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Effects of inhaled human insulin on airway lining fluid composition in adults with diabetes

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ABSTRACT: Inhaled human insulin (Exubera® (human insulin of rDNA origin) Inhalation Powder) causes small, early and reversible changes in pulmonary function in subjects with diabetes mellitus. The present study assessed whether changes occur in cellular and soluble constituents of airway lining fluid consistent with inflammation as a possible cause for Exubera®-associated lung function alterations.

Two 31-week, open-label, sequential design phase 2 studies were conducted, one with 20 subjects with type 1 and one with 24 subjects with type 2 diabetes. After run-in, all subjects received subcutaneous insulin for 12 weeks, followed after 1 week by 12 weeks of Exubera®. Bronchoalveolar lavage fluid cell counts and protein constituents were determined at baseline, after 12 weeks of subcutaneous insulin and after 12 weeks of Exubera®.

Baseline cellular and soluble constituents of lavage fluid were similar to those reported for nondiabetic adults. Exubera® produced no consistent clinically or statistically significant changes in total or differential lavage fluid cell counts or protein concentrations, even though Exubera®-associated changes in pulmonary function are known to be fully manifest within 12 weeks.

Therefore, 12 weeks of Exubera® treatment is not associated with evidence of pulmonary inflammation. The treatment effects on lung function observed in Exubera® trials are not caused by lung inflammation.

KEYWORDS: Bronchoalveolar lavage, bronchoscopy, Exubera®, glycaemic control, pulmonary function tests

Inhaled human insulin (EXU; Exubera® (human insulin of rDNA origin) Inhalation Powder) was approved for use in adult patients with type 1 or 2 diabetes mellitus in the USA and European Union in January 2006.[1] Clinical trials with EXU have shown similar efficacy and tolerability to those of subcutaneous (SC) insulin in subjects with diabetes [1–4]. EXU is a pre-meal insulin with a time–action profile closer to meals than SC regular insulin [5].

Clinical trials have also revealed that subjects treated with EXU have a small decline in some pulmonary function measurements relative to those treated with SC insulin [1–4, 6, 7]. These treatment group differences in forced expiratory volume in one second (FEV_1) and diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$) were observed within 1–3 weeks of initiation of therapy, were fully manifest within 12 weeks of therapy, were nonprogressive, and reversed on discontinuation of EXU [3, 4, 6, 7]. A similar magnitude of change in FEV_1 and $D_{L,CO}$ has been reported for a different inhaled insulin product [8].

The aetiology of this decline is at present unclear, although indirect evidence suggests that it is unlikely to be secondary to lung inflammation. Extensive noninvasive evaluation, including high-resolution computed tomography, has failed to demonstrate any associated radiographic abnormalities of the lung parenchyma. Furthermore, the time-course, pattern and prompt reversibility of the EXU-associated changes in pulmonary function are distinct from

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[1]On October 18, 2007, Pfizer Inc. announced that it was returning the worldwide rights for Exubera® to Nektar, the company from which it licensed the inhaled insulin technology.

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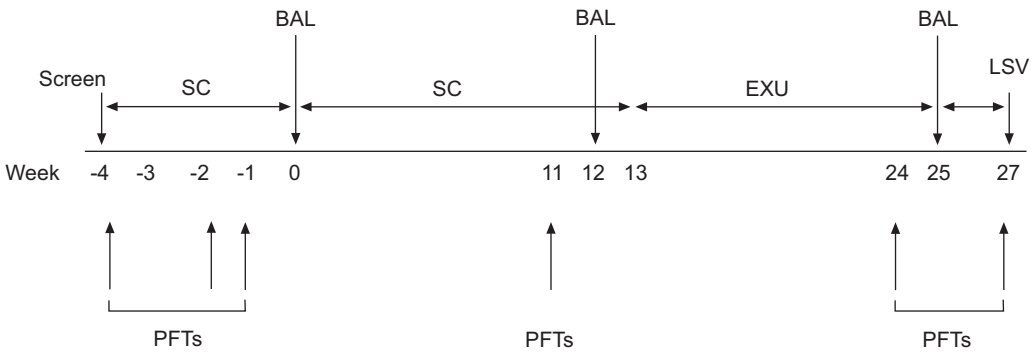

FIGURE 1. Study design. SC: subcutaneous insulin; BAL: bronchoalveolar lavage; PFT: pulmonary function test; EXU: Exubera®; LSV: last subject visit.

the known time-course and pattern of FEV_1 changes produced from stimuli associated with chronic inflammation; however, no studies have directly examined whether the EXU-associated changes in lung function are caused by lung inflammation.

