ORIGINAL ARTICLE

Does insulin lispro preserve the physiological defences to hypoglycaemia during intensive insulin therapy with a conventional basal bolus regimen?

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Aim: Insulin lispro used in an intensive basal/bolus regimen produces equivalent glycaemic control to human-soluble insulin but reduces rates of hypoglycaemia. We tested the hypothesis that the use of rapid-acting analogues might prevent the development of defective hypoglycaemic counterregulation during intensive insulin therapy.
Methods: Ten patients with type 1 diabetes (four female, mean age 33 ± 3 years, diabetes duration 12 ± 2 years) participated in an open, randomized cross-over study, with 2 months run-in and 4-month treatment periods using either lispro or human-soluble insulin before meals and human NPH insulin (NPH) at night. The total of reported hypoglycaemic episodes (lispro vs. soluble, 123 vs. 128) and HbA_{1c} (6.1 ± 0.2 vs. $6.6 \pm 0.2\%$) were similar during both treatments. At the end of each period, we measured symptomatic, counterregulatory and cognitive responses, and glycaemic thresholds during hypoglycaemia, induced with a hyperinsulinaemic clamp (blood glucose of 5, 4.5, 3.5 and 2.5 mmol/l).
Results: We found similar overall responses of adrenaline, cortisol, growth hormone and total symptom score. Glycaemic thresholds for rises in adrenaline (3.1 ± 0.2 vs. 3.1 ± 0.2 mmol/l, $p = 0.76$), cortisol (2.2 ± 0.1 vs. 2.2 ± 0.1 mmol/l, $p = 0.16$), growth hormone (3.3 ± 0.15 vs. 2.9 ± 0.2 mmol/l, $p = 0.13$), symptoms (3.2 ± 0.2 vs. 3.3 ± 0.1 mmol/l, $p = 0.051$) and impaired cognitive function (3.0 ± 0.2 vs. 3.0 ± 0.2 mmol/l, $p = 0.20$) were also similar.
Conclusion: Four months of intensive treatment, with insulin lispro used pre-prandially and isophane at night, produced relatively preserved but equivalent physiological responses to hypoglycaemia as those on soluble insulin. Longer periods of treatment or alternative regimens may be necessary to demonstrate beneficial effects on hypoglycaemic physiological responses.

Keywords: insulin analogues, type 1 diabetes, hypoglycaemia, intensive insulin therapy, glucose counterregulation
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Introduction

The risk of hypoglycaemia is a major factor preventing many patients with type 1 diabetes from maintaining their blood glucose close to normal. This is largely as a result of the limitation of current conventional insulin therapy which, following subcutaneous injection, produces excessive insulin concentrations, particularly in the post-absorptive period [1]. The increased frequency of severe hypoglycaemic episodes during intensive insulin therapy is partly a result of inappropriate hyperinsulinaemia, but also because periods of hypoglycaemia themselves impair the physiological defence to hypoglycaemia. Repeated hypoglycaemia reduces physiological responses to further episodes by resetting the glucose level at which the autonomic nervous system is activated to a lower concentration [2–4]. The subsequent counterregulatory failure

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and diminished symptomatic responses, in the presence of cerebral dysfunction owing to neuroglycopenia, also contribute to the syndrome of hypoglycaemia unawareness [5].

A number of clinical studies have demonstrated recently that incorporating rapid acting insulin analogues into a basal bolus insulin regimen reduces the frequency of hypoglycaemic episodes, particularly in those undertaking intensive insulin therapy [6]. This reduction is relatively modest in patients with moderately controlled diabetes but the benefit increases progressively in those with tight glucose targets [7,8].

Programmes of hypoglycaemia avoidance can reverse physiological defects in the hypoglycaemic response and restore awareness of hypoglycaemia, at least in part [9–11]. These require patients to avoid most, if not all, hypoglycaemia for a period of weeks. Therefore, we reasoned that the use of rapid-acting analogues might prevent the development of defective hypoglycaemic counterregulation during intensive insulin therapy. We tested this hypothesis in a study involving 10 patients with type 1 diabetes who were participating in a clinical trial of the rapid-acting insulin analogue, insulin lispro.

Patients and Methods

Originally, we approached all 130 subjects participating in a multicentre clinical trial to join the present study of which 13 agreed but only 10 completed both arms.

