Absorption and Metabolic Effect of Inhaled Insulin

Intrapatient variability after inhalation via the Aerodose Insulin Inhaler in patients with type 2 diabetes

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OBJECTIVE — To compare the intrapatient variability of the pharmacokinetic and pharmacodynamic responses to inhaled regular insulin (INH) delivered via the Aerodose Insulin Inhaler with that of subcutaneously injected regular insulin (SC) in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — A total of 15 patients with type 2 diabetes (nonsmokers, 10 men, aged 47–77 years) received two 240-unit doses of INH, delivered via a clinical Aerodose Insulin Inhaler and two 24-unit doses of SC under euglycemic clamp conditions on four separate study days. Glucose infusion rates (GIRs) and serum insulin concentrations were monitored over the following 8 h. Comparisons of intrapatient coefficients of variation (CV) were used to assess the reproducibility of INH versus SC.

RESULTS — INH showed a bioavailability (0–8 h postdosing) of 16% and biopotency of 13% relative to SC. Comparison of the CVs (%) for area under the curve for serum insulin and GIR between INH and SC showed no significant differences between the treatments during 0–3 h (19% for INH versus 23% for SC) or 0–8 h (22% for INH versus 16% for SC). INH exhibited a shorter time to peak insulin concentration (T_{max} [mean ± SD] 76 ± 51 vs. 193 ± 66 min) and shorter time to peak metabolic effect (T_{GIRmax} 170 ± 53 vs. 244 ± 75 min) compared with SC ($P < 0.001$). No adverse events were observed.

CONCLUSIONS — Comparable dosing reproducibility and shorter time to peak action of INH compared with SC suggest that INH delivered via the Aerodose Insulin Inhaler can provide reliable preprandial dosing of insulin in patients with type 2 diabetes.

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Delivery of inhaled regular insulin (INH) as a practical alternative to subcutaneously injected regular insulin (SC) has awaited development of specialized and portable aerosol delivery devices (1–3). In this regard, the Aerodose Insulin Inhaler (Aerogen, Mountain View, CA) is being developed to deliver insulin to patients with diabetes. Previously, we demonstrated that compared with SC, the Aerodose Insulin Inhaler delivers biologically active insulin to the lung with an earlier time to maximal serum insulin concentration and maximal metabolic effect, a relative bioavailability and biopotency in the range reported by others for INH, and with no acute effects on lung function (4,5).

A key prerequisite for demonstrating the clinical viability of INH is to show that repeated administration of the same INH dose results in pharmacokinetic (PK) and pharmacodynamic (PD) responses with a treatment-to-treatment variance similar to that observed for SC. The aim of our study was, therefore, to compare the intrapatient variability of duplicate treatments of INH delivered via the clinical Aerodose Insulin Inhaler with that of duplicate treatments of SC in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study design

A single-center, open-label, randomized, active-controlled, two-treatment, two-sequence cross-over study was conducted to compare two same-dose INH treatments and two same-dose SC treatments. A total of 25 patients with type 2 diabetes were screened for the study; 16 patients were enrolled and 15 patients completed the study. One patient withdrew his consent for personal reasons after a single INH treatment and a single SC treatment. The age of the 10 men and 5 women who completed the study was 47–77 years, BMI was 29.6 ± 2.8 kg/m^2 (mean ± SD); all patients were nonsmokers and had normal lung function (forced expiratory volume in 1 s [FEV_1] at screening was 3.5 ± 0.9 l, 113 ± 18% of predicted value). At screening, all study candidates were tested for their ability to perform a 5-s slow deep breath followed by a 5-s breath-hold breathing maneuver, which is required for dosing via the Aerodose Insulin Inhaler. Recordings of flow rate versus time, obtained from inhalations through a model Aerodose Insulin Inhaler

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Abbreviations: AUC, area under the curve; CV, coefficient of variation; FEV_1, forced expiratory volume in 1 s; GIR, glucose infusion rate; GIR-AUC, AUC for glucose infusion rate; INH, inhaled regular insulin; Ins-AUC, AUC for insulin; PD, pharmacodynamic; PK, pharmacokinetic; RIA, radioimmunoassay; SC, subcutaneously injected regular insulin.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

