

Dissociation of Lung Function Changes with Humoral Immunity during Inhaled Human Insulin Therapy

John G. Teeter and Richard J. Riese

Pfizer Global Research and Development, New London, Connecticut

Rationale: Inhaled human insulin (INH; Exubera [human insulin (recombinant DNA origin) Inhalation Powder]) causes small changes in pulmonary function and increases in insulin antibodies compared with subcutaneous (SC) insulin.

Objectives: To investigate the relationship between changes in pulmonary function and insulin antibodies and acute effects of INH on lung function.

Methods: In a 24-wk multicenter study, 226 patients with type 1 diabetes were randomized to receive daily premeal INH or SC insulin for 12 wk (comparative phase), followed by SC insulin for 12 wk (washout phase).

Measurements: Spirometry tests were conducted and insulin antibody levels were measured throughout the study. Acute insulin-induced changes in lung function were calculated as the difference between FEV₁ before, and 10 and 60 min after, insulin.

Main Results: There was a temporal dissociation between pulmonary function changes and insulin antibody generation. Small treatment group differences in changes in FEV₁ from baseline, favoring SC insulin, were fully manifest by 2 wk of INH therapy, did not increase during the remainder of the comparative phase, and resolved within 2 wk of INH discontinuation. By contrast, insulin antibody levels remained low for the first 2 wk with INH, increased during Weeks 2 to 12, and gradually declined during washout. There was no evidence of acute insulin-induced alterations in lung function 10 and 60 min postinhalation.

Conclusion: The small lung function changes observed with INH therapy are not mediated by the humoral immune response, or associated with acute decrements in lung function immediately after insulin inhalation.

Keywords: Exubera; insulin antibodies; spirometry

Inhalable forms of human insulin are being developed to facilitate the uptake of insulin therapy and to improve diabetes outcomes (1). Inhaled human insulin (INH; Exubera [human insulin (+DNA origin) Inhalation Powder]; Pfizer Inc., New York, NY; Nektar Therapeutics, San Carlos, CA) is a novel, noninvasive, pulmonary dry-powder human insulin and delivery system approved in the United States and European Union for the treatment of adult patients with type 1 or type 2 diabetes mellitus. In several phase 2/3 studies, INH has demonstrated comparable efficacy and tolerability to subcutaneous (SC) insulin in patients with type 1 or type 2 diabetes, and superior glycemic control to oral antidiabetic agents in patients with type 2 diabetes (2–5).

Small but consistent treatment group differences in pulmonary function tests (PFTs) have been reported with INH (2, 3). Previous controlled studies, using nonstandardized PFT methodology and equipment, have shown that these differences occur

early after treatment initiation, are clinically insignificant, are nonprogressive with up to 2 yr of INH therapy, and are reversible on treatment discontinuation (4). However, no studies to date have investigated the short-term effects of INH after dosing.

Several studies have demonstrated higher levels of insulin-reactive antibodies with INH compared with SC insulin (6). The greatest antibody responses were observed in patients with type 1 diabetes and the smallest responses were observed in non-insulin-using (patients who had not received insulin in any form before INH study entry) patients with type 2 diabetes. Peak antibody levels across all groups were generally observed after 6 to 12 mo of insulin therapy. There were no correlations between antibody binding and glycemic control (measured on the basis of glycosylated hemoglobin [HbA_{1c}]), insulin dose requirements, hypoglycemic events, incidence of allergy or other hypersensitivity reactions, or pulmonary function. Furthermore, additional studies showed no correlation between levels of insulin-reactive antibodies, and postprandial glucose control or duration of insulin action (7). To date, the temporal relationship between changes in pulmonary function and levels of insulin antibodies with INH has not been investigated.

This study was designed to investigate the efficacy and safety profile of INH in patients with type 1 diabetes. The objectives of this work are to evaluate the short-term effects of INH administration (12 wk of therapy) on lung function, the relationship between PFT responses and insulin-reactive antibodies associated with INH treatment, and whether repeated administration of INH could cause an acute decrement in lung function 10 and 60 min postinhalation. To achieve these objectives, highly standardized PFT methodology and a validated assay for the measurement of insulin antibodies were used in this multicenter study. Some of the results of this study have been previously reported in abstract form (8, 9).