The main trial was measuring the effect of quick-acting insulin analogues as part of a basal bolus regimen during intensified insulin therapy in patients with type 1 diabetes. The full details of the design have been described previously [8]. Briefly, the study utilized an open, randomized cross-over design. Subjects initially entered a 2-month run-in during which their glucose control was optimized with multiple injections of human soluble insulin (Humulin S, Eli Lilly, Indianapolis, IN, USA) before the three main meals and human NPH insulin (Humulin I) at bedtime. Patients were in regular contact with the diabetes nurses who recommended changes in insulin dose based on home blood-glucose profiles, with the intention of achieving DCCT (Diabetes Control and Complications Trial) glycaemic targets. Details of hypoglycaemic episodes were recorded in patient diaries.

Those who achieved tight glucose control (HbA1c $< 8\%$ at the end of the run-in period) were then randomized to receive either insulin lispro (Humalog) for 4 months followed by soluble insulin for an additional 4 months, or soluble insulin therapy for 4 months followed by insulin lispro for 4 months.

The principle end-points of the main trial were the number of hypoglycaemic episodes (symptoms or signs associated with hypoglycaemia experienced by the patient, or observed by another person, or alternatively a home blood-glucose measurement of less than 3 mmol/l), and secondary end-points included HbA_{1c} (measured by HPLC ion exchange chromatography, non-diabetic range, 3.8–5.5%).

The 10 subjects gave written consent to participate in the study, which was approved by the Research Ethics Committees of our three institutions. The subjects (five female) had the clinical features of type 1 diabetes and had no serious microvascular complications. They had a mean age of 33 ± 3 years, a mean duration of diabetes of 12 ± 2 years and a body mass index (b.m.i.) of 25.6 ± 0.8. None had a history of hypoglycaemia unawareness or recurrent severe hypoglycaemia.

The Measurement of Physiological Responses to Experimental Hypoglycaemia

Each of the 10 patients underwent a 'slow fall' glucose clamp [10] on three occasions: at the end of the run-in period, and during the final week of the periods taking insulin lispro or soluble insulin. Patients were admitted the evening before study and fasted from midnight. A cannula was sited in an antecubital vein of the non-dominant arm and insulin infused overnight to maintain blood glucose between 5 and 10 mmol/l.

At 0700 hours, a retrograde cannula was inserted into a dorsal hand vein of the non-dominant arm, the hand placed in a hand box heated to 50 °C and a hyperinsulinaemic clamp started [human soluble insulin (Humulin S)], infused at a fixed rate of 60 mU/M^2/min). A 20% glucose infusion was adjusted every 5 min according to blood glucose measurements obtained from a dorsal hand vein. Blood glucose was measured by a glucose oxidase method (Yellow Springs Instrument Co, Yellow Springs, OH, USA).

Blood glucose was held at 5 mmol/l for 40 min then lowered in 40-min steps to 4.0, 3.5, 3.0 and 2.5 mmol/l. At each step, glucose fell for the first 20 min and was then clamped at target for 20 min.

The following measurements were made at 10 min intervals.

Symptom Scores

Symptom scores were obtained by asking patients to rate a series of individual symptoms from 1 (not present) to 7 (very severe) [12]. Symptoms used were: sweating, tremor, pounding heart, hunger (autonomic), difficulty

Table 1 Mean glucose values (± s.e.m.) in mmol/l at each glucose plateau for the three glucose clamps. There were no significant differences at any plateau

Target glucose	5.0	4.0	3.5	3.0	2.5
run-in	6.3 ± 0.6	4.2 ± 0.2	3.5 ± 0.02	3.0 ± 0.04	2.5 ± 0.02
lispro	5.7 ± 0.3	4.0 ± 0.03	3.5 ± 0.1	3.0 ± 0.03	2.5 ± 0.04
human-soluble	6.7 ± 0.7	4.3 ± 0.2	3.5 ± 0.04	3.1 ± 0.02	2.5 ± 0.03
two-way anova	F = 0.91	F = 2.23	F = 0.01	F = 0.64	F = 0.98
	p = 0.41	p = 0.13	p = 0.99	p = 0.54	p = 0.39

speaking, confusion, drowsiness, clumsiness, odd behaviour (neuroglycopenic), headache, nausea (malaise); and itching (dummy).

Four-Choice Reaction-time Test

Cognitive function was measured using a serial 4-choice reaction-time test, measuring 500 responses for each test [12]. The mean time for correct responses was recorded for each test. Patients were trained on at least four occasions through the evening before each stepped clamp and again, immediately before the clamp, to ensure stable performance before inducing hypoglycaemia and eliminate any effect of improving performance as a result of practice during the study.