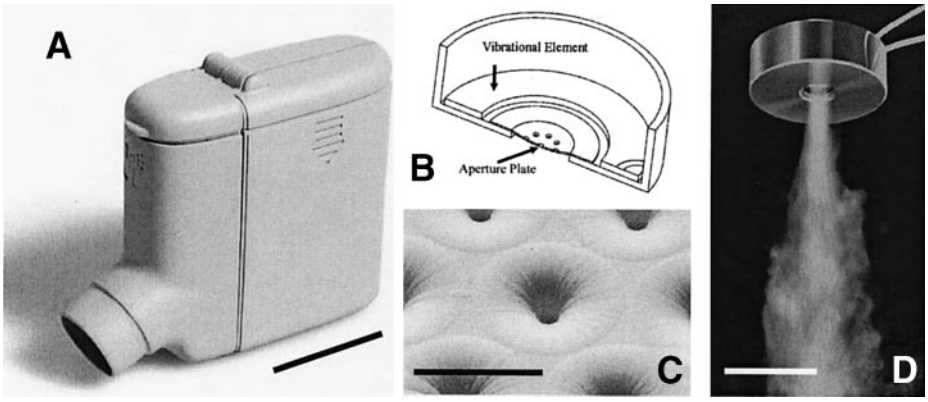

Figure 1—A: *The Aerodose Insulin Inhaler (bar = 3 cm).* B: *Schematic of a cross-section through the aerosol generator showing the aperture plate.* C: *Magnified view of the aperture plate (bar = 100 μm).* D: *When liquid insulin is placed on the concave surface of the vibrating aperture plate, an aerosol is generated (bar = 1 cm).*

attached to a spirometer and software program (2120 Spirometer and Spirotach IV software; Vitalograph, Hamburg, Germany), showed that all subjects were able to perform this breathing maneuver.

Patients were admitted into the research unit the evening before the day of treatment. After overnight fasting, an antecubital venous catheter was inserted into the right arm of each patient for infusion of glucose and insulin. A catheter was also inserted retrogradely into a vein in the left hand for collection of blood for continuous blood glucose monitoring using the Biostator (Medizintechnik, Ulm, Germany). The hand was placed in a warming device throughout the experiments. Another catheter was inserted in an antecubital vein of the left arm to collect blood samples for measurement of blood glucose, serum insulin, and plasma C-peptide levels.

After catheterization, a euglycemic clamp was established at a target blood glucose level of 6.1 mmol/l by intravenous glucose infusion. This baseline glucose level was achieved for at least 2 h before dosing with INH or SC. A baseline intravenous insulin infusion (0.3 mU · kg^{-1} · min^{-1}) was administered for the duration of the clamp to suppress endogenous insulin secretion. If the initial blood glucose value at baseline was >8.3 mmol/l, a small intravenous insulin bolus was administered to accelerate the normalization of blood glucose to the target value. In this case, the baseline period was extended to 4 h. At the end of the baseline period, either INH or SC was administered. On two of the four study days, patients inhaled 240 units of insulin (Humulin R, U-500; Eli Lilly, Indianapolis, IN) delivered via the Aerodose Insulin Inhaler. The INH dose was scaled up 10-fold compared with the SC dose to deliver an intended dose of 24 units SC equivalent based on an approximate 10% relative bioavailability obtained in previous studies with the clinical Aerodose Insulin Inhaler in normal subjects (4,5). On the other two study days, patients received 24 units SC (Humulin R, U-100) into the anterior abdominal wall by means of a syringe (Microfine IV; Becton-Dickinson, Heidelberg, Germany). The glucose infusion rate (GIR) and blood glucose, serum insulin, and plasma C-peptide levels were measured for 8 h from time 0 ($t = 0$ min), which was defined as the end of INH or SC dosing. At −3 h before drug administration, vital signs (systolic and diastolic blood pressure, respiration rate, heart rate, and body temperature) and spirometry (FEV_1 and forced vital capacity) measurements were recorded. These measurements were repeated at −0.5 h before and at 4 and 8 h after administration of insulin. All adverse events that were reported (between study days) and observed (during study days) were recorded. Blood samples for serum insulin measurements were collected via an indwelling catheter at −2, −1.5, −1, and −0.5 h as well as at 0, 5, 10, 20, 30, 40, 50, and 60 min and then every 30 min to 8 h postdosing. Samples for plasma C-peptide measurements were taken at −45, −15, 0, 10, 20, 40, and 60 min and then every 60 min to 8 h postdosing. Serum insulin concentrations were measured using a commercially available radioimmunoassay (RIA) kit (DPC-Biermann, Bad Nauheim, Germany); the mean intra-assay and interassay coefficient of variation [CV] was 6.0 and 3.5%, respectively. Plasma C-peptide levels were also measured using a commercially available human C-peptide RIA kit (Linco Research, St. Charles, MO); the mean intra-assay and interassay CV was 4.8 and 12.3%, respectively. RIAs were performed at the IKFE (Mainz, Germany).

