcine insulin [9–14]. As a novel insulin preparation, insulin aspart needed to be compared with human insulin to examine the comparative symptomatic and counterregulatory hormonal responses to hypoglycaemia. Studies in healthy volunteers have shown that the overall variability of action of insulin aspart was comparable to that of human insulin [15]. In everyday life, a fall in blood glucose triggers a hierarchy of responses at different glycaemic thresholds [16]. Autonomic activation is characterised by the rapid development of haemodynamic changes, sweating, tremor and the coincidental onset of autonomic symptoms of hypoglycaemia [17].

The present study was designed to compare the blood glucose threshold for autonomic activation during hypoglycaemia induced by insulin aspart and soluble human insulin. Secondary objectives were to compare the counterregulatory, symptomatic and physiological responses to hypoglycaemia induced by insulin aspart and soluble human insulin.

Research design and methods

Subjects

Patients with Type 1 diabetes were recruited from the diabetes outpatient clinic at the Royal Infirmary of Edinburgh. Men and women, aged 18–45 years, with Type 1 diabetes of relatively short duration (between 1 and 6 years), were screened for inclusion in the trial. They had good glycaemic control, with nine patients using twice daily regular and isophane insulins, and the remainder were on multiple insulin injection therapy (regular insulin before meals with isophane at bedtime), for a minimum of 1 year. They were not overweight and had normal autonomic function [18]. None of the patients had evidence of diabetic complications, including retinopathy, peripheral or autonomic neuropathy or the presence of microalbuminuria, and all were normotensive (blood pressure $<140/90$ mmHg). Exclusion criteria included concomitant administration of beta adrenoceptor blocking drugs, systemic corticosteroids, anxiolytic drugs, antidepressant drugs, hypnotic agents or angiotensin-converting enzyme inhibitors, and a history of impaired awareness of hypoglycaemia or severe recurrent hypoglycaemia. The trial had the approval of the local medical ethics advisory committee and was performed in accordance with the Helsinki Declaration and appropriate Food and Drug Administration regulations. All participants gave written, informed consent.

Study design

A double-blind, randomised, two-period crossover trial was conducted in a single centre. Each patient attended the centre on four occasions: a pre-trial visit to establish suitability, two study days (separated by a 3–6-week washout period), and a post-trial visit. None of the patients experienced severe hypoglycaemia within the 3 weeks preceding the study day and/or symptomatic hypoglycaemia and/or a recorded blood glucose level of less than 3.5 mmol L^{-1} within 5 days of either study day. On the study days, the patient received an infusion of soluble human insulin (Actrapid®, Novo Nordisk A/S, Denmark), at a rate of 2–6 U h^{-1} and an infusion of 10% dextrose to achieve and maintain relative euglycaemia (blood glucose 5.0–8.0 mmol L^{-1}) before the induction of acute hypoglycaemia in the early afternoon. The cognitive tests were performed several times during the pre-hypoglycaemia period to minimise practice effects. Acute hypoglycaemia was induced by intravenous infusion of insulin aspart or soluble human insulin (100 U ml^{-1} at a rate of 2 mU kg^{-1} min^{-1} into an antecubital vein in the dominant arm). Each patient received one of the agents (in a random sequence) on each study day. The infusion continued until the onset of acute autonomic activation, or until blood glucose levels fell to 2.0 mmol L^{-1}, or until florid symptoms of hypoglycaemia had developed that were subjectively uncomfortable for the patient. Once the autonomic reaction had occurred, hypoglycaemia was reversed by the infusion of dextrose (10%, 200 ml h^{-1}) to restore normoglycaemia within 90 min.

Efficacy measurements

The primary efficacy endpoint was the blood glucose concentration at the time of the autonomic reaction (R). This was defined as the blood glucose value when an abrupt rise in heart rate (15% from baseline) and/or the onset of sweating was observed. The secondary efficacy endpoints were counterregulatory hormonal responses to hypoglycaemia, haemodynamic autonomic responses, hypoglycaemia symptom scores, cognitive function tests and blood glucose response. Safety measurements included general physical examination, fundoscopy, electrocardiogram (ECG), autonomic function, blood pressure and heart rate, haematology, biochemistry, glycaemic control, urinalysis and adverse events.