Bronchoalveolar lavage (BAL) is a well-tolerated, minimally invasive technique that samples cells and solutes from the lower respiratory tract and is used as a standard diagnostic procedure in pulmonary medicine [9, 10]. BAL fluid (BALF) assessments provide a direct indication of the cellular response to inflammatory stimuli within the lung. Changes in absolute cell counts within BALF, shifts in leukocyte differential (proportion of lymphocytes, neutrophils, eosinophils or macrophages) in BALF, or changes in BAL protein composition can reveal subtle inflammatory reactions in the lungs.

The current authors conducted two studies (one in subjects with type 1 and one in subjects with type 2 diabetes), with the primary objective being to assess the effects of EXU on airway lining fluid composition in comparison with SC insulin, and secondarily to assess routine efficacy and safety parameters during 12 weeks of short-acting SC insulin followed by 12 weeks of EXU treatment. BAL was performed *via* a standardised protocol in all patients. Since the onset of the lung function alterations associated with EXU therapy occurs within 1–3 weeks of initiation of therapy and the changes are fully evident within 12 weeks of therapy [6], the 12-week treatment duration in these studies was an optimal time interval for the assessment of whether or not inflammation plays a role in mediating these changes.

METHODS

Patients

For inclusion in the study, subjects aged 18–55 yrs with type 1 diabetes or aged 30–55 yrs with type 2 diabetes had to have normal lung function, defined as follows: $D_{L,CO}$ <120% and >80% of predicted; total lung capacity <120% and >80% pred; forced vital capacity (FVC) and FEV_1 >80% pred; and FEV_1/FVC >70%. They also had to have glycosylated haemoglobin (HbA1c) between 5.5 and 11.0%, fasting insulin C-peptide <0.2 pmol·mL^{-1} with type 1 diabetes and >0.2 pmol·mL^{-1} with type 2 diabetes, and body mass index $\leqslant$30 kg·m^{-2} with type 1 diabetes and $\leqslant$35 kg·m^{-2} with type 2 diabetes. Subjects needed to have been on a stable insulin regimen for 2 months prior to screening. Those with a history of smoking (>5 pack-yrs), atopy or pulmonary disease, brittle diabetes, or a predisposition to severe hypoglycaemia were excluded. All subjects with type 2 diabetes were using SC insulin at study entry.

Study design

Two 31-week, nonrandomised, comparator-controlled, open-label, sequential design, multicentre phase 2 studies were conducted, one in subjects with type 1 and one in subjects with type 2 diabetes (fig. 1). Following screening, subjects began a 4-week run-in period, during which diabetes control was optimised by SC insulin therapy consisting of two to three pre-meal daily doses of short-acting insulin together with administration of intermediate/long-acting insulin [6]. During the study, subjects received the SC insulin regimen for 13 weeks, followed by pre-meal EXU with intermediate/long-acting SC insulin for 12 weeks, followed by a 2-week wash-out period. Patient consent and site information are provided in the online data supplement.

Measurements

Fibreoptic bronchoscopy was performed at baseline (week 0), after 12 weeks of SC therapy (week 12) and following 12 weeks of EXU (week 25) using a standardised protocol. BAL was performed on subjects who had fasted overnight and prior to the first daily dose of short-acting insulin using standard bronchoscopic techniques *via* a transoral approach. Visual inspection of the trachea and bronchi bilaterally was performed prior to lavage, and the mucosal surface was graded using the Bronchitis Index scale [11]. BAL was performed in one nondependent segment (anterior segment of right upper lobe, right middle lobe, left lingula) by instillation of five lavages of 20 mL normal saline. Lavages were collected with low suction and pooled. If the total lavage fluid collected after all five aliquots was <50 mL, a second site in the same lung was lavaged and the fluid combined. Bronchoscopists were instructed to use the same segment for each subsequent BAL procedure. BAL was postponed if the subject had an intercurrent respiratory tract illness at that time.

Initial processing of the BAL fluid was conducted at the local sites. Total cell counts were performed using cell preparations of BAL fluid diluted 1:1 with trypan blue on a haemacytometer. Cytoprep slides were stained with the leukostat stain kit (Fisher Scientific, Pittsburgh, PA, USA). Differential cell counts were performed at a central laboratory (by M.C. Liu) as described previously [12]. BALF albumin, fibrinogen and total

protein assays were conducted at IBT Reference Laboratories (Lenexa, KS, USA).