At the end of the clamp, each patient was given a meal and released from the research unit after being determined clinically stable. Patients returned to the research unit for subsequent treatments at intervals ranging from 3 to 20 days.

Aerodose Insulin Inhaler

The clinical version of the Aerodose Insulin Inhaler used in this study is a small, hand-held, breath-actuated inhaler that contains Aerogen's proprietary aerosol generator (Fig. 1*A* and *B*). The aerosol generator consists of a domed aperture plate containing numerous precision-formed holes (Fig. 1*B* and *C*) surrounded by a vibrational element (Fig. 1*B*). When liquid insulin is placed on the concave surface of the aperture plate, vibration of the plate results in a micropumping action that creates a fine-droplet, low-velocity aerosol that is suited for deep lung delivery (Fig. 1*D*) (6). Breath activation of the aerosol generator is triggered by an inspiratory flow rate >15 l/min. The Aerodose Insulin Inhalers used in this study emitted aerosol with a volume median diameter droplet size of 3.8 ± 0.1 μm and were configured to deliver aerosol during the first 4 s of each 5-s inhalation. On average, 16 slow, deep 5-s

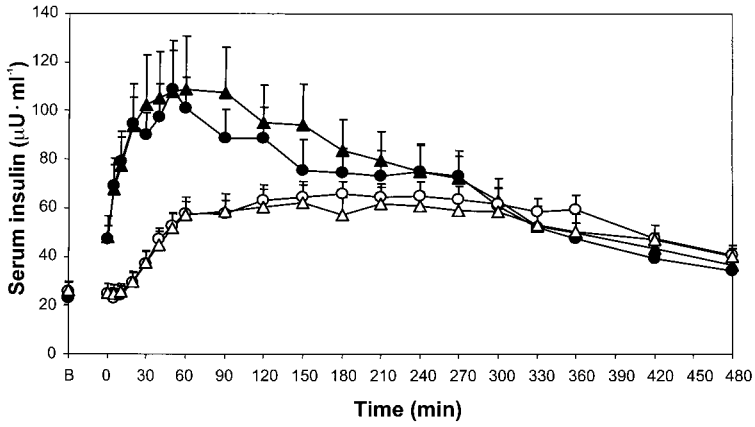

Figure 2—*Serum insulin levels in 15 patients with type 2 diabetes after two administrations of INH (240 units; solid symbols) on two study days and two administrations of SC (24 units; open symbols) on two other study days. The mean of baseline insulin values is also shown (time point B). Solid circles represent the first dose of INH; solid triangles represent the second dose of INH; open circles represent the first dose of SC; open triangles represent the second dose of SC. Data are means ± SE.*

inhalations, each inhalation followed by a 5-s breath-hold, were used to deliver the INH dose (mean dosing time for INH was 5.9 ± 1.7 min).

Statistical methods

After correction for baseline, a polynomial function (sixth order) was fitted to each individual GIR profile obtained for estimation of PD summary measures. Baseline GIR was calculated as the mean of all GIRs recorded for 2 h preceding the insulin dosing. Serum insulin level was also corrected for baseline, but a form-fitting function was not necessary. Baseline insulin was calculated as the mean of values taken at −1.5, −1.0, and −0.5 h before dosing. The area under the curve (AUC) for insulin (Ins-AUC) and GIR (GIR-AUC) was calculated using the trapezoidal rule. PK and PD summary measures were analyzed using ANOVA, with period, treatment, sequence, and patient nested within sequence as factors. For each subject, the relative bioavailability and biopotency of INH was calculated from the ratio of the AUC values (Ins-AUC and GIR-AUC, respectively) of INH relative to the average of the two SC treatments, normalized to the dose [$(AUC_{INH}/AUC_{SC}) \times (Dose_{SC}/Dose_{INH}) \times 100$]. The average of the two INH administrations provided the relative bioavailability and biopotency for that subject. CVs (CV = SD/mean × 100) were used to describe intrapatient variability for SC and INH treatments across PK and PD summary measures. CVs were calculated based on the results obtained for the two INH treatments and two SC treatments. The treatment differences in CVs among the 15 patients were compared using Wilcoxon's signed-rank test.