METHODS

Study Design and Patients

Subjects aged between 25 and 65 yr, diagnosed with type 1 diabetes for more than 1 yr, and with HbA_{1c} values between 5.5 and 11.0% at screening, a stable SC insulin schedule involving at least two injections daily, and a body mass index not exceeding 30 kg/m², were included in the study. Patients were excluded if they had a history of any active lung disease, smoked within 6 mo of randomization, or presented abnormal pulmonary function at screening (carbon monoxide diffusing capacity [DL_{CO}] less than 70% or more than 120% predicted, total lung capacity [TLC] less than 70% or greater than 130% predicted, FEV₁ less than 70% predicted, and an FEV₁/FVC ratio less than 70%), major organ system disease, or clinically significant abnormalities on laboratory screening. The equations of Miller and coworkers (10) and Hankinson and coworkers (11) were used to establish baseline percentage predicted lung function for DL_{CO} and FEV₁, respectively. A 12% race correction in DL_{CO} was applied for subjects of African American heritage (12).

This open-label, 24-wk, parallel-group, outpatient study consisted of a 3-wk baseline run-in phase and a 24-wk treatment phase. During the baseline run-in phase, all subjects received an SC insulin regimen. This consisted of two or three times daily administration of insulin lispro or regular insulin, plus once- or twice-daily administration of Ultralente or neutral protamine Hagedorn (NPH) insulin or once-daily

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Correspondence and requests for reprints should be addressed to John G. Teeter, M.D., Pfizer Global Research and Development, 50 Pequot Avenue, M56025-A4223, New London, CT 06320. E-mail: john.g.teeter@pfizer.com

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administration of insulin glargine before bedtime. Before randomization, patients received instruction regarding the use of the insulin inhalation device. Subjects were randomized via a computer-generated randomization scheme to receive an INH regimen or to continue receiving SC insulin for 12 wk (comparative phase). The INH regimen consisted of premeal INH (2) plus a single bedtime dose of Ultralente insulin, NPH insulin, or insulin glargine. Subjects randomized to receive SC insulin in the comparative phase maintained the regimen established during the run-in period. During the second 12 wk of the study (washout phase), all subjects received the SC insulin regimen that had been established during the run-in period. This study was approved by each site's institutional review board.

The initial total daily dose of INH, estimated according to the following formula,

$$\text{Body weight (kg)} \times 0.15 \text{ mg/kg} = \text{total daily dose (mg)}$$

was administered as three equivalent preprandial doses ([total daily dose]/3) throughout the day. Administration was preceded by a blood glucose test and the dose was adjusted weekly to achieve a mean fasting glycemic target of 80 to 140 mg/dl. Patients could adjust doses when preprandial glucose was outside the target range.

Study Parameters

This study was designed to investigate the efficacy and safety profile of INH in patients with type 1 diabetes. The efficacy of study treatment was assessed by the change from baseline in HbA_{1c} at the end of the comparative (Week 12) and washout (Week 24) phases of the study, reported in more detail elsewhere (13).

This article focuses on the acute effects of INH on airway function and the relationship between PFT changes and insulin antibody responses. Comprehensive PFTs including spirometry, lung volumes, and diffusing capacity were performed at screening and at Weeks -2, -1, 0, 1, 2, 3, 4, 6, 8, 12, 14, 16, 20, and 24 during the study. Baseline values were established as the average of the Weeks -2, -1, and 0 observations before administration of the study drug. This separation of screening and baseline PFT measurements was conducted to minimize regression to the mean in the initial stages of the trial secondary to screening. Treatment group differences in change from baseline PFTs were assessed at protocol-defined intervals during the study. Subjects were tested in the fasting condition between 6:00 A.M. and 10:00 A.M., before receiving their first daily dose of short-acting insulin. FEV₁ was also measured before (PRE) and 10 and 60 min after (POST) short-acting insulin administration at Weeks 0, 4, 8, and 12. Acute insulin-induced changes in lung function were evaluated by calculating the difference between (POST - PRE) FEV₁ (Δ FEV₁) before, and 10 and 60 min after, insulin (INH or SC) administration.

PFT methodology employed in this study involved standardized equipment, training of testing personnel, and centralized data collection. A Collins CPL lung function analyzer (Ferraris Respiratory, Louisville, CO), customized with standardized software to provide real-time quality control prompts and common analytic procedures at the time of testing, was deployed to each site. All study coordinators performing PFTs underwent an intensive 2-d training seminar at Quantum Research, Inc. (Toledo, OH), before study initiation and were required to demonstrate proficiency in a written test, and to have performed testing of acceptable quality on at least five healthy volunteers before the testing of any study subjects. All studies were performed in accordance with the guidelines established by the American Thoracic Society (14, 15). All data were centrally collected at Quantum Research, Inc., and assessed for quality. Sites received feedback within 24 h of any reported tests of unacceptable quality and recommendations for improvement were offered.