Pulmonary function tests (spirometry, lung volumes by helium dilution and $D_{L,CO}$) were conducted at screening (week -4) and at weeks -2, -1, 0, 11, 24 and 27, using standardised methodology [6, 7, 13]. HbA1c and fasting plasma glucose (FPG) levels were collected at screening, baseline (week 0), and at weeks 11 and 24. General safety monitoring, including adverse events and hypoglycaemic events, was conducted throughout the trial.

Statistical methods

The primary end-points in the present study were lung lining fluid cell count and differentials within subjects after 12 weeks of EXU therapy compared with 12 weeks of SC ((EXU-SC)-(SC-baseline)). For the BALF parameters of total cell count and differential cell counts, a linear statistical model that included centre, sex, atopic disease status and smoking history (never-*versus* ex-smoker) was used to calculate adjusted least squares means for the change from baseline (week 0) to week 12 (SC), the change from week 12 to week 25 (EXU), and the difference between the changes for EXU and SC, along with its 95% confidence interval.

The primary analysis set consisted of all patients who had cell differentials from all three BALF samples. The full analysis set consisted of all patients who received any study treatment and had at least one BAL cell differential. Although the sample size of 20 patients was determined from practical considerations, this sample size was estimated to provide a 95% confidence interval for the estimate of the mean within subject difference between treatment arms that is $\pm$3.9% for lymphocytes and $\pm$1.0% for neutrophils. This estimate was based on data derived from a normal (nondiabetic), nonsmoking population [9]. For additional statistical methods, see online data supplement.

RESULTS

In total, 24 patients with type 1 diabetes and 26 patients with type 2 diabetes were treated during the SC insulin phase; 21 patients with type 1 and 24 patients with type 2 diabetes were subsequently treated during the EXU phase (table 1). A total of 20 patients with type 1 diabetes and 24 patients with type 2 diabetes completed all three bronchoscopy procedures and were included in the primary analysis set. The patient demographics at screening are shown in table 2. Discontinuations are described in the Adverse events section of the online supplement.

BALF total cell counts, leukocyte differential and protein concentrations

Changes in total leukocyte count and BALF leukocyte differential were small after 12 weeks of SC insulin or 12 weeks of EXU and showed no apparent trends favouring either treatment group among subjects with type 1 (fig. 2a) or type 2 (fig. 2b) diabetes. In patients with type 1 diabetes, analysis of the changes in total cell count and leukocyte differential during the SC insulin and EXU treatment periods showed no statistically significant or clinically meaningful differences between the SC insulin and EXU treatment periods (table 3). In patients with type 2 diabetes, analysis of the changes in total cell count, lymphocytes or eosinophils during treatment periods showed no statistically significant or clinically meaningful differences between the SC insulin and EXU treatment periods (table 3); however, for macrophages and neutrophils, there was a small but statistically significant treatment group difference, with an increase in percentage of neutrophils associated with SC insulin administration and an increase in percentage of macrophages associated with EXU therapy (table 3). These differences appear to be due to changes from week 0 to week 12 (SC insulin treatment period), not from changes between weeks 12 and 25 (EXU), and result from a small increase in neutrophils (percentage of total cells) that occurred between weeks 0 and 12. The mean observed values for both parameters returned to near baseline mean values by week 25. Among patients with type 1 or type 2 diabetes, there was no change in BALF albumin, fibrinogen or total protein concentrations associated with either SC insulin or EXU treatment (figs 3a and b).

Pulmonary function

Small decreases in FEV_1 and $D_{L,CO}$ were observed in patients with either type 1 or type 2 diabetes during the EXU treatment period (table 4). Patients with type 1 diabetes experienced a mean decline in FEV_1 from week 11 to week 24 (EXU treatment period) of 0.067 L and a mean decline in $D_{L,CO}$ from week 11 to week 24 of 0.699 $mL \cdot min^{-1} \cdot mmHg^{-1}$. Patients with type 2 diabetes exhibited a decline in FEV_1 from week 11 to week 24 of 0.027 L, and a decline in $D_{L,CO}$ of 0.902 $mL \cdot min^{-1} \cdot mmHg^{-1}$ during the same EXU treatment period. These changes are entirely consistent with previous, larger EXU studies, which more fully characterised EXU-associated alterations in lung function [1–4, 6, 7]. In the larger pulmonary safety studies the mean EXU-associated declines in FEV_1 were ~0.030–0.040 L and in $D_{L,CO}$ were ~0.4–0.6 $mL \cdot min^{-1} \cdot mmHg^{-1}$. The less consistent changes observed in FEV_1 and $D_{L,CO}$ during the SC treatment and 2-week follow-up phases are probably secondary to the expected variability associated with these measurements in a relatively small group of patients.