RESULTS

Pharmacokinetics

Serum insulin levels after each of two administrations of INH and two administrations of SC are shown in Fig. 2. Baseline serum insulin levels before exogenous insulin administration were similar between the INH and SC treatment groups (t = baseline [B], Fig. 2, $P = 0.115$). Upon completion of dosing (designated $t = 0$ min), serum insulin levels were significantly higher for INH than for SC (Fig. 2, $P < 0.001$), indicating rapid insulin absorption after inhalation. A significantly earlier time to early half-maximal serum insulin levels (early $T_{50\%}$) and time to maximal insulin level (T_{max}) were also observed for INH compared with SC (Table 1, $P < 0.001$). Maximal serum insulin levels (C_{max}) were also higher for INH than for SC and were accompanied by greater AUCs for INH compared with SC for the first 3 h (Ins-AUC_{0-3}) and for the total clamp duration (Ins-AUC_{0-8}) (Table 1). The intrapatient CVs of the PK summary measures with INH and SC are shown in Table 1 (in parentheses). No significant differences in the CVs were observed between INH and SC treatments for the PK summary measures. A mean relative bioavailability for INH, compared with SC, of 16% was observed over the 8-h postdose clamp period. When $t = 0$ was defined as the start of dosing, T_{max}, early $T_{50\%}$, T_{GIRmax}, and early $T_{GIR50\%}$ increased by ~6 min. However, AUC and comparison of CV between treatments were not significantly affected (data not shown). Decreases in C-peptide levels (treatment averages) of 11 and 7% were observed for INH and SC, respectively, by the end of the clamp (t = 480 min), as compared with baseline. Within treatments, these changes from baseline were not statistically significant (data not shown).

Pharmacodynamics

The GIRs after INH and SC treatments are shown in Fig. 3 (GIR was averaged every 15 min). Maximal GIR values (GIR_{max}) were significantly greater for INH than for SC ($P < 0.05$, Table 1). A greater GIR_{max}

Table 1—*PK and PD summary measures after inhalation of INH and SC*

Summary measures	INH	SC
PK		
Ins-AUC_{0-3} (mU · ml^{-1} · min^{-1})	12 ± 8 (19 ± 10)	4.9 ± 2.1* (23 ± 15)
Ins-AUC_{0-8} (mU · ml^{-1} · min^{-1})	22 ± 14 (22 ± 15)	14 ± 4* (16 ± 6)
Early $T_{50\%}$ (min)	10 ± 8 (40 ± 33)	54 ± 27* (20 ± 27)
T_{max} (min)	76 ± 51 (40 ± 24)	193 ± 66* (31 ± 31)
C_{max} (μU/ml)	96 ± 58 (12 ± 8)	47 ± 14* (17 ± 13)
PD		
GIR-AUC_{0-3} (mg/kg)	687 ± 263 (19 ± 16)	421 ± 198* (22 ± 17)
GIR-AUC_{0-8} (mg/kg)	1,684 ± 511 (21 ± 18)	1,333 ± 434* (19 ± 13)
Early $T_{GIR50\%}$ (min)	44 ± 32 (32 ± 22)	78 ± 28* (24 ± 24)
T_{GIRmax} (min)	170 ± 53 (46 ± 24)	244 ± 75* (24 ± 24*)
GIR_{max} (mg · kg^{-1} · min^{-1})	5.5 ± 1.6 (21 ± 15)	4.3 ± 1.4 (23 ± 11)

Data are means ± SD (intrapatient variability of summary measures [CV]). *$P < 0.05$ for INH vs. SC.