Measurements of Counterregulatory Hormones (at 20 min intervals)

First, 5 ml of blood was added to lithium heparin tubes containing 0.1 ml of EGTA glutathione and, after separation, the plasma was stored at −80° C. Plasma adrenaline and noradrenaline were analysed by high-performance liquid chromatography (HPLC) with electrochemical detection [13]. Cortisol, growth hormone and free plasma insulin (measured hourly) were measured using a double-antibody radioimmunoassay (RIA) [14]. All the assays were performed in a single laboratory.

Statistical Analyses

Continuous data, expressed as mean ± s.e.m. were analysed using ANOVA methods, with responses of counterregulation calculated and compared using the summary measure area under the curve (AUC). We also calculated the glycaemic thresholds of activation of each physiological response, defined as the prevailing blood glucose when the response exceeded two standard deviations of five basal euglycaemic measurements for two consecutive time-points. If the response did not exceed this level, then, for statistical purposes, the threshold was defined as 2.0 mmol/l. A significant increase in symptom score was defined as an increase of four for total symptom score at two consecutive time-points. A significant deterioration in cognitive function was defined as an increase in reaction time of 5% [10]. A total of 10 patients in a cross-over design gave us 80% power to identify a difference in the glycaemic threshold in adrenaline of 0.5 mmol/l, assuming a standard deviation of 0.2. A two-sided analysis was carried out with significance set at 0.05.

Results

Glycaemic Control

HbA_{1c} was 6.1 ± 0.3% after 2 months of the run-in period, not significantly different (f = 2.96, p = 0.077) from

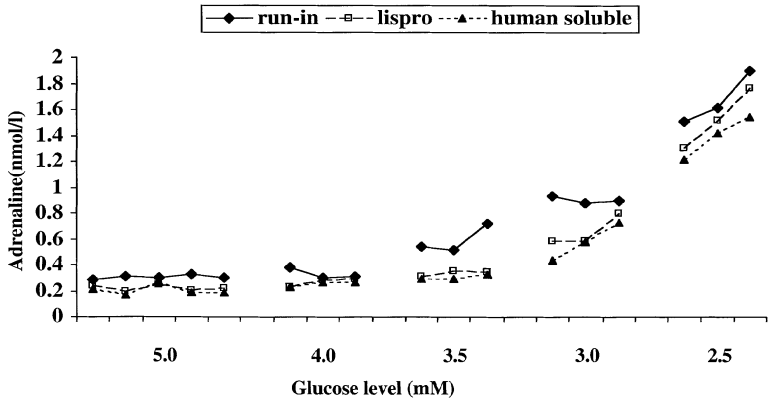

Fig. 1 Changes in adrenaline concentration during the glucose clamps. There were no significant differences between the curves at any time-point.

Table 2 Areas under the curves and glycaemic thresholds for physiological responses measured during the three glucose clamps

Physiological response (units AUC)	Area under the curve				Glycaemic threshold (mmol/l)			
	Run-in	Lispro	Human-soluble	Result of anova	Run-in	Lispro	Human-soluble	Result of ANOVA
Adrenaline (nmol/min/l)	138 ± 31	105 ± 19	96 ± 29	F = 1.1 p = 0.34	3.3 ± 0.2	3.1 ± 0.2	3.0 ± 0.2	F = 0.3 p = 0.76
Noradrenaline (nmol/min/l)	267 ± 38	246 ± 21	259 ± 28	F = 0.38 p = 0.69	2.8 ± 0.2	2.6 ± 0.2	2.7 ± 0.2	F = 0.28 p = 0.76
Growth hormone (iu/min/l)	5940 ± 1760	4050 ± 940	4430 ± 1570	F = 1.16 p = 0.33	3.0 ± 0.1	3.3 ± 0.2	2.9 ± 0.2	F = 2.25 p = 0.13
Cortisol (mmol/min/l)	75 ± 9	59±5	70 ± 12	F = 1.23 p = 0.31	2.5 ± 0.2	2.2±0.1	2.2 ± 0.1	F = 2.01 p = 0.16
Total symptom score (score/min)	3979 ± 290	4170 ± 320	4006 ± 270	F = 1.00 p = 0.39	2.8 ± 0.1	3.2 ± 0.1	3.3 ± 0.1	F = 3.51 p = 0.051
Reaction time (ms/min)	93 000 ± 9500	86 000 ± 6000	87 000 ± 8000	F = 0.44 p = 0.65	2.7 ± 0.1	3.0 ± 0.2	3.0 ± 0.2	F = 1.82 p = 0.20

HbA_{1c} levels after 4 months of treatment with insulin lispro (6.1 ± 0.2%) or soluble insulin (6.6 ± 0.3%).