To assess whether the changes in lung function were associated with changes in total cell counts and leukocyte counts, scatter plots were created correlating the change in FEV_1 with the changes in total cell counts and leukocyte subset counts during the EXU treatment phase. There were no consistent correlations observed between the change in FEV_1 and changes in total cell counts and leukocyte subset counts in the BALF in patients with either type 1 or 2 diabetes mellitus (fig. 4). These data provide further evidence that inflammation is not mediating the changes in lung function observed during EXU therapy.

Airway evaluations

Bronchoscopic airway evaluation total scores revealed near normal values in subjects with type 1 or type 2 diabetes (table 1 in the online supplement). The mean total score at baseline was 1.30 in patients with type 1 diabetes and 2.04 in patients with type 2 diabetes. No significant changes were observed at week 12 or week 25 in either patient population, indicating there was no change following SC insulin or EXU therapy. The lack of changes seen in the airway evaluation is consistent with the lack of changes observed in BAL total cell counts, leukocyte differentials and protein composition.

TABLE 1 Disposition of patients with type 1 or type 2 diabetes

	Type 1 diabetes		Type 2 diabetes	
	SC insulin	EXU	SC insulin	EXU
Screened	42		48	
Assigned to treatment				
Treated	24	21	26	24
Completed	21	20	24	24
Discontinued	3	1#	2	0
Analysed for BAL (primary analysis set)	20	20	24	24
Analysed for safety (adverse events)	24	21	26	24
Discontinuations				
Not related to study drug	3	1	2	0
Other	2	0	1	0
Subject defaulted	1	1	1	0
Total	3	1	2	0
Study visit completion				
Baseline (week 0)	23		25	
SC insulin treatment (week 12)	22		24	
EXU insulin treatment (week 25)	21		24	

Data are presented as n. SC: subcutaneous; EXU: Exubera®; BAL: bronchoalveolar lavage. #: patient completed the week 25 visit and was lost to follow-up in the 2-week run-out phase.

Efficacy

HbA1c and FPG measurements taken at the end of each treatment period showed ongoing maintenance of glycaemic control (table 2 in the online supplement).

DISCUSSION

The present study is the first to directly assess whether a potential inflammatory response can play a role in mediating the small, early, nonprogressive and reversible changes in lung function observed during EXU therapy [1–4, 6, 13]. Since the onset of these EXU-associated changes in lung function occurs within 1–3 weeks of initiation of therapy and the changes are fully manifest within 12 weeks of therapy, the 12-week EXU treatment duration in the current study is the optimal time period to assess the potential cause of these effects. The present data clearly show no measurable inflammatory response within the lung during EXU therapy, indicating that inflammation is not playing a role in the EXU-associated changes in lung function.

TABLE 2 Patient demographics at screening: full analysis set

	Type 1 diabetes	Type 2 diabetes
Subjects n	24	26
Males	17 (71)	21 (81)
Females	7 (29)	5 (19)
Age yrs	37.9 (18–58)	45.1 (21–59)
Body mass index kg·m^{-2}	26.4 (22.3–32.3)	30.2 (24.3–37.0)
Diagnosis yrs	18.2 (2.9–46.2)	9.7 (1.4–35.1)
HbA1c %	7.8±1.2	8.2±1.2
C-peptide pmol·mL^{-1}	0.166±0.001	0.528±0.334

Data are presented as n (%), mean (range) or mean±SD, unless otherwise stated. HbA1c: glycosylated haemoglobin.

The BALF total cell count and leukocyte differentials from the diabetic populations in the present study fell within the range found in normal, nonsmoking individuals [9, 14–18] for all treatment periods. This indicates that the presence of diabetes itself does not alter the cellular composition of the lung lining fluid in nonsmoking patients without lung disease.

There was no consistent change in BALF total cell counts, leukocyte differentials or protein composition following 12 weeks of EXU therapy in patients with type 1 or type 2 diabetes. In contrast, active cigarette smokers exhibit a 2.5- to 7-fold increase in BALF total cell counts that is secondary to a marked influx of macrophages [9, 14, 19]. Subjects with asthma display an increase in eosinophils or neutrophils [20, 21]. In allergic subjects with asthma, segmental allergen challenge causes an 8.6-fold increase in BALF total counts, an 8.4-fold increase in fibrinogen concentration, and eosinophils increase from $\sim 0.0 \times 10^6$ cells·mL^{-1} to 0.7×10^6 cells·mL^{-1} [22]. This increase in eosinophils is markedly greater than the number of eosinophils observed at any time-point during the current study.