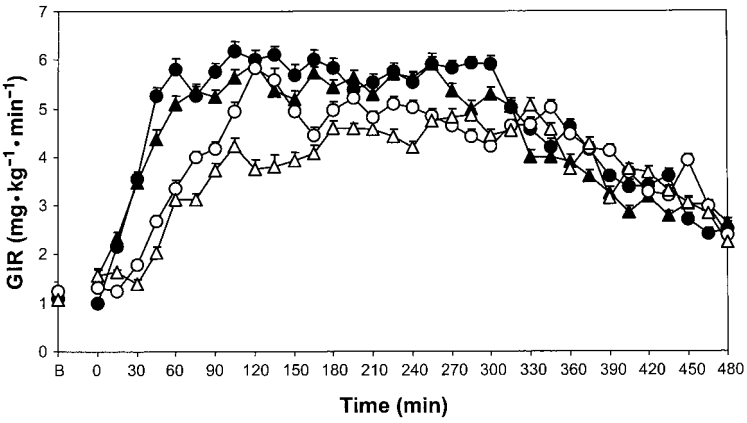

Figure 3—*GIRs registered in 15 patients with type 2 diabetes after two administrations of INH (240 units; solid symbols) on two study days and two administrations of SC (24 units; open symbols) on two other study days. GIRs have been averaged over 15-min periods. The mean of baseline GIR values is also shown (time point B). Solid circles represent the first dose of INH; solid triangles represent the second dose of INH; open circles represent the first dose of SC; open triangles represent the second dose of SC. Data are means ± SE.*

was accompanied by a significantly greater overall metabolic effect for INH (GIR-AUC_{0-3} and GIR-AUC_{0-8}, Table 1) than for SC. Time to half-maximal metabolic action (early $T_{GIR50\%}$) and time to maximal metabolic effect (T_{GIRmax}) were significantly shorter ($P < 0.05$) for INH compared with SC (Table 1). A relative biopotency of 13% was observed for INH over the duration of the 8-h postdose clamp period.

Comparisons of the intrapatient CVs of the PD summary measures (Table 1) showed no differences between INH and SC, except for T_{GIRmax}, where the CV was significantly lower for SC than for INH ($P < 0.05$).

Safety

INH and SC were well tolerated and no drug- or device-related adverse events were observed. In this study, more than a ±10% change in FEV_1 from baseline was considered clinically relevant. Using this definition, no clinically relevant differences in FEV_1 between treatments were observed at baseline, at 4 h post-treatment, or at 8 h post-treatment. A statistically significant ($P < 0.05$), although not clinically relevant, increase in mean FEV_1 from baseline was observed at 8 h for both INH and SC. One patient had a 12.4% reduction from baseline at 4 h post-treatment during the second INH treatment, returning to within ±10% of baseline level at 8 h post-treatment. However, because a similar reduction (12.5%) and time course of recovery were also observed during an SC treatment, the former was not considered clinically relevant. No instances of coughing were observed during dosing with either INH or SC.

CONCLUSIONS — To our knowledge, this is the first report of a head-to-head comparison of INH and SC intrapatient variability under glucose clamp conditions in patients with type 2 diabetes. The glucose clamp technique provides the purest view of the glucose-lowering effect of exogenous insulin. This experimental paradigm is warranted to adequately compare the repeatability of metabolic action of a novel mode of insulin delivery, such as INH, with that of SC. The Aerodose Insulin Inhaler delivered insulin with an intrapatient variability, as described by the CV in both the overall systemic appearance of insulin (Ins-AUC_{0-8}) and total metabolic effect (GIR-AUC_{0-8}), that was comparable to that obtained by SC. The CVs for Ins-AUC_{0-8} and GIR-AUC_{0-8} for INH and SC obtained in this study ranged from 16 to 22%. The similarity of the CVs between INH and SC indicates that the Aerodose Insulin Inhaler can deliver insulin with a clinically acceptable within-patient dose-to-dose reproducibility.