During the euglycaemic clamp phase and throughout each hypoglycaemia test, arterialised whole blood samples were taken for measurement of glucose and counterregulatory hormonal levels. Blood glucose was estimated at 5-min intervals until R was identified, the time clock

was then set to zero, and measurements continued until the end of the study at the same time intervals. The timing of samples from R allowed for observation of inter-individual variation in the time taken for blood glucose to fall to the threshold level to trigger the autonomic reaction [19]. Blood for measurement of the counter-regulatory hormonal responses was taken at baseline, baseline plus 20 min, R, and at 15-min intervals following R until R + 60 min. Heart rate and sweating were monitored continuously, with serial measurements of blood pressure at 10-min intervals. The hypoglycaemic symptom score was calculated using a validated symptom questionnaire [20]. The change in individual symptom scores was determined by subtracting the baseline value from the score for each symptom at each time point. A seven-point scale (1 = symptom absent, 7 = symptom experienced with great intensity) was used to score the presence and intensity of the principal autonomic, neuroglycopenic and non-specific (e.g. malaise) symptoms of hypoglycaemia as described in the Edinburgh Hypoglycaemia Scale [20]. The common autonomic symptoms included in this scale are sweating, pounding heart, shaking and hunger. Symptom scores were completed at baseline and at 10-min intervals until R, at R, and at 15-min intervals thereafter.

Methods

Plasma glucose was estimated using a Yellow Springs Analyser (Yellow Springs, OH, USA), plasma adrenaline and noradrenaline by high-performance liquid chromatography (HPLC) using electrochemical detection [21], cortisol using a heterogeneous competitive magnetic separation assay (Technicon Immuno 1 assay), plasma adrenocorticotrophic hormone (ACTH) by radioimmunoassay [22], growth hormone (GH) by immunoradiometric assay (IRMA) using labelled monoclonal antibody and solid-phase polyclonal antibody detection [23], and plasma pancreatic polypeptide and glucagon by standard radioimmunoassays [24,25]. The intra-assay and inter-assay coefficients of variation of determination were within 15% for catecholamines and glucagon, and within 10% for GH, pancreatic polypeptide and ACTH. Insulin was analysed using a radioimmunoassay (Pharmacia RIA 100, Uppsala, Sweden, validated for insulin aspart).

The cognitive function tests used were the Trail Making Test B and the Digit Symbol Test. The Trail Making Test B is a divided attention task from the Halsted-Reitan Neuropyschological Battery and the Digit Symbol Test is part of the Wechsler Adult Intelligence Scale – Revised [26,27].

Heart rate was monitored continuously using precordial electrodes; sweating was monitored continuously from an area of abdominal skin below the right costal margin by ventilated capsule hygrometry using a Dew Point Sensor (Michell, Cambridge, UK) as described previously [28]; and blood pressure was recorded with a digital automated sphygmomanometer (OMRON/HEM70SCP, Omron Corporation, Japan).

Statistical analysis

Sample size power calculations indicated that 16 participants would yield sufficient power to detect a difference of 0.5 mmol L^{-1} in the blood glucose threshold primary endpoint [28], so 20 participants were recruited to allow for up to four withdrawals.

The primary efficacy endpoint (blood glucose level at the time of autonomic reaction) was logarithmically transformed and subjected to an analysis of variance (ANOVA) for a two-period crossover design. The statistical method included treatment as a fixed effect and subject as a random effect.

