The effect of the optimal use of rapid-acting insulin analogues on insulin secretion in Type 2 diabetes

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Abstract

The abnormal glucose tolerance of Type 2 diabetes is characterized by post-prandial hyperglycaemia. We aimed to examine whether the restoration of a more physiological insulin profile using rapid-acting insulin analogues might, through effects on glucose toxicity, improve endogenous insulin secretion rate (ISR) and secondly improve markers of vascular risk. Eighteen people with insulin-treated Type 2 diabetes were recruited into a single centre, cross-over, open-labeled study. The order of pre-meal unmodified human insulin or insulin aspart was randomized: treatment periods lasted at least 8-12 weeks after which ISR was assessed by stepped low-dose glucose infusion and fasting markers of vascular risk measured. Glucose control was good (HbA1c 6.94 ± 0.12 (±SE) vs 7.07 ± 0.13 %, NS) with insulin aspart and human insulin. Mean post-prandial self-monitored blood glucose concentration was also good particularly with insulin aspart (7.5 ± 0.41 vs 8.19 ± 0.34 mmol/l) but the difference did not reach statistical significance. Over 160 min ISR did not differ between insulin aspart and human insulin and there was also no change in various markers of vascular risk. In conclusion a meal-time+basal insulin regimen gave close to normal post-prandial blood glucose control with both the insulin aspart and human insulin regimens, such that no difference in ISR or markers of vascular risk could be demonstrated.
1. Introduction

The earliest abnormality of glucose tolerance in the development of Type 2 diabetes is post-prandial hyperglycaemia. After an initial compensatory increase in pancreatic insulin secretion the developing β-cell failure results in impaired glucose tolerance characterized by post-prandial hyperglycaemia. With progressive β-cell failure, insulin secretion continues to diminish and overt diabetes develops [1]. The cause of this failure of the pancreas to produce insulin is not fully understood but a direct effect of glucose toxicity on the ability of the pancreatic β-cells to secrete insulin is thought to exacerbate the problem as glucose levels rise [2-4]. Restoration of a more physiological insulin profile resulting in a reduction in post-prandial hyperglycaemia could, theoretically, reduce or reverse the toxic effect of raised blood glucose levels on the pancreatic β-cell. It is hypothesized that a reduction in post-prandial hyperglycaemia could, through effects on glucose toxicity, result in an improvement in endogenous insulin secretion in Type 2 diabetes. Rapid-acting insulin analogues have been consistently shown to reduce post-prandial blood glucose levels in both Type 1 [5-9] and Type 2 diabetes [10-13]. Therefore we aimed to examine whether the restoration of a more physiological insulin profile using insulin aspart could result in an improvement in endogenous insulin secretion when compared to unmodified human insulin in people with insulin-treated Type 2 diabetes.
In this study endogenous insulin secretion was assessed using a low-dose stepped glucose infusion. The low-dose stepped glucose infusion has been shown to be a sensitive method of detecting small changes in insulin secretion [14,15]. This allowed comparison of endogenous insulin secretion rate in people with Type 2 diabetes using rapid-acting insulin analogue and human insulin treatment.

A further aim of this study was to assess the effect of a reduction in levels of post-prandial hyperglycaemia on a wide variety of metabolic and haemostatic parameters. We have previously found that a small reduction of 0.8 mmol/l in post-prandial blood glucose levels, which was achieved when insulin aspart was compared with human insulin as part of a double blind crossover study, does not have a significant effect on the levels of various metabolic parameters [16]. An open-label design was used since it was hoped that it might make target post-prandial blood glucose levels in the insulin analogue group easier to attain in order to achieve a larger difference in post-prandial blood glucose levels between the two groups. In addition the length of each treatment period was increased from 6 weeks in our previous study to at least 8-12 weeks.
2. Participants and methods

2.1. Participants

Eighteen insulin-treated people with Type 2 diabetes were recruited. Their clinical characteristics are given in Table 1. All patients gave written informed consent to participate in the study, which was carried out according to the principles of the Declaration of Helsinki and was approved by the local Ethics Committee.

