In both fasting normal and diabetic subjects, nasally administered insulin achieves significant falls in plasma glucose concentrations. Repeated administration before and during a meal has been necessary to lower postprandial glycaemic excursion in subjects with NIDDM. We have studied the use of Novolin® Nasal which employs a non-irritant, lecithin-based enhancer as a vehicle for human insulin, on postprandial glucose profiles in NIDDM subjects to determine efficacy, optimal dose frequency, and tolerability. Seventeen NIDDM subjects (15 men, 2 women) participated in a randomized, partially blinded, placebo-controlled, crossover trial of three active treatment regimens (nasal insulin, 120 U at 0 min, 60 U at 0 and +20 min or 120 U at +20 min) in relation to a standardized mixed meal given at 0 min. All active treatments significantly reduced postprandial glucose concentrations compared to placebo. Intranasal insulin given at 0 min at a dose of 60 U or 120 U resulted in a 50% reduction in postprandial incremental glucose compared to placebo over the first 2 h, whereas treatment with 60 U both at 0 and 20 min lead to a 70% reduction over the 240 min postprandial period. Post-prandial intravenous insulin was the least effective. There were no episodes of symptomatic hypoglycaemia. Local tolerability was excellent with only four reports of transient nasal irritation out of a total of 68 doses. The delivery device was accurate with intra-device CV of delivered dose of 4.8%. We conclude that nasal insulin is effective in reducing postprandial glycaemia in subjects with NIDDM and is well tolerated. Repeated dosing achieved the greatest reduction in postprandial glycaemic responses to a mixed meal.

**KEY WORDS** Nasal insulin Mixed meal test NIDDM

**Introduction**

Early prandial insulin availability is necessary to maintain normal blood glucose responses to a carbohydrate challenge. This is universally deficient in subjects with non-insulin dependent (Type 2) diabetes mellitus (NIDDM) and impaired glucose tolerance (IGT), resulting in exaggerated postprandial blood glucose excursions. Subcutaneous soluble insulin fails to mimic early prandial plasma insulin concentrations because of its relatively slow rate of absorption and consequent delayed onset of action leading to inadequate control of postprandial hyperglycaemia with the added risk of delayed hypoglycaemia. Restoration of early prandial insulin concentrations in NIDDM subjects by intravenous insulin infusion has been shown to improve subsequent blood glucose excursion but does not offer a therapeutic alternative for clinical use. The usefulness of insulin delivered via the nasal mucosa has been investigated for a number of years. Absorption is more rapid than that of subcutaneous insulin but despite the use of enhancers its bioavailability remains much lower than insulin delivered by either the subcutaneous or intravenous route.

The nasal administration of insulin in the fasting state to both normal and diabetic subjects results in consistent reductions in blood glucose concentrations. However, the reported effects on postprandial glycaemia and long-term blood glucose control in diabetic subjects have been inconsistent and inadequate. In subjects with insulin-dependent (Type 1) diabetes mellitus (IDDM), administration of nasal insulin prior to meals has been shown to reduce postprandial glycaemic excursion in a dose-dependent manner. A similar reduction in postprandial blood glucose excursion is observed over the first 90 min when compared with subcutaneous insulin, but with a reduced incidence of subsequent postprandial hypoglycaemia. The long-term (3-4
months) replacement of preprandial subcutaneous soluble insulin with nasal insulin in subjects with insulin-dependent diabetes mellitus (IDDM) has been reported to result in either stable$^{15}$ or worsening of glycaemic control.$^{17}$ In subjects with NIDDM, however, despite the achievement of ‘physiological’ postprandial insulin concentration, postprandial glycaemic excursion has been shown to be unaltered by a single dose of insulin (15 U) given intranasally at the commencement of a test meal.$^{18}$ Significant reductions in postprandial glycaemia have, however, been demonstrated if the dose of insulin (30 U) given intranasally is repeated two or three times during the course of the meal.$^{12}$ These observations emphasize that the acute reproduction of postprandial insulin concentrations similar to those found in normal subjects is unlikely to overcome the marked insulin resistance present in the majority of subjects with NIDDM.