Serum samples for measurement of insulin antibodies were collected at Weeks 0, 1, 2, 4, 8, 12, 14, 16, 20, and 24. A quantitative radioligand-binding assay, as previously described (7), was used to measure insulin antibodies in human serum. Free and antibody-bound insulin was first removed by an acid-charcoal extraction step, after which the insulin-extracted samples were incubated overnight with radiolabeled insulin. The insulin-antibody complexes were precipitated and radioactivity was measured. Data are expressed as microunits of insulin bound per milliliter of test serum. The assay was validated and optimized for accuracy and reproducibility. The assay had a lower limit of quantitation

of 2.1 μ U/ml and all data below the lower limit of quantitation were assigned a value of 1.05 μ U/ml.

Statistical Analyses

In this article, the primary analysis population is analyzed, which includes all randomized subjects who had at least two FEV₁ measurements between screening and randomization, had at least two postbaseline FEV₁ measurements, and received randomized study drug for at least 50% (i.e., 6 wk) of the required comparative phase treatment duration.

Assuming a treatment group difference of 0, a common standard deviation of 0.150 to 0.200 L (FEV₁), a confidence level of 71 to 85%, and 80% power, the sample size of 100 subjects per treatment group provided this study with the precision to detect differences in change from baseline FEV₁ of 0.040 L.

The descriptive statistics for PFT measurements included observed value, percent of predicted value at baseline, and change from baseline for all postbaseline visits. Insulin antibody data were summarized using descriptive statistics.

Treatment comparison based on group means of the FEV₁ data was done according to the PROC MIXED procedure for repeated measure analysis in SAS (SAS Institute, Cary, NC). The variance-covariance structure was the Spatial Power form, which assumes higher correlation between neighboring time points than between time points farther apart. This analysis provided information on the profile of group mean change in FEV₁ during early INH treatment. The primary analysis model for FEV₁ included variables for treatment, assessment time point, sex, and country, as well as the continuous covariates of age, baseline FEV₁, and height.

RESULTS

Patient Disposition, Baseline Characteristics, and Efficacy

A total of 353 subjects were screened for this study and 226 patients were randomized. A summary of patient disposition is presented in Figure 1.

The baseline demographic and clinical characteristics of subjects were comparable in both treatment groups at study entry (Table 1).

The decline from baseline in HbA_{1c} was comparable for both treatment groups at the end the comparative and washout phases of the study (0.5% for both treatment groups at both time points).

Changes in FEV₁ and Insulin-reactive Antibody Levels

Decreases from baseline FEV₁ were observed in both treatment groups during the comparative period. Total declines by Week 12 were 0.065 and 0.053 L in the INH and comparator groups, respectively. Small treatment group differences in change from baseline FEV₁ ranged from 0.006 L (or 6 ml favoring INH) seen at Week 6, to a maximum of -0.043 L (or 43 ml favoring SC insulin) seen at Week 2, with the difference at Week 12 being -0.009 L (or 9 ml favoring SC insulin). The mean treatment group differences observed remained small, representing 1.3% of the baseline FEV₁ in the INH cohort at Week 2, and 0.3% of the baseline FEV₁ at Week 12. The treatment group differences resolved within 2 wk during the washout phase (Figure 2 and Table 2).

Treatment group differences in change from baseline FEV₁ and insulin antibodies are presented in Figure 2. With INH therapy, mean insulin antibody levels were low during the first few weeks (23.3 μ U/ml at Week 2), increased steadily thereafter, reached a maximum at Week 12 (132.5 μ U/ml), and declined gradually during washout (50.3 μ U/ml at Week 24). In patients treated with SC insulin, mean antibody levels remained relatively constant throughout the study (baseline, Week 12, and Week 24 values: 17.4, 19.5, and 21.6 μ U/ml, respectively; Figure 2).

The treatment group differences in FEV₁ were fully manifest within the first 2 wk of therapy and remained stable thereafter,