2.2 Methods

A single centre, randomized, open-label, crossover design was used. Following recruitment participants were converted from their usual twice-daily insulin regimen to a pre-meal plus basal regimen using unmodified human insulin (Human Actrapid, Novo Nordisk, Bagsvaerd, Denmark) before each main meal and human NPH insulin (Insulatard, Novo Nordisk) before bed. All patients remained on this regimen for the run-in period lasting for a minimum of 1 week and a maximum time period of 4 weeks. A third party not involved in conducting the study randomized patients to either human soluble insulin or insulin aspart (NovoRapid, Novo Nordisk) before main meals for each treatment period. Patients remained on each pre-meal insulin for treatment periods lasting at least 8-12 weeks and at the end of each treatment period an in-patient study morning took place in order to assess insulin secretion and a fasting blood sample was taken for biochemical markers. Patients were then crossed-over to the second treatment arm, with the alternative pre-meal insulin and an identical study day took place at the end of the second treatment period.
Participants maintained a minimum of weekly contact with the investigator, when insulin doses were adjusted and were asked to carry out one seven-point glucose profile per week. Target blood glucose levels were set as 4.0-6.0 mmol/l pre-prandially and 5.0-7.5 mmol/l post-prandially in the absence of hypoglycaemia.

Participants were instructed to inject pre-meal insulin subcutaneously 5 min before breakfast, lunch and dinner when injecting insulin aspart, and 30 min before meals when taking human soluble insulin. All participants remained on bed-time NPH and concomitant medication was continued throughout the study.

At randomization and 1-2 weeks before each study morning pre-breakfast serum or plasma levels of the following metabolic and biochemical variables were measured: total cholesterol, HDL cholesterol, triglycerides, fibrinogen, plasminogen activator inhibitor-1 (PAI-1), and E-selectin. Albumin excretion rate was estimated using the results of three timed overnight urine collections. HbA1c was also measured at baseline and at the end of each treatment period.

2.3 Study morning protocol

Participants arrived at the Investigation Unit at 0800 h following a 12-h fast and having omitted their NPH insulin the previous evening. A sampling cannula was inserted into one forearm or hand vein and this forearm was maintained in a hand warming box for 15 min before any samples were taken and for the duration of the procedure in order to ensure arterialization of the venous sample.
At 0845 h (-15 min) a fasting blood sample was taken for plasma glucose estimation. Only patients with plasma glucose concentrations between 5.0 and 9.0 mmol/l at 0845 h continued the study morning. In addition, on the second study morning plasma glucose level at 0845 h was no greater than 2.5 mmol/l different from the first study morning. Participants whose plasma glucose levels did not meet these criteria were asked to return another day.

At 0900 h a baseline blood sample was taken for plasma glucose and plasma C-peptide levels. A stepped low-dose glucose infusion (20% dextrose) was commenced at 0900 h. The stepped low-dose glucose infusion is based on a previously described method [14,15]. Glucose was infused at 2.0 mg/kg/min and maintained for 40 min, increasing to 4.0, 6.0 and 8.0 mg/kg/min, each step for 40 min. After commencing the glucose infusion blood samples were taken for plasma glucose and C-peptide at 10 min intervals for the first 20 min of each 40-min period and thereafter at 5 min intervals.

2.4 Biochemical analysis

Plasma glucose was measured using a glucose oxidase method (Yellow Springs glucose analyser, Yellow Springs Instrument Company, Yellow Springs, OH, USA). Plasma C-peptide concentrations were measured by an enzyme-linked immunosorbent assay using a commercial kit (Dako Diagnostics, Ely, UK). Standard laboratory methods were used to measure serum total cholesterol, HDL cholesterol, and triglyceride concentrations. Fibrinogen concentration was determined using an automated analyser by standard techniques (ACL 3000 Coagulometer, Instrumentation Laboratory,
Lexington, MA, USA). PAI-1 and E-selectin levels were measured by enzyme-linked immunoassay kits (Innogenetics, Ghent, Belgium and R & D Systems, Minneapolis, MN, USA). Urinary albumin concentration was measured by radioimmunoassay [18] and the median AER used.

DCCT-aligned HbA$_{1c}$ (non-diabetic < 6.1%) was determined by HPLC (Eurogenetics, Hampton, UK).