Hypoglycaemia

Symptomatic hypoglycaemia was no different during treatment with insulin lispro compared with soluble insulin. The 10 subjects experienced a total of 123 episodes during treatment with insulin lispro, and 128 when taking soluble insulin. None of the subjects experienced a severe hypoglycaemic episode during the study.

Blood Glucose

Mean glucose values at each plateau were similar during the three clamps and are shown in table 1.

Insulin Levels

There were no significant differences between mean insulin concentrations during the clamp at the end of the run-in (234 ± 30 pmol/l), after 4 months of insulin lispro (240 ± 30 pmol/l) or after treatment with soluble insulin (204 ± 18 pmol/l, F = 0.81, p = 0.46).

Counterregulatory Hormones

There was no significant difference for any of the measured counterregulatory hormones: adrenaline (figure 1), noradrenaline, cortisol or growth hormone in terms of AUC or glycaemic threshold at the end of the run-in, after 4 months of insulin lispro or after treatment with soluble insulin (table 2). The mean differences and 95% confidence intervals between responses (AUC) and glycaemic thresholds, at the end of the two treatment periods, were: adrenaline (9.6 nmol/min/l, −27.4 to 46.5, p = 0.57, 0.1, −0.4 to 0.6, p = 0.66), noradrenaline (−12.8 nmol/min/l, −64.6 to 39.0, p = 0.59, −0.1, −0.6–0.4, p = 0.66), cortisol (−10 980 mmol/min/l, 12 160 to −38 500, p = 0.39, 0.05, 0.09 to −0.15, p = 0.59), growth hormone (−383 iu/min/l, −3800 to 3040, p = 0.80, 0.4, −0.006 to 0.81, p = 0.053).

Symptom Scores

There were no significant differences in total symptom scores during hypoglycaemia at during any of the three clamps in terms of AUC or glycaemic threshold at the end of the run-in, after 4 months of insulin lispro or after treatment with soluble insulin (table 2). The mean differences and 95% confidence intervals between responses (AUC) and glycaemic thresholds, at the end of the two treatment periods, were 164 score/min, −429 to 757, p = 0.55, −0.05, −0.48 to 0.38, p = 0.80.

Cognitive Function

There were no significant differences in the increase in four-choice reaction time during hypoglycaemia during any of the three clamps in terms of intervals between responses (AUC) or glycaemic threshold at the end of the run-in, after 4 months of insulin lispro or after treatment with soluble insulin (table 2). The mean differences and 95% confidence AUC and glycaemic thresholds, at the end of the two treatment periods, were −624 ms/min, −27 800 to 26 500, p = 0.96, 0, −0.64 to 0.64, p = 1.

Discussion

We believe this is the first study that has measured physiological responses to hypoglycaemia following a period of treatment using a rapid-acting insulin analogue in a commonly used intensive regimen (a pre-meal rapid-acting preparation and isophane insulin as basal replacement before bed). This regimen has been shown to reduce hypoglycaemia [7], particularly at night [8]. We found no differences in the glycaemic threshold, or magnitude of counterregulatory responses, symptom scores or cognitive impairment during experimental hypoglycaemia, after 4 months of intensive insulin therapy, using either insulin lispro or soluble insulin. Therefore, the data indicate that insulin lispro, when used as the pre-meal insulin during intensive insulin therapy, does not affect physiological defences to hypoglycaemia.

The limitations of the study design may have contributed to these negative findings. One possibility is that the study might have been insufficiently powered to detect a difference in hypoglycaemic response. It was not practical to perform hyperinsulinaemic clamps in all 132 patients who participated in the main study. We calculated that studying 10 patients on three occasions using a cross-over design would give us sufficient power to detect a difference in the glycaemic threshold in adrenaline of 0.5 mmol/l, a clinically relevant difference. The differences in glycaemic thresholds with either soluble or lispro insulin, and other physiological responses, were generally small. Furthermore, the AUC of the counterregulatory hormones and the summary measure we used to assess the magnitude of response were similar after treatment on either insulin. These data did not indicate a clinically important trend or suggest that with increased numbers we would have found significant differences.