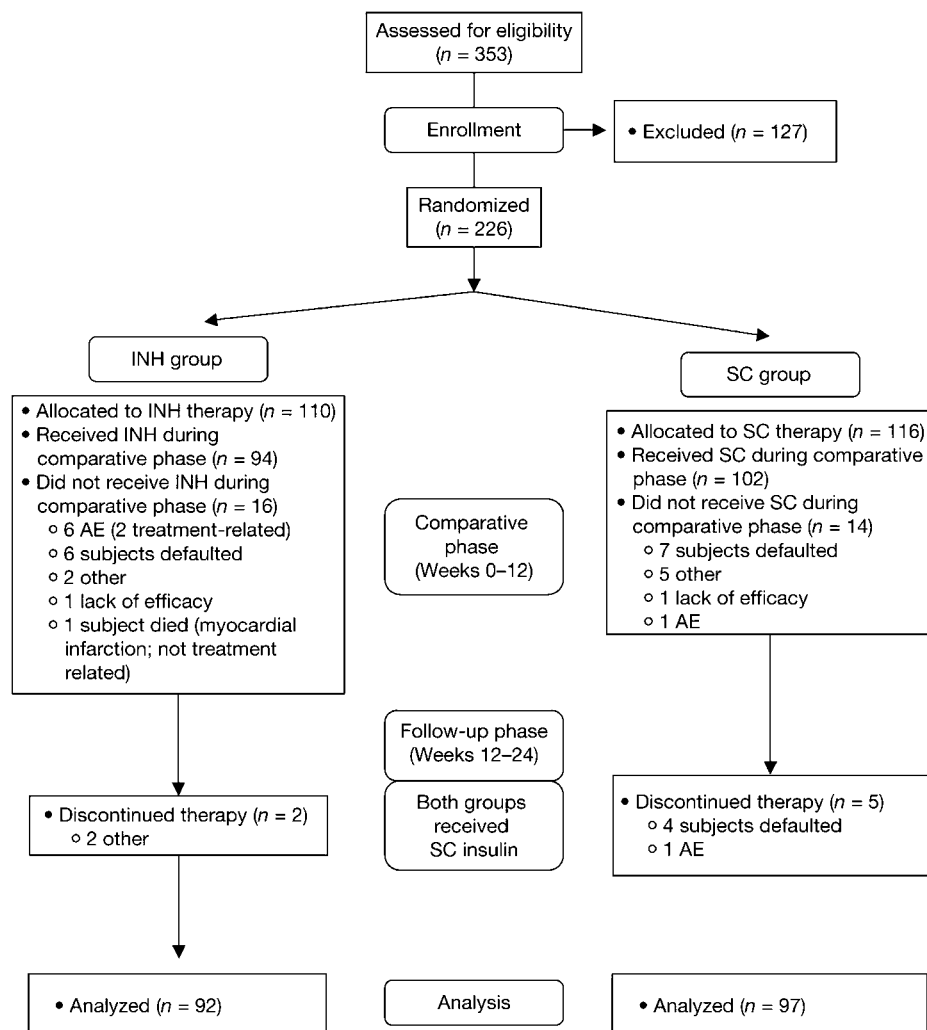


Figure 1. Subject disposition. AE = adverse event; INH = inhaled human insulin; SC = subcutaneous.

well before peak insulin antibody levels were achieved (Figure 2). Similarly, the treatment group differences in FEV₁ resolved within 2 wk of treatment discontinuation whereas the insulin antibody levels declined gradually throughout the washout. Thus, these data show a clear temporal dissociation between INH-induced changes in FEV₁ and insulin-reactive antibody production, suggesting that the two phenomena are not causally related.

To explore whether intrasubject changes in FEV₁ possibly correlate with increases in insulin-reactive antibody generation in this study, scatter plot analyses were generated to investigate

changes in FEV₁ and insulin antibody levels from baseline to end of comparator phase, and from baseline to end of washout phase (Figure 3). These analyses showed no observed correlation between changes in FEV₁ and production of insulin antibodies. These changes were consistent with the observations on the temporal dissociation of FEV₁ and insulin antibody levels as described above, and provide clear evidence that the small INH-induced changes in lung function were not a result of the humoral immune response to INH.

Acute Insulin-induced Alterations in Lung Function

Short-term changes in FEV₁ 10 and 60 min after INH administration from pre-INH FEV₁ are shown in Table 3. Overall, these changes remained small and were well within the normal variation observed with repeated spirometry testing. There was no measurable worsening of acute insulin-induced alterations in lung function observed over the course of this study. Distributions of the acute change in FEV₁ 10 and 60 min postinhalation at the end of the comparator phase (Week 12) were similar between treatment groups (Figure 4). Thus, there was no evidence of acute decrements in lung function 10 and 60 min post-INH inhalation after 12 wk of daily drug exposure.