The main limitation to the use of nasally administered insulin is its low bioavailability, typically in the order of 8–15 % of the administered dose.$^{16–12}$ The need to use specific enhancers (for example, bile salt and polyethylene ether derivatives) to facilitate insulin absorption$^{10,14,15}$ has resulted in significant nasal irritation,$^9$ thereby limiting acceptability by the user.

We have investigated the effects of Novolin® Nasal (Novo Nordisk AS, Bagsvaerd, Denmark) which employs a non-irritant, lecithin-based enhancer (with di-decanoyl-alpha-phosphatidylcholine, DDPC) on postprandial plasma glucose profiles in subjects with NIDDM. The primary aim of the investigation was to establish the efficacy of this formulation of nasal insulin in reducing postprandial blood glucose excursion in relation to a test meal. Additional aims were to investigate the effects of timing and frequency of dosing on postprandial glycaemic excursion and to assess the nasal tolerance to the preparation.

**Patients and Methods**

The trial was a placebo-controlled, randomized, part blind, crossover, single centre study in NIDDM patients approved by the local ethical committee. Seventeen islet cell antibody negative NIDDM subjects (15 men and 2 women) were recruited and gave written informed consent to take part. Subjects were of mean ±SD age 57.7 ± 6.8 years, with body mass index 27.7 ± 3.8 kg m$^{-2}$; glycosylated haemoglobin 8.6 ± 1.3 % and time since diagnosis 7.3 ± 4.23 years. All had fasting C-peptide concentrations greater than 0.17 nmol l$^{-1}$. Of the 17 subjects, 1 (male) was treated by diet alone while the remainder (15 men and 1 woman) were treated by diet and a sulphonylurea preparation.

Prior to study, and within 1 week of its conclusion, all subjects underwent a full medical, biochemical, and haematological screen. In addition, each subject was examined by a consultant otorhinolaryngologist to exclude any clinically significant nasal pathology. All subjects attended our investigation unit prior to the first study day for training in the use of the pen delivery device for nasal administration of the test preparations.

The structure of each study day was identical, with the NIDDM subjects being admitted to the investigation unit after a 10 h overnight fast. An intravenous cannula was inserted into an antecubital vein for sampling purposes and baseline blood samples for plasma glucose (PG) and immunoreactive insulin (IRI) were taken at times designated −30, −10, and 0 min. Blood was also taken for glycosylated haemoglobin (HbA$\textsubscript{1c}$) measurement at time −30 min and sulphonylurea medication (where appropriate) was administered at this time. At 0 min a standardized mixed meal was given to each subject to be consumed over 10 min, consisting of breakfast cereal (15 g), milk (200 ml), fruit juice (250 ml), bread (60 g), butter (9 g), and meat (50 g): total energy content 500 kcal, 60 % (76.4 g) carbohydrate, 20 % (11.6 g) fat and 20 % (25.5 g) protein.

Each subject underwent three different treatment regimens with nasal insulin and one treatment with placebo. These treatments were self-administered, given in random order, and using a new device on each of the four study days which were each separated by one week. Subjects continued with their normal isocaloric diets and sulphonylurea preparations (where applicable) between study days. The treatments consisted of nasal insulin 120 U given at 0 min or at + 20 min, a split dose of 60 U at 0 and + 20 min with placebo given only at 0 min. The trial was blinded for the subject and investigator except for the day on which the split dose of nasal insulin was administered. Prior to subject dosing, pen devices were weighed before and after three trial dose administrations to allow assessment of the accuracy of the delivery system. Pen devices with a coefficient of variation (CV) of greater than 10 % in the administered doses were discarded prior to the study period. Blood samples for PG and IRI were taken at 0 min, every 5 min from + 10 min until + 75 min, thereafter every 15 min until + 120 min and then every 30 min until + 240 min. Samples were separated rapidly in a refrigerated centrifuge and the plasma stored at −20 °C until assay.