The intrapatient variability found for the Aerodose Insulin Inhaler compares well with dose reproducibility data obtained with a variety of liquid and dry powder insulin inhalers (7–9). In a recently reported glucose clamp study in patients with type 1 diabetes (8), the AERx (Aradigm, Hayward, CA) Insulin Diabetes Management System was shown to deliver liquid insulin aerosol with an intrapatient variability of 26% for Ins-AUC and 34% for GIR-AUC. A comparable intrapatient variability has also been reported for this device and the same patient population in non–glucose clamp studies (10,11), and recently, a CV of 14% for Ins-AUC was reported for the AERx delivery system in normal subjects (12). Dose reproducibility for dry powder inhalers has also been reported. For example, Pfützner et al. (13) showed that Technosphere insulin was delivered via the MedTone Inhaler (Pharmaceutical Discovery, Elmsford, NY) to patients with type 2 diabetes with an intrapatient CV for GIR_{max} and T_{GIRmax} of 34 and 20%, respectively. These authors did not report estimates of Ins-AUC and GIR-AUC intrapatient variability. Numerical estimates for intrapatient variability for the Exubera (Pfizer, Groton, CT) dry powder insulin inhaler have not been reported to date, although Gelfand et al. (7) have stated that the metabolic reproducibility of INH delivered via this device in a nonclamp study is as good as that of SC. It should be noted that a potential caveat in the comparison of results between the present and other studies is that differences in experimental conditions (e.g., methods for calculating reproducibility, clamp versus nonclamp) may contribute to the differences in intrapatient variability values obtained among studies.

Data in the literature for dose reproducibility for SC absorption and action are surprisingly sparse, especially for patients with type 2 diabetes. Early studies (14) assessed PK intrapatient variability by monitoring clearance of radiolabeled insulin from the injection site, making comparison with the present study difficult. However, later studies (15–17) of regular insulin performed under glucose clamp conditions have yielded intrapatient CVs for Ins-AUC and GIR-AUC from 11 to 44%, a range that encompasses the AUC CV values obtained for SC in the present study.

As reported in other studies of INH (1,2,8,9,18–21), we found that the times to maximal values for both serum insulin levels (T_{max}) and metabolic action (T_{GIRmax}) were shorter for INH compared with SC. One explanation for this finding is that the more rapid rate of appearance of insulin in the circulation observed for

INH is the result of the immediate access of inhaled insulin to the circulation across a thin alveolar epithelium-endothelium barrier (3). From a therapeutic standpoint, this characteristic of INH should allow dosing closer to the time of a meal, thereby shortening and possibly eliminating the waiting period between insulin dosing and mealtime indicated for SC. Whether INH will allow better coverage of prandial insulin requirements in patients with diabetes will require further investigation.

The relative bioavailability and biopotency values of 16 and 13%, respectively, observed here were within the range (5–30%) reported by others (1,8,9,20,22) for INH delivered via other specialized inhalers but was also greater than that seen in normal subjects in our previous studies (4,5). The increase in relative bioavailability and biopotency values observed in this study might be the result of either a greater efficiency of pulmonary delivery and absorption of INH and/or a lower efficiency of SC absorption in patients with type 2 diabetes. We are unaware of any data showing that diabetes results in an increase in lung permeability. A more likely explanation is that the increased adiposity in patients with type 2 diabetes and the associated decrease in their efficiency of SC absorption relative to leaner normal subjects (23) results in a relative decrease in the denominator (see STATISTICAL METHODS) and, therefore, a higher relative biopotency and bioavailability. The greater than anticipated relative bioavailability obtained in this study and the associated increase in the absorbed INH dose compared with SC resulted in significantly greater AUCs and peak serum insulin and peak GIR for INH. Although beyond the scope of the present study, the mechanisms underlying the low bioavailability of INH in general, as well as the fate of the unabsorbed insulin, warrant further investigation.

The greater relative bioavailability compared with biopotency (16 vs. 13%, respectively) was the result of the greater Ins-AUC values achieved for INH versus SC. The dose-response curve for insulin is curvilinear, such that increasing levels of insulin result in proportionately smaller increases in the blood glucose–lowering effect of insulin (24,25). The lower biopotency value is, therefore, the result of a proportionally lower GIR-AUC obtained at the greater Ins-AUC for INH.

INH was well tolerated in this study. No inhaler- or drug-related adverse events, including no clinically significant changes in lung function, were observed throughout the study for either treatment arm. An assessment of the long-term safety of the Aerodose Insulin Inhaler will require take-home studies.

In conclusion, an intrapatient variance for INH comparable with SC, combined with a shorter time to maximal metabolic effect, suggests that the Aerodose Insulin Inhaler is well suited for delivery of preprandial insulin in patients with type 2 diabetes.

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