To determine whether the changes in FEV₁ before and after insulin administration correlated with increases in insulin-reactive antibody generation in this study, scatter plot analyses were generated. There was no observed correlation between

TABLE 1. BASELINE CHARACTERISTICS OF PATIENTS AT STUDY ENTRY

	INH	SC
Age (yr), mean (range)	39.7 (20–65)	40.6 (23–63)
Sex, M/F	61/49	63/53
Height, cm/BMI, kg/m ²	170.7/25.5	171.3/25.5
FEV ₁ , L (% predicted)	3.370 (92.3)	3.299 (91.1)
Mean HbA _{1c} , %	7.5	7.5

Definition of abbreviations: BMI = body mass index; F = female; HbA_{1c} = glycosylated hemoglobin; INH = inhaled human insulin; M = male; SC = subcutaneous insulin.

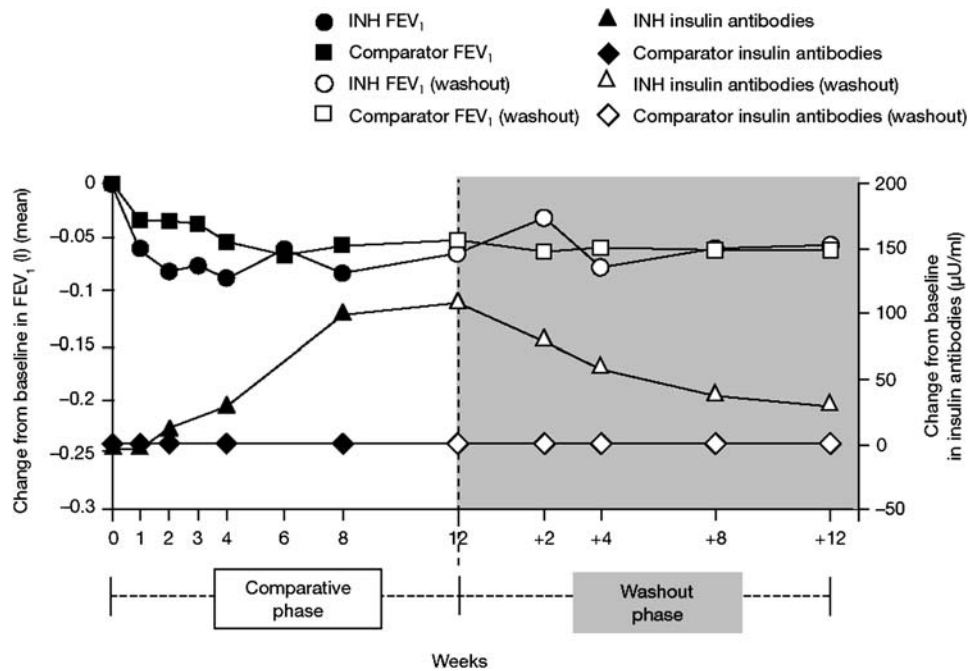


Figure 2. Observed mean change from baseline in preinsulin dosing FEV₁ and change from baseline in mean serum insulin antibody levels in INH and SC insulin-treated subjects throughout the study.

changes in acute insulin-induced alterations in lung function ($[\text{POST}_{\text{Week 12}} - \text{PRE}_{\text{Week 12}}] - [\text{POST}_{\text{Week 0}} - \text{PRE}_{\text{Week 0}}]$) and production of insulin-reactive antibodies (Figure 5).

DISCUSSION

This is the first study examining the detailed time course of pulmonary function changes and insulin antibody production after initiation of INH treatment. This is also the first study investigating the short-term effects of INH therapy by highly standardized PFT methodology, and investigating whether INH induces acute changes in FEV₁ immediately after dosing during 12 wk of therapy. These data show a clear temporal dissociation

between INH-induced pulmonary function changes and insulin antibody generation. This temporal dissociation combined with the lack of acute effects on lung function immediately after INH inhalation, and the lack of correlation between pulmonary function declines and insulin antibody production, provide strong evidence that changes in pulmonary function are not mediated by the humoral immune response to INH.

Both the INH- and comparator-treated subjects experienced a decline in FEV₁ over time in this study. The magnitude of the decline from baseline in both groups (65 ml in INH subjects and 53 ml in comparator subjects at Week 12) was similar to that observed in prior studies (2, 3), but greater than expected for

TABLE 2. CHANGE FROM BASELINE FEV₁, AND TREATMENT GROUP DIFFERENCES IN CHANGES FROM BASELINE FEV₁, FOR INHALED AND SUBCUTANEOUS INSULIN GROUPS