For the counterregulatory hormonal responses to hypoglycaemia, the area under the hormone concentration–time curve (AUC), the maximum hormone concentration (C_{max}) and the time to maximum hormone concentration (t_{max}), were derived from the hormone profiles. AUC and C_{max} were logarithmically transformed before being subjected to an ANOVA, and t_{max} was assessed using the Wilcoxon signed rank test. For the autonomic responses (heart rate, blood pressure and sweating), endpoints were defined as the increase from baseline to the maximum value in the interval from 0 to response time R + 60 min. For each endpoint, soluble human insulin and insulin aspart were compared by an ANOVA. For the hypoglycaemia symptom scores, the baseline-adjusted total symptom score at time R for soluble human insulin and insulin aspart were compared using the Wilcoxon signed rank test. For the cognitive function tests, endpoints were the time to complete the Trail Making Test B, and the Digit Symbol Test score at time R. Soluble human insulin and insulin aspart were compared by an ANOVA for each endpoint. For the blood glucose response, the endpoint was the estimated slope of the blood glucose profile from the start of the infusion to time R. The blood glucose responses of soluble human insulin and insulin aspart were compared by an ANOVA. The safety data were analysed for significant changes from pre- to post-trial visits using the Wilcoxon signed rank test.

The analysis population consisted of all patients randomised to treatment. A significance level of 5% was used throughout the analyses. SAS version 6.09 (Statistical Analysis Systems, SAS Institute, Raleigh, NC, USA) on a UNIX platform was used for all statistical programming.

Results

16 out of 17 randomised subjects completed the study. Baseline patient characteristics are summarised in Table 1.

Efficacy

Overall analysis of both primary and secondary efficacy endpoints indicated that there were no significant

Table 1. Clinical characteristics of patients with Type 1 diabetes participating in the study ($n=17$)

	Mean ± SD	Range
Age (years)	28.8 ± 6.6	18.0–44.1
BMI[a] [kg $(m^2)^{-1}$]	23.6 ± 1.8	20.1–26.0
Duration of diabetes (years)	2.4 ± 1.3	1.0–5.0
HbA_{1c}[b] (%)	6.7 ± 0.7	5.5–7.8
Total insulin dose[c] (IU)	39.4 ± 15.1	10–76

[a]BMI, body mass index.
[b]HbA_{1c}, glycated haemoglobin A_{1c}, non-diabetic range: 4.0 – 5.8%.
[c]Dose at visit 1.

differences between insulin aspart and soluble human insulin with respect to hypoglycaemia threshold, generated symptoms and counterregulatory responses.

Mean blood glucose profiles for insulin aspart and soluble human insulin are nearly identical for the two insulin types (Figure 1). The difference in blood glucose concentration at autonomic reaction (R) for insulin aspart and soluble human insulin was not significant (NS): 1.88 ± 0.39 mmol L^{-1} vs 1.89 ± 0.43 mmol L^{-1} (mean ± SD). Time R (median and interquartile range) was similar for both insulin types: 50 (39–60) min vs 50 (45–60) min (NS).

Furthermore, no significant difference between insulin aspart and soluble human insulin was shown for any of the secondary efficacy endpoints. Analysis of AUC, C_{max} and t_{max} for the counterregulatory hormones adrenaline, noradrenaline, glucagon, cortisol, ACTH, GH and pancreatic polypeptide showed no differences between the two insulin types (Table 2). For both insulins, a pronounced adrenaline response occurred during hypoglycaemia, which peaked around R (Figure 2A). Mean C_{max} of adrenaline was 2.84 nmol L^{-1} for insulin aspart and 3.69 nmol L^{-1} for soluble human insulin (NS); t_{max} occurred at 53.8 min for insulin aspart and 53.4 min for soluble human insulin (NS). The magnitude of the noradrenaline response was smaller, but occurred