**Novolin® Nasal**

This was supplied courtesy of Novo Nordisk AS, Bagsvaerd, Denmark, in a disposable pen-shaped device containing 3 ml of Human Insulin solution (50 000 U l$^{-1}$). Each device was able to deliver 20–70 U in increments of 10 U as an aerosolized spray into each nostril. Placebo was supplied on identical devices without the addition of human insulin.

**Analytical Methods**

Plasma glucose was assayed on an autoanalyser (Chemlab Instruments Ltd, Hornchurch, Essex, UK) using an enzymatic colorimetric method with intra-assay and inter-assay coefficients of variation (CV) of less than 2 %.
Plasma immunoreactive insulin was measured by a modification of the technique of Heding, using a second antibody to separate free and antibody bound insulin. The intra-assay and inter-assay CVs were 4.6% and 7.3%, respectively. HbA1c (normal range 5.5–7.8%) was measured by column chromatography (Test-Combination HbA1c, BCL Ltd, Lewes, Sussex, UK).

Statistical Methods

The plasma glucose and immunoreactive insulin profiles are summarized as median absolute and incremental areas under curves (AUC). Incremental values (Δ) were calculated by subtracting the median basal value from all subsequent values.

Results

Metabolic Parameters

Figure 1 shows the absolute median glucose and insulin profiles for the three treatment regimens and the placebo study day. The variation in median fasting plasma glucose concentration between treatment regimes was not statistically significant (p = 0.91). The insulin profiles demonstrate that peak IRI concentrations brought about by the split treatment regimen (60 U at 0 and + 20 min) were almost double those of the other regimens despite the same net dose of insulin. Table 1 gives the absolute median AUC glucose concentrations for placebo and treatment days. Treatment at 0 min, irrespective of dose, resulted in lower median AUC (0–60 min) glucose concentrations, although this failed to reach significance for the split dose regimen. All active treatments reduced median AUC (0–120 and 0–240 min) glucose concentrations compared to placebo, although the split dose regimen resulted in the greatest falls in AUC glucose over both 120 and 240 min. Figure 2 shows the incremental glucose excursion for the active treatment regimens and the placebo study day. The three treatment regimens significantly reduced incremental glucose excursion following the mixed meal compared to the placebo. Treatment commencing at time 0, irrespective of dose, lead to reductions of 40–50% of Δ AUC plasma glucose at 60 min and 50–60% of Δ AUC plasma glucose at 120 min compared to placebo (p < 0.001 in each case). The administration of the split dose of nasal insulin at 0 and + 20 min, however, resulted in the greatest reduction (≈ 70%, p < 0.005 compared to placebo) in Δ AUC plasma glucose over 240 min with median plasma glucose values reduced below basal concentrations at this point although no subjects developed symptomatic hypoglycaemia (see Figure 2).

Nasal Irritation

Local tolerability of this preparation of nasal insulin was excellent with only four reports of mild transient nasal irritation out of a total of 68 dosing episodes. Irritation began 20 min after dosing and resolved without any intervention within the following 20-min period. No subject left the trial because of persisting or recurrent nasal symptoms and no clinically significant mucosal

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0–60 min</th>
<th>0–120 min</th>
<th>0–240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>659 (256)</td>
<td>1388 (589)</td>
<td>2519 (1307)</td>
</tr>
<tr>
<td>0 min</td>
<td>649 (310)</td>
<td>1336 (763)</td>
<td>2176 (1383)</td>
</tr>
<tr>
<td>20 min</td>
<td>601 (223)</td>
<td>1358 (332)</td>
<td>2227 (926)</td>
</tr>
<tr>
<td>0 + 20 min</td>
<td>652 (211)</td>
<td>1216 (450)</td>
<td>2011 (964)</td>
</tr>
</tbody>
</table>

Absolute AUC plasma glucose values (mmol L⁻¹) presented as median and (semiquartile range). Comparisons to placebo by the Wilcoxon signed rank test (p values are two sided). * p = 0.03, b p = 0.02, † p = 0.01, d p = 0.007, ‡ p = 0.005.