	Mean (SD) Change from Baseline FEV ₁ (L)		Mean (90% CI) Adjusted† (INH-SC)
	INH	SC	
Baseline*	3.37 (0.814)	3.30 (0.701)	
Comparative Phase			
Week 1	-0.053 (0.113)	-0.029 (0.116)	-0.019 (-0.051, 0.013)
Week 2	-0.085 (0.149)	-0.036 (0.101)	-0.043 (-0.075, -0.011)
Week 3	-0.077 (0.134)	-0.035 (0.117)	-0.035 (-0.067, -0.003)
Week 4	-0.088 (0.154)	-0.055 (0.131)	-0.028 (-0.060, 0.003)
Week 6	-0.061 (0.145)	-0.066 (0.114)	0.006 (-0.026, 0.038)
Week 8	-0.080 (0.145)	-0.056 (0.141)	-0.019 (-0.050, 0.013)
Week 12	-0.065 (0.149)	-0.053 (0.144)	-0.009 (-0.041, 0.023)
Washout Phase			
Week 2	-0.032 (0.149)	-0.063 (0.154)	0.041 (0.007, 0.076)
Week 4	-0.078 (0.140)	-0.060 (0.147)	-0.012 (-0.046, 0.022)
Week 8	-0.060 (0.145)	-0.062 (0.149)	0.006 (-0.029, 0.040)
Week 12	-0.057 (0.136)	-0.062 (0.156)	0.014 (-0.021, 0.049)

Definition of abbreviations: CI = confidence interval; INH = inhaled human insulin; SC = subcutaneous insulin.

* Observed value reported at baseline.

† Adjusted model includes terms for treatment, week, country, age, height, sex, and baseline pulmonary function.

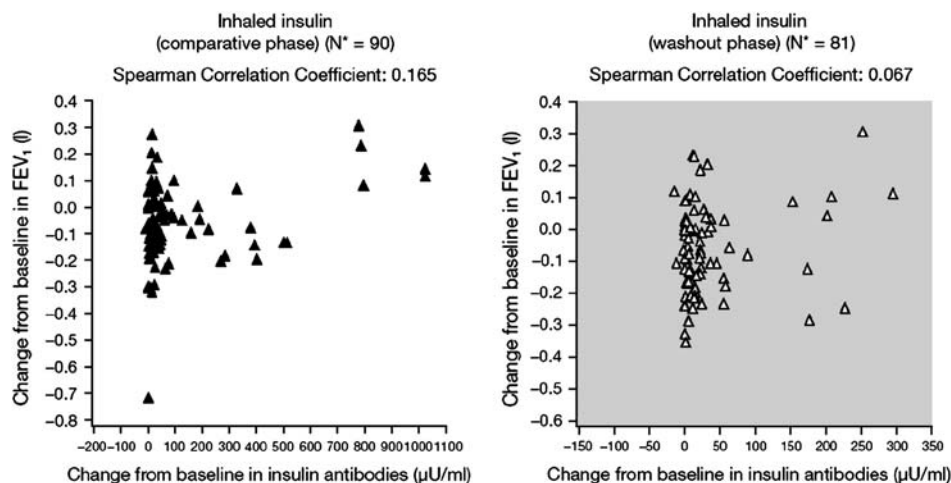


Figure 3. Scatterplots of change from baseline in insulin antibody versus change from baseline in FEV₁ at the end of the comparative and washout phases of the study. *N = number of subjects with both change from baseline in insulin antibodies and change from baseline in FEV₁.

normative declines observed in the normal population (16, 17). One potential explanation for this increased decline in both groups is a regression to the mean. However, this explanation is unlikely to completely explain the findings because the screening spirometry was independent of measurements that were included in the baseline calculation, and the baseline FEV₁ was the mean of three independent measurements. A second potential explanation is the relationship between decline in lung function and the diagnosis of diabetes (18–26). Indeed, one longitudinal study in 125 patients with type 2 diabetes over a 7-yr period showed an average annual decline in FEV₁ of 71 ml/yr (24). Finally, improved glycemic control exhibited by subjects in clinical trials might potentially improve airway tonicity and lead to a decrease in FEV₁. This hypothesis is supported by data in rats showing increased function of inhibitory, neuronal M2 muscarinic receptors in the airways after induction of diabetes (27).

Small treatment group differences in change from baseline FEV₁, favoring SC insulin, were evident at most time points in this study. The magnitudes of the group mean differences (+6 to –43 ml) were similar to those in previous INH studies (2–4), and represent less than 1.3% of the baseline FEV₁ in the INH cohort. Although previous studies had identified this small, INH-induced signal in lung function, this was the first study to precisely identify the time course of the onset and offset of the pulmonary function decline. The treatment group differences were fully manifest within 1 to 2 wk of INH administration, and completely resolved within 2 wk of INH cessation. This time course of

lung function changes argues against a primary role of adaptive immunity in mediating these changes.