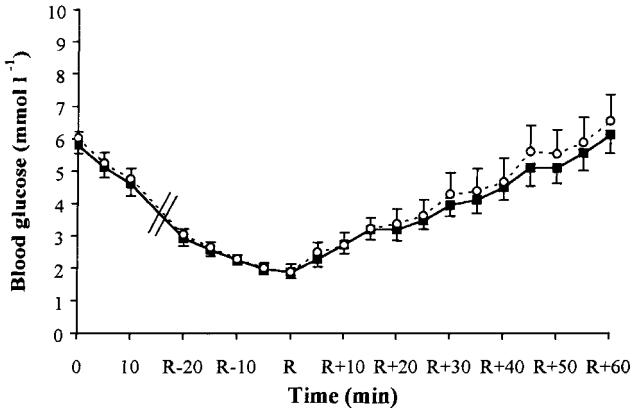

Figure 1. Mean (±2 SEM) blood glucose profiles for insulin aspart (■) and soluble human insulin (○) ($n=16$). R = onset of acute autonomic reaction; // = gap in time on *x*-axis

Table 2. Analysis of the endpoints (AUC, C_{max}, t_{max}) for the counterregulatory hormones ($n=16$)

Parameter	Insulin aspart (mean ± SD)	Human insulin	Estimate of insulin aspart vs human insulin[a]	95% CI
Adrenaline				
AUC[b] (nmol L^{-1})	1.07 ± 0.46	1.20 ± 0.65	0.964	0.668; 1.392
C_{max} (nmol L^{-1})	2.84 ± 1.52	3.69 ± 2.13	0.797	0.514; 1.234
t_{max} (min)	53.8 ± 15.7	53.4 ± 10.9	0.000	–10.000; 8.500
Noradrenaline				
AUC (nmol L^{-1})	1.95 ± 0.56	1.94 ± 0.65	1.015	0.904; 1.141
C_{max} (nmol L^{-1})	2.44 ± 0.63	2.54 ± 0.99	0.990	0.839; 1.168
t_{max} (min)	64.1 ± 20.3	48.8 ± 33.0	15.000	–5.000; 37.000
Glucagon				
AUC (nmol L^{-1})	65.31 ± 32.27	61.98 ± 29.91	1.046	0.981; 1.115
C_{max} (nmol L^{-1})	84.06 ± 41.84	82.81 ± 45.39	1.027	0.937; 1.125
t_{max} (min)	53.9 ± 28.0	55.3 ± 23.2	–2.500	–20.000; 15.000
Cortisol				
AUC (nmol L^{-1})	450.16 ± 122.48	490.96 ± 144.33	0.919	0.840; 1.006
C_{max} (nmol L^{-1})	640.88 ± 177.97	684.31 ± 166.18	0.929	0.825; 1.045
t_{max} (min)	62.6 ± 34.2	71.9 ± 31.0	–9.000	–31.500; 7.500
ACTH				
AUC (nmol L^{-1})	9.43 ± 7.81	9.33 ± 6.90	0.968	0.583; 1.609
C_{max} (nmol L^{-1})	23.94 ± 21.57	22.38 ± 18.94	1.011	0.660; 1.548
t_{max} (min)	47.3 ± 30.5	49.2 ± 25.0	–4.000	–20.000; 16.000
GH				
AUC (nmol L^{-1})	23.60 ± 10.61	27.35 ± 15.93	0.906	0.732; 1.122
C_{max} (nmol L^{-1})	53.52 ± 23.33	59.41 ± 31.83	0.941	0.733; 1.207
t_{max} (min)	71.6 ± 29.8	73.4 ± 30.3	0.000	–17.500; 12.000
Pancreatic polypeptide				
AUC (nmol L^{-1})	309.73 ± 173.36	354.02 ± 338.97	0.937	0.682; 1.289
C_{max} (nmol L^{-1})	715.63 ± 384.51	774.06 ± 789.37	1.020	0.718; 1.449
t_{max} (min)	62.2 ± 20.3	69.4 ± 14.7	–6.500	–19.500; 5.000

$p > 0.05$ for all measurements.
[a]The estimate is a ratio for AUC and C_{max}, and a difference for t_{max}.
[b]AUC = area under the curve.