2.5 Calculation of insulin secretion rates

Estimation of insulin secretion rate (ISR) was carried out using a previously described purpose written programme [15] based on the two-compartment model of C-peptide kinetics developed by Polonsky and colleagues [14,17]. The two compartment model for C-peptide distribution is shown in Fig. 1 and is an indirect method of calculating ISR based on peripheral C-peptide concentrations. This is possible since C-peptide does not undergo first pass metabolism in the liver, is secreted in equimolar quantities to insulin and has a constant renal clearance. The model assumes that once secreted C-peptide distributes into a central intravascular compartment (C$_t$) consisting of the plasma space and tissues in rapid equilibration with plasma from which sampling occurs at time t, and a peripheral extravascular compartment at time t (Y$_t$). Rate constants $k_1$ and $k_2$ describe the rate at which C-peptide passes from the plasma to the extravascular compartment ($k_1$) and back again to the plasma compartment ($k_2$). C-peptide is also irreversibly metabolized from the central compartment and excreted by the kidneys by another rate constant, $k_3$. C-peptide kinetic parameters ($k_1$, $k_2$, $k_3$ and the volume of distribution, $V_D$) were estimated based on previously published work [15,19]. It is assumed that plasma C-peptide decay follows a pattern that can be described by the
sum of two exponential decay curves. The half-lives for each known as the long half-life and the short half-life can be estimated from age, sex, BMI and diabetic status. The volume of distribution of C-peptide is also necessary to model C-peptide decay and calculate the C-peptide kinetic parameters and is estimated from body surface area [20] and sex. C-peptide kinetic parameters were obtained for this population of study patients.

Endogenous ISR at time $t$ ($S_t$) can be calculated by solving differential equations for $S_t$ (15). As part of that process it was necessary to model the concentration of C-peptide in the central compartment on a curve to allow manipulation of the function $C_t$.

ISR estimation and C-peptide modelling was performed with a purpose written programs using Microsoft Excel (Seattle, WA, USA).

2.6 Statistical analysis

The amount of insulin secreted over each 40-min time period at each glucose infusion rate was determined for unmodified human insulin and insulin aspart by calculating the area under the ISR curve using the trapezoid rule. Student’s $t$-test (paired) was used to test for differences in ISR between the two insulins using the Statistical Package for Social Science (SPSS, Chicago, IL, USA).

For the various metabolic markers of vascular risk skewed data were normalized by logarithmic transformation and statistical comparison was by paired Student’s $t$-test using Graph Pad Instat (San Diego, CA, USA). Results
are expressed as mean ± SE for normally distributed data and median (range) for AER. Statistical significance was judged against a \( p \) value of 0.05.

3. Results

3.1 Patient disposition

Twenty four people were originally recruited to this study. Two patients were withdrawn by the investigator prior to randomization because of concern about compliance. Of the 22 patients randomized a further patient was withdrawn by the investigator because of compliance issues and two patients withdrew due to changes in their personal circumstances. One patient had poor venous access and intravenous cannulation was not possible on one of the study days necessitating withdrawal. Eighteen patients therefore completed the study.

3.2 Treatment periods

Participants remained on insulin aspart for an average of 111 ± 31 days and on human insulin for an average of 107 ± 34 days (mean ± SD). The length of the treatment period between the two groups did not differ.

3.3 Insulin doses

The total dose of pre-meal insulin taken per day did not differ with insulin aspart compared to human soluble insulin (mean ± SD: 68.0 ± 8.2 vs 68.6 ± 9.2 U/day). Likewise, the dose of bedtime NPH insulin given did not change.
between the two periods (43.7 ± 5.0 vs 40.2 ± 3.9 U/day). No patient required a second dose of NPH insulin.

3.4 Blood glucose control

Glycated haemoglobin fell during the study (randomization vs insulin aspart vs human insulin (7.71 ± 0.13 vs 6.94 ± 0.12 vs 7.07 ± 0.13 %, both p<0.01). There was not a significant difference in HbA₁c between insulin aspart and human insulin at the end of each treatment period. HbA₁c improved from 7.71 % at randomization to 6.89 % at the end of the first treatment period but had risen slightly to 7.12 % by the end of the second treatment period. There was thus an order effect between the first and second treatment periods.