Generation of insulin antibodies during INH therapy is a recognized phenomenon that occurs more frequently and at higher levels in patients with type 1 diabetes (2, 3, 7). The data presented in this article significantly add to the existing literature by examining the detailed time course of insulin antibody formation, and how this time course compares with temporal changes in lung function both during treatment and after cessation of therapy. There was a clear temporal dissociation of insulin antibody formation and INH-induced treatment group differences in FEV₁. This is especially evident after cessation of therapy, where the diminution of insulin antibody levels lags far behind resolution of mean FEV₁ differences between the randomized cohorts. This result is further reinforced by the lack of correlation in insulin antibody levels and lung function, a finding that is consistent with previously published literature (6). Thus, there is no evidence of a causal relationship between insulin antibody formation and lung function changes in INH-treated subjects.

Allergen-induced airway responsiveness is an important component of asthma and is characterized by acute changes in FEV₁ after administration of specific allergens or nonspecific bronchoconstricting agents (28–30). Importantly, inhalation of insulin in this trial did not provoke acute changes in FEV₁ even after 12 wk of daily use, indicating that airway smooth muscle contraction is not occurring acutely, and is an unlikely explanation for mediating the small changes in lung function observed with INH

TABLE 3. ACUTE CHANGES IN FEV₁ 10 AND 60 MINUTES AFTER INSULIN DOSING AT BASELINE AND AT WEEKS 4, 8, AND 12

	Week 0	Week 4	Week 8	Week 12
INH				
Mean PRE FEV ₁ , L (n)	3.370 (99)	3.280 (98)	3.301 (97)	3.309 (96)
Mean 10-min ΔFEV ₁ *, L (n)	0.014 (99)	0.002 (87)	0.005 (91)	–0.006 (93)
Mean 60-min ΔFEV ₁ †, L (n)	0.017 (98)	0.029 (85)	0.007 (90)	0.004 (93)
SC				
Mean PRE FEV ₁ , L(n)	3.299 (103)	3.264 (98)	3.252 (102)	3.252 (97)
Mean 10-min ΔFEV ₁ *, L (n)	–0.006 (102)	0.012 (86)	0.006 (100)	0.004 (97)
Mean 60-min ΔFEV ₁ †, L (n)	0.002 (102)	0.032 (85)	0.015 (99)	0.025 (97)

* 10-min POST FEV₁ – PRE FEV₁.

† 60-min POST FEV₁ – PRE FEV₁.