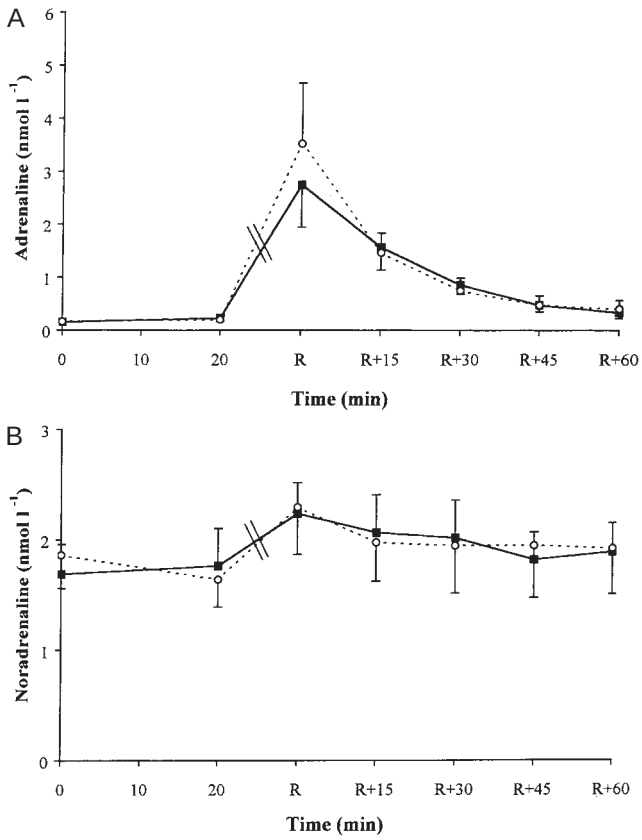

Figure 2. Mean (±2 SEM) adrenaline (A) and noradrenaline (B) profiles for insulin aspart (■) and soluble human insulin (○) ($n=16$). R = onset of acute autonomic reaction; \\ = gap in time on *x*-axis

around the same time (Figure 2B). Mean C_{max} of noradrenaline was 2.44 nmol L^{-1} for insulin aspart and 2.54 nmol L^{-1} for soluble human insulin (NS); t_{max} occurred at 64.1 min for insulin aspart and 48.8 min for soluble human insulin (NS). Other hormonal responses peaked later, although the incremental rise in plasma glucagon was very modest. Thus, there was no evidence of a delay in, or of a diminished counterregulatory response to, insulin aspart compared with soluble human insulin.

Analysis of the endpoints for autonomic responses (changes in diastolic and systolic blood pressure, heart rate and sweating), blood glucose slope, cognitive tests and hypoglycaemia symptom score showed no significant differences between the two insulin types (Table 3).

Safety

No serious adverse events were reported during the study. One patient withdrew due to a syncopal episode which occurred before administration of any study drugs. A total of 16 patients experienced 11 adverse events; five following treatment with insulin aspart and six following treatment with soluble human insulin. All adverse events were considered to be mild and not related to the trial products, and all resolved with little or no intervention.

Discussion

The present study found no differences in the timing or magnitude of physiological, symptomatic or counter-regulatory hormonal responses to acute hypoglycaemia induced by insulin aspart compared with human soluble insulin in patients with Type 1 diabetes of relatively short duration. These conclusions are supported by the similar results observed for the two insulin types with respect to pharmacodynamics, counterregulatory hormonal responses, the amount of insulin administered, hypoglycaemic potency, blood glucose slope, hypoglycaemia symptom scores, autonomic endpoints and results of cognitive function tests. These results suggest that insulin aspart is unlikely to provoke different hypoglycaemic responses than human insulin when used in clinical practice. Similar observations were reported for another rapid-acting insulin analogue, insulin lispro, in a study of similar design, also performed in patients with Type 1 diabetes of similar duration [14], and in a comparative study with a different design by Torlone and coworkers [29].