A more important limitation of our design was that we could not guarantee that those patients we studied would suffer less hypoglycaemia. Furthermore, the technology available prevented us from measuring the incidence of hypoglycaemia with any accuracy. We selected patients prospectively and, clearly, it was not possible to predict who would suffer fewer episodes. This subgroup of patients reported similar numbers of mild, hypoglycaemic episodes on either insulin. As antecedent hypoglycaemia contributes to impaired counterregulation during intensive insulin therapy [2,15], this might explain why counterregulatory responses were no different. There was a trend towards a lower HbA_{1c} at the end of the period on lispro, although this did not reach statistical significance in this small group. Nevertheless, it is interesting that these particular patients tended to improve their glycaemic control rather than exhibit a fall in episodes of hypoglycaemia. Clinical trials of quick-acting insulin analogues have generally demonstrated unchanged levels of HbA_{1c} with lower rates of hypoglycaemia, although a few have reported the opposite. This may reflect the use of different protocols for insulin adjustment during the conduct of the trials, or the way in which individual patients use the new preparations. If patients were able to use insulin analogues to improve glycaemic control and maintain hypoglycaemic counterregulation, then this would be an important clinical benefit, as improving metabolic control can impair these responses. This needs to be established in a study powered to detect improvements in glycaemic control. However, the generally modest improvements in glycaemic control seen in previous studies suggest that an experimental study along these lines would require large and probably impractical numbers.

However, reported rates of hypoglycaemia do not reflect the 'hypoglycaemic burden' as episodes of hypoglycaemia are often unrecognized [16], particularly at night [17]. Furthermore, the reported incidence of hypoglycaemia does not necessarily predict the physiological response to a hypoglycaemic challenge. Tsui *et al.* compared the counterregulatory response in 10 patients with type 1 diabetes after 3 months of CSII (continuous subcutaneous insulin infusion) with either soluble or lispro insulin [18]. They reported a fall of 0.5% in HbA_{1c} and a 35% reduction in the incidence of hypoglycaemia with insulin lispro, yet found no difference in peak counterregulatory responses. Thus, although these limitations may explain why we failed to show a difference, they do not negate our conclusion that including a quick-acting insulin analogue in a typical basal bolus regimen does not affect physiological responses to hypoglycaemia.

A notable feature of the present study was that patients had near normal HbA_{1c} values yet, in either treatment arm, did not demonstrate major impairments in hypoglycaemic physiological responses. There was no substantial lowering of glucose levels associated with the onset of endocrine or symptomatic responses characteristic of counterregulatory failure. There is a strong association between intensive insulin therapy, low HbA_{1c} levels and the risks of severe hypoglycaemia and defective counterregulation, although the link is not straightforward [15,19,20]. The primary aim of the research nurses in this study was to keep glucose at DCCT targets but they also worked with patients to limit the frequency of hypoglycaemia. This might have prevented the development of impaired counterregulation. Alternatively, the selection of patients without a history of severe hypoglycaemia may have excluded those in whom intensive therapy would most probably provoke defective counterregulation.

The potential benefit of the rapid-acting insulin analogues might best be examined in those with known defects in physiological defences to hypoglycaemia.

Replacing pre-prandial soluble insulin with lispro, without addressing the limitations of current basal insulins, may be insufficient, on its own, to maintain or improve glucose counterregulation. Lalli *et al.* reported reduced hypoglycaemia over 1 year of intensive insulin therapy in 28 patients with type 1 diabetes who used insulin lispro, compared with 28 who remained on soluble insulin [21]. They tested physiological responses to hypoglycaemia in a subset of 14 (whose rates of hypoglycaemia were not reported) and demonstrated that glucose thresholds for physiological responses were set at a higher concentration in those randomized to lispro. The regimen consisted of a mixture of pre-meal lispro and isophane insulin, adjusted to avoid hypoglycaemia and maintain tight control. Perhaps additional NPH with insulin lispro pre-prandially, smoothed the insulin profile, reduced hypoglycaemia and preserved physiological responses. Alternatively, a longer treatment period may be necessary to demonstrate significant differences.

We need to explore which regimens and insulin combinations can best exploit the pharmacokinetic benefits of rapid-acting analogues. Furthermore, the introduction of new long-acting insulin analogues that provide a flatter insulin profile and stable basal supply when compared with current medium-acting insulins [22], may produce more physiological insulin replacement, less hypoglycaemia and preserved hypoglycaemic defences.

We conclude that a rapid-acting insulin analogue does not preserve or improve physiological responses to hypoglycaemia when used in a traditional basal bolus regimen over 4 months. Whether a longer period of treatment or different combinations of rapid-acting analogues and medium acting insulin would demonstrate benefit remains to be determined by further work.

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