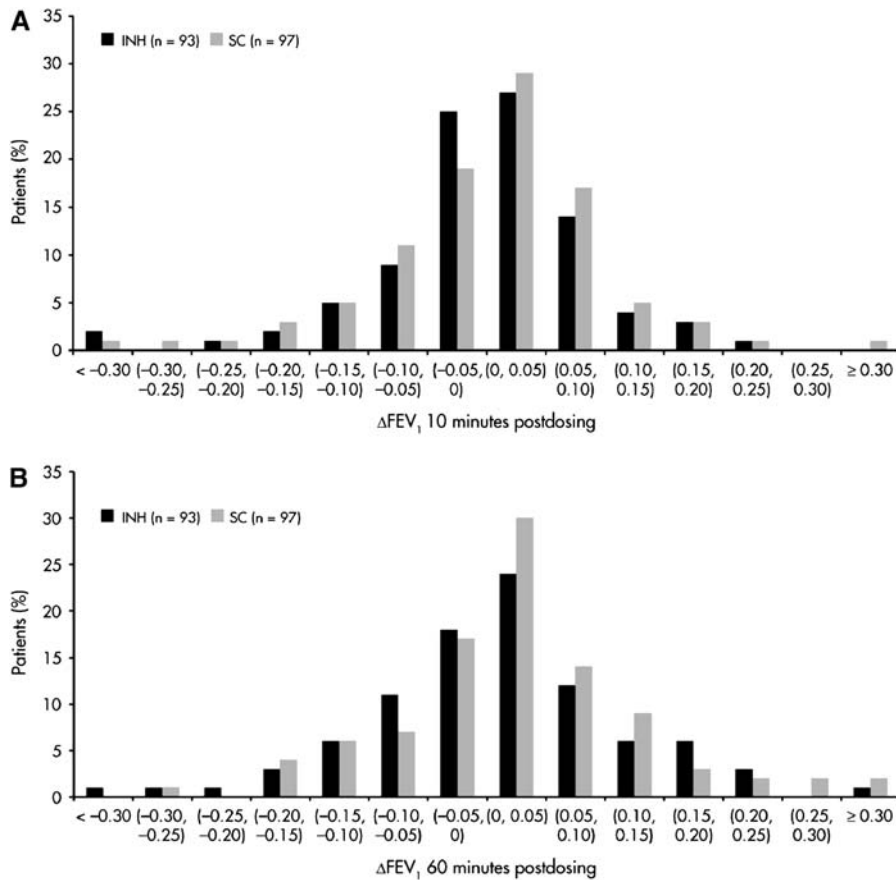


Figure 4. Distribution of the acute change in FEV_1 (ΔFEV_1 , L) 10 min (A) and 60 min (B) after insulin dosing at Week 12.

therapy. These findings are consistent with the observation that the immunoglobulin subtype of insulin antibodies is IgG, and not IgE, which is commonly associated with allergen-induced asthma (6, 31).

There are several limitations to the current study that must be recognized when interpreting these data. First, the comparative phase of this study was only 12 wk in duration. It is possible that INH effects within the lung may occur after a longer term exposure that is not evident at the 12-wk time point. Arguing against this are longer term data showing no increase in treatment group differences in FEV_1 , beyond those found at 6 mo of therapy, when INH is administered continuously for up to

2 yr (4). Second, a more sensitive measurement of airway responsiveness can be obtained in methacholine or histamine respiratory challenge tests. Third, subjects included in this study had relatively normal lung function (FEV_1 greater than 70% of predicted), hence the study did not examine effects of INH in patients with moderate-to-severe or uncontrolled asthma or chronic obstructive pulmonary disease. Finally, direct measurements of inflammatory mediators or markers in induced sputum or bronchoalveolar lavage fluid were not performed. It is possible that chronic inhalation of protein may induce low levels of inflammation detectable only by more invasive methods. Future studies are required to address these important issues.

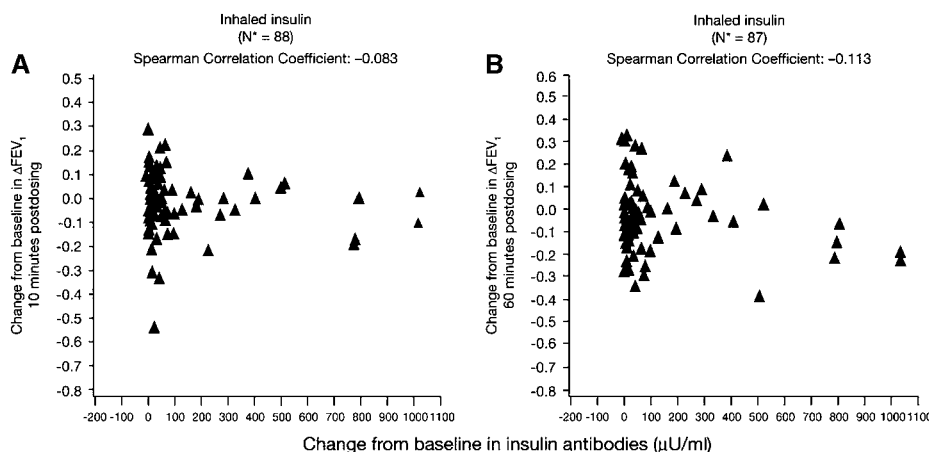


Figure 5. Scatterplots of change from baseline in insulin antibody versus change from baseline in ΔFEV_1 (L) 10 min (A) and 60 min (B) after INH inhalation during the comparative phase: $(POST_{Week\ 12} - PRE_{Week\ 12}) - (POST_{Week\ 0} - PRE_{Week\ 0})$. A: *N = number of subjects with both change from baseline in insulin antibodies and change from baseline responsiveness FEV_1 , 10 min after short-acting insulin dosing. B: *N = number of subjects with both change from baseline in insulin antibodies and change from baseline responsiveness FEV_1 , 60 min after short-acting insulin dosing.

In conclusion, the small, treatment group differences in lung function observed with INH therapy are temporally dissociated from and not correlated with generation of insulin antibodies. Also, there is no evidence that daily inhalation of INH gives rise to acute changes in lung function 10 and 60 min postinhalation for up to 12 wk of therapy. These findings provide additional insight into the possible mechanisms of INH-induced lung changes and support the safety profile of this novel mode of insulin delivery.

Conflict of Interest Statement: J.G.T. is an employee of Pfizer, Inc. R.J.R. is a full-time employee of Pfizer, Inc.

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