The patients included in this study had Type 1 diabetes for a period of 1–5 years. Diabetes of this relatively short

Table 3. Analysis of the endpoints for autonomic responses ($n=16$)

Parameter	Insulin aspart (mean ± SD)	Human insulin	Estimate of insulin aspart vs human insulin[a]	95% CI
Autonomic responses				
Change in systolic blood pressure (mmHg)	20.2 ± 16.0	15.2 ± 6.5	5.000	−4.212; 14.212
Change in diastolic blood pressure (mmHg)	12.0 ± 14.0	5.9 ± 5.6	6.063	−1.968; 14.093
Change in heart rate (beats min^{-1})	22.1 ± 13.5	18.7 ± 6.1	3.375	−3.095; 9.845
Change in sweating (H_20 ppmV)	631.3 ± 657.0	981.3 ± 950.2	−350.000	−842.237; 142.237
Blood glucose slope				
Blood glucose slope (mmol L^{-1} min^{-1})	−0.081 ± 0.018	−0.083 ± 0.020	0.002	−0.0079; 0.0111
Cognitive tests and hypoglycaemia symptom score				
Hypoglycaemia symptom score	24.4 ± 20.5	27.2 ± 18.9	−2.000	−10.500; 5.000
Trail Test B	64.4 ± 32.1	69.6 ± 34.7	−5.188	−16.954; 6.579
Digit Symbol Test (s)	45.8 ± 18.3	40.0 ± 17.4	5.813	−1.406; 13.031

$p > 0.05$ for all measurements.
[a]The estimate is an estimated difference between insulin aspart and human insulin (insulin aspart minus human insulin).

duration was chosen to avoid the problem of significant counterregulatory hormonal deficiencies (other than glucagon), which are common with a longer duration of diabetes. Potential differences between soluble human insulin and insulin aspart cannot be excluded in patients with Type 1 diabetes of much longer duration, who may have acquired abnormalities of counterregulatory hormonal deficiencies and impaired awareness of hypoglycaemia.

In the present study, induction of hypoglycaemia by continuous infusion of insulin was chosen because it simulates the development of acute hypoglycaemia that occurs in the everyday life of the person with insulin-treated diabetes, and this method enables identification of the blood glucose threshold for the autonomic response to hypoglycaemia. Furthermore, variations in the responses to the insulins could be assessed accurately after intravenous administration. This would have been problematic with subcutaneous administration, as the absorption profiles of both insulins via the subcutaneous route are substantially different. The physiological changes induced by acute autonomic stimulation generate most of the autonomic symptoms of hypoglycaemia that have been shown previously to commence at the time of the autonomic reaction, coinciding with the onset of awareness of hypoglycaemia [17,28]. Once the glycaemic threshold for autonomic activation is reached, the resulting autonomic response and symptoms quickly attain maximal intensity. This is unlike the effect of progressive neuroglycopenia inducing cognitive impairment and neuroglycopenic symptoms, which become more profound as blood glucose declines further.

Although there is inter-subject variability in the glycaemic threshold for autonomic activation, the study design has the benefit of allowing reproducible autonomic stimulation in all participants. This is in contrast to the hyperinsulinemic glucose clamp technique, which examines hypoglycaemic responses at various blood glucose levels, and does not result in a sudden and identifiable onset of autonomic activation, the magnitude of which is not always reproducible. The disadvantage of the insulin infusion technique is that blood glucose falls too quickly to determine glycaemic thresholds for the counter-regulatory hormonal responses to hypoglycaemia [14]. However, the temporal pattern and magnitude of individual counterregulatory hormones can be observed and compared. No safety concerns about insulin aspart were raised during the study.

In conclusion, the results of the present study indicate that insulin aspart and soluble human insulin elicit the same counterregulatory and symptomatic responses to hypoglycaemia and have similar safety profiles in patients with Type 1 diabetes of relatively short duration.

Acknowledgements

This research study was supported by a research grant from Novo Nordisk A/S. We are grateful for the assistance of Professor K.D. Buchanan for assays of glucagon and pancreatic polypeptide, Dr C. Gray for assays of cortisol, ACTH and GH, and Dr I. Morton for catecholamine assays.

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