Self-monitored pre-breakfast blood glucose concentrations were comparable with insulin aspart and human insulin (7.0 ± 0.4 vs 6.5 ± 0.2 mmol/l, NS) (Fig.2). Post-prandial blood glucose concentrations 2 hours after breakfast tended to be lower with insulin aspart but this did not reach statistical significance (8.6 ± 0.7 vs 9.6 ± 0.5 mmol/l, p=0.074). After lunch and dinner post-prandial blood glucose concentrations were within the target range of 5.0-7.5 mmol/l in both groups with no significant difference between the two pre-meal insulins. Although mean post-prandial blood glucose concentration after the three main meals of the day was at the upper end of the target range for insulin aspart and above target for human insulin this was not a statistically significant difference (7.5 ± 0.4 vs 8.2 ± 0.3 mmol/l, p=0.142). Before dinner blood glucose concentrations were lower with human insulin (6.6 ± 0.6 vs 5.4 ± 0.4 mmol/l, p=0.044) but before bed blood glucose values tended to be lower with insulin aspart (6.3 ± 0.4 vs 7.3 ± 0.3 mmol/l, p=0.058).
3.5 Biochemical outcome measures

All measurements of biochemical outcome are shown in Table 2.

There were no significant differences in pre-breakfast serum lipids, fibrinogen, E-selectin and AER following treatment with insulin aspart or human insulin. Concentrations of lipids, fibrinogen, E-selectin and AER did not change significantly between randomization and at the end of each treatment period on either insulin, in spite of the significant improvement in HbA₁c.

Levels of PAI-1 fell between randomization and following treatment with insulin aspart (97.7 ± 4.9 vs 86.8 ± 5.7 µg/l, p=0.044). There was no change in PAI-1 levels compared to baseline following treatment with human insulin (97.9 ± 4.9 vs 95.6 ± 5.3 µg/l).

3.6 Insulin secretion rate

The parameters \( V_D \), \( k_1 \), \( k_2 \), and \( k_3 \) were estimated as (mean ± SD) 4.65 ± 0.44 l, 0.061 ± 0.004, 0.048 ± 0.001, 0.059 ± 0.002 respectively for the insulin aspart group and 4.64 ± 0.44 l, 0.061 ± 0.003, 0.048 ± 0.0001, 0.058 ± 0.002 for the unmodified human insulin group. Analysis of C-peptide parameters showed no significant differences between the two groups.

Plasma glucose and plasma C-peptide concentrations during the stepped glucose infusion are shown in Fig. 3.

Plasma glucose concentrations progressively rose and formed a plateau at each stage of the stepped glucose infusion. There was no difference in plasma glucose concentrations between the two groups at each step of the glucose infusion.
Plasma C-peptide concentrations followed a similar pattern to plasma glucose concentrations tending to rise and plateau as glucose infusion rates progressively increased. Again plasma C-peptide concentrations did not vary between insulin aspart and human insulin at each stage of the stepped glucose infusion.

The area under the curve (AUC) of the insulin secretion curve for each insulin is shown in Fig. 4. The AUC is equivalent to the amount of insulin secreted over each time period. There was no difference in total insulin secretion or insulin secretion at each stage of the stepped low-dose glucose infusion. Over the 160 min time period insulin secretion in those patients taking insulin aspart was $63.1 \pm 9.0$ nmol and $66.6 \pm 9.1$ nmol with human insulin (NS).

4. Discussion

The present study was of open-label design in contrast to our previous study [16]. The different pharmacokinetics and pharmacodynamics of insulin aspart and human insulin are well documented [21,22]. It was postulated that an open-label, as compared to a double-blind study, would allow target post-prandial blood glucose concentrations to be achieved more easily in the insulin aspart group compared to the human insulin group since pre-meal insulin analogue doses could be increased to reduce post-prandial hyperglycaemia without the risk of between of late interprandial hypoglycaemia associated with human insulin.

Target post-prandial blood glucose control was achieved in the insulin aspart group with a mean post-prandial blood glucose concentration of 7.5 mmol/l across the three main meals of the day. However, this was only 0.7 mmol/l
less than the mean post-prandial blood glucose concentration achieved with human insulin and was not a statistically significant difference. In fact, for two of the three meals of the day mean post-prandial blood glucose concentrations were in the target range with both insulin aspart and human insulin. Our studies have highlighted the difficulty in achieving a large difference in post-prandial blood glucose levels between the two groups when human soluble insulin is compared to a rapid-acting insulin analogue in people with good glucose control overall. In fact, our studies have somewhat surprisingly demonstrated that very good overall and post-prandial glycaemic control can be obtained in patients with insulin-treated Type 2 diabetes who are intensively managed using a pre-meal plus basal insulin regimen with frequent blood glucose monitoring and regular contact with a health care professional, regardless of whether human soluble insulin or a rapid-acting insulin analogue is used as pre-meal insulin therapy. This is pertinent since it is recognised that glycaemic variability, in addition to but independent of HbA$_{1c}$, is a significant cause of oxidative stress in the development of diabetic complications ( ). Other studies have achieved greater differences in post-prandial blood glucose control between human soluble insulin and rapid-acting insulin analogues in Type 2 diabetes [10,11] but at notably worse levels of overall glycaemic control than was achieved in our studies. A comparison between NPH insulin alone and a rapid-acting insulin analogue in patients with insulin-treated Type 2 diabetes should cause a more marked difference in post-prandial blood glucose concentrations than could be achieved with the use of unmodified human insulin.
A greater difference in post-prandial blood glucose levels between the two arms of our studies would have more strongly tested the hypotheses of the studies. However, the use of surrogate markers do not always provide unequivocal answers to clinical questions and the evidence so far from our two studies and a further study in which lipid profile was examined [11] have yet to show any changes in markers of vascular risk associated with improved post-prandial glycaemic control.