![Figure 1. Absolute plasma glucose and insulin profiles; placebo treatment.](image1)

![Figure 2. Incremental plasma glucose profiles; placebo treatment.](image2)
changes were noted on the follow-up rhinological examination in any subject.

Accuracy of Delivery System

The pen devices used in the study delivered the nasal insulin solution with an intra-device CV of delivered volume of 4.8% (95% CI 4.3–5.4%). Three devices only were discarded as the CV of delivered volume was greater than 10%.

Discussion

Attempts to minimize postprandial glycaemic excursion in subjects with NIDDM using subcutaneous soluble insulin are limited by the inappropriate absorption characteristics of the available preparations. Intravenous insulin, used to mimic early prandial insulin concentrations, although capable of producing a beneficial effect on postprandial glucose levels, is impractical. Previous studies with nasal administration of insulin demonstrated that bioavailability needed to be improved by the inclusion of a variety of enhancers, many of which were associated with significant nasal irritation.

In addition, the influence of the nasally administered insulin on postprandial glycaemia in NIDDM subjects has been shown to be dependent on the dose, timing, and frequency of administration. The presence of insulin resistance in NIDDM also necessitates the production of supraphysiological insulin concentrations in acute intervention studies in order to achieve reductions in postprandial glycaemic excursion.

The current study examined the delivery of nasal insulin via Novolin® Nasal which employs a non-irritant lecithin-based enhancer and a pen-based delivery system. All active treatment regimes resulted in significant reductions in absolute AUC glucose concentrations over 120 and 240 min after the meal; however, dosing at the onset of the meal (either 60 U or 120 U) resulted in the greatest reduction in AUC plasma glucose concentrations over the initial 60 and 120 min postprandial period. Administration of the nasal insulin (120 U) at + 20 min only, although capable of reducing incremental AUC glucose concentration compared to placebo, had the weakest effect over the 120 and 240 min periods, with reductions in the order of 20–30% only. Repetition of the reduced dose (60 U) of nasal insulin at + 20 min had the greatest effect on both absolute and incremental AUC glucose concentrations over the 4 h postprandial period, associated with the most rapid return to basal plasma glucose concentrations although no subject experienced symptomatic hypoglycaemia. Interestingly we observed an almost doubling of peak insulin (IRI) concentrations during the split dose nasal insulin regimen compared to the single dose regimens. As the total dose delivered was identical in all active treatments, this effect may be a result of the enhancer itself priming the nasal mucosa to a further dose of insulin.

Nasal irritation occurred after approximately 6% of the doses of nasal insulin which compares favourably to rates in an earlier report using the same preparation in normal healthy subjects. Studies using bile salts as enhancers have reported nasal irritation in up to 100% of subjects studied where the use of lauroyl-9 (a polyethylene ether derivative) has been associated with nasal irritation in 25–50% of subjects depending on concentration. In addition, the accuracy of the delivery devices appeared to be good with an intra-device coefficient of variation of 4.8% although no other published study has commented on the accuracy of the delivery devices.

This study has shown that this preparation of nasal insulin is well tolerated by NIDDM subjects in the short term and that all dose regimens investigated had significant effects in reducing postprandial regimens compared to placebo. If the United Kingdom Prospective Diabetes Study reports a reduction in diabetic complications as a result of improved blood glucose control then nasal insulin may continue to be developed in view of its potential to replace the deficient early phase insulin release in subjects with NIDDM. The long-term acceptability to the patient of using the preparation twice with each meal and the potential cost involved in providing nasal insulin formulations with low bioavailability will also need careful consideration.

References