It is well recognised that an acute increase in blood glucose levels results in endothelial dysfunction in both normal and diabetic subjects (). In the present study E-selectin was measured as a marker of endothelial dysfunction and improved post-prandial glycaemic control was not associated with any change in E-selectin levels. In contrast Ceriello et al examined the effect of improved post-prandial blood glucose control on endothelial dysfunction in subjects with Type 2 diabetes by measuring flow-mediated dilation of the brachial artery (). In this study, there was greater preservation of flow-mediated dilation in the patients with Type 2 diabetes who had been given pre-prandial insulin aspart than those given unmodified human insulin. This study suggests that post-prandial hyperglycaemia is accompanied endothelial dysfunction which can be modified by insulin aspart use in contrast to the present study.

A large, prospective, long-term, randomized controlled trial looking at clinically useful patient orientated endpoints such as myocardial infarction, stroke or death is perhaps the only definitive way of establishing whether reducing post-prandial blood glucose concentrations with rapid-acting insulin analogue use could be of real clinical benefit to patients.
At all stages of the low-dose stepped glucose infusion plasma glucose and C-peptide levels were very similar in both groups and there was no difference in ISR. There are several possible reasons for this. Without achieving significantly improved post-prandial glycaemic control in the insulin aspart group, in comparison to human insulin, we were unable to fully test our original hypothesis.

A second possibility is that participants could not increase their endogenous ISR enough for differences between the two different insulin treatments to be detectable. The UKPDS clearly showed that β-cell failure is progressive with 50% of patients requiring insulin therapy 6 years after diagnosis increasing to 80% after 9 years [23,24]. The mean duration of diabetes in our cohort of patients was over 13 years and all were insulin-treated implying that all had significant pancreatic failure with low levels of islet β-cell function and resultant low endogenous insulin secretion. It is possible that in our group of patients with longstanding Type 2 diabetes endogenous insulin secretion was too poor to be significantly augmented by subtle therapeutic changes. It would be interesting to repeat the present study using patients with Type 2 diabetes who have recently commenced insulin therapy since these patients have greater endogenous insulin reserve.

Whilst no change in endogenous ISR was detected with insulin aspart therapy it may be that the improved blood glucose profile seen with rapid-acting insulin analogue use resulted in a reduction in exogenous insulin requirements. However, since HbA1c significantly improved during the study with an associated increase in exogenous insulin doses, it is not possible to test this hypothesis.
A possible criticism of this study is that participants were not supervised the night before insulin secretion measurement took place. Patients were asked to omit their night time dose of NPH and to fast for 12 h prior to attending the Investigation Unit at 0800 h the following morning. Preferably patients would have been admitted to the Investigation Unit the evening before each study day. Ideally an intravenous insulin infusion would have been used to lower fasting plasma glucose to similar levels in both groups and eliminate potentially confounding effects of differences in the basal glucose concentration prior to commencing the graded glucose infusion. However a previous study showed consistent measurement of ISR when basal plasma glucose levels were between 5-9 mmol/l regardless of whether insulin was given before the graded glucose infusion or not [14].

In summary, we were able to obtain good glycaemic control using an intensified pre-meal plus basal regimen with either insulin aspart or unmodified human insulin as pre-meal insulin. Insulin aspart has not been shown to have a consistent advantage in reducing markers of vascular risk or improving endogenous ISR compared to unmodified human insulin in our studies.
References


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