Significant alterations in BALF have also been observed in patients with interstitial lung processes and following inhalation of ozone and diesel exhaust. Cell counts rose by 52% in subjects with idiopathic pulmonary fibrosis and by 91% in subjects with interstitial lung disease secondary to connective tissue disorders [14]. Subjects with acute lung injury and acute respiratory distress syndrome exhibited an 8-fold increase in BALF total cell counts, indicative of a brisk neutrophil influx

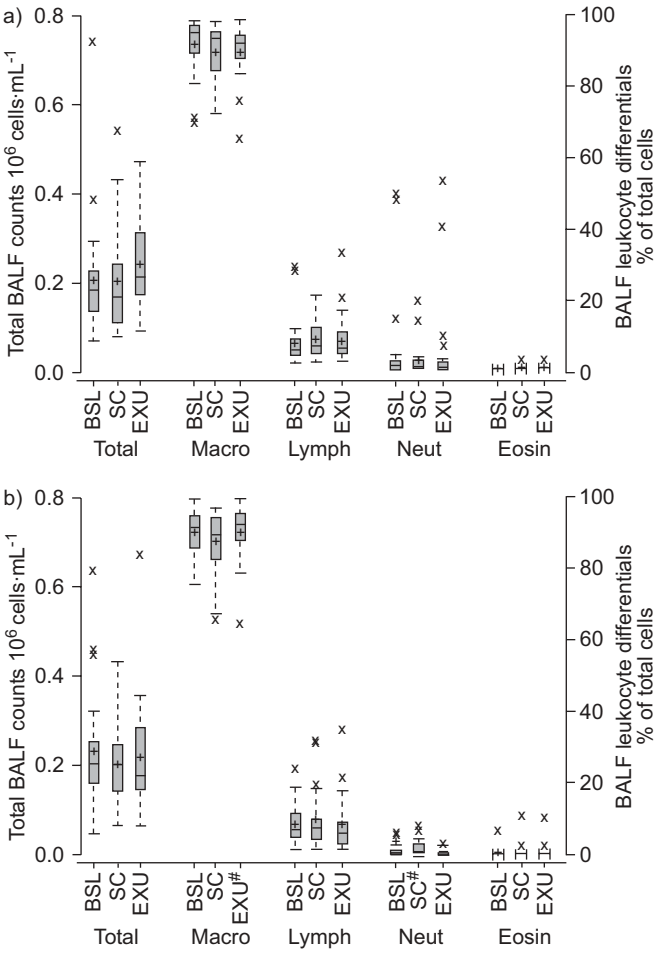

FIGURE 2. Box plot showing total bronchoalveolar lavage fluid (BALF) cell counts and leukocyte cell differentials at baseline (BSL) and following 12 weeks of treatment with either subcutaneous (SC) insulin or Exubera® (EXU) in subjects with a) type 1 (primary analysis set; n=20) or b) type 2 (primary analysis set; n=24) diabetes. The box plot represents the median and the 25th and 75th percentiles (+: mean). Whiskers extend from the box to the farthest point within 1.5 times the interquartile range; values beyond those points are represented by an X. Macro: macrophages; Lymph: lymphocytes; Neut: neutrophils; Eosin: eosinophils. #: statistically significant treatment group difference with an increase in percentage of neutrophils associated with SC administration and an increase in percentage of macrophages associated with EXU therapy (table 3).

within the lung, and a 35-fold increase in BALF total protein concentration, indicating a marked increase in alveolar permeability [23]. Smaller, but significant, increases in BALF neutrophils, total protein and fibrinogen occur 1–24 h following inhalation of ozone [24–27]. Inhalation of diesel exhaust results in significant increases in bronchial wash neutrophils and BALF lymphocytes [28]. Importantly, the current study shows no neutrophil infiltration as a result of EXU therapy, indicating the absence of an acute inflammatory response.

Semiquantitative grading of the airway mucosal surface using the Bronchitis Index scale [11] failed to reveal any clinically significant changes during EXU therapy. These findings are consistent with the lack of alterations in BALF total cell counts, leukocyte differentials and protein composition. They are also in contrast to the airway evaluation scores of 8.5 in asymptomatic smokers and 13.2 in patients with chronic bronchitis, compared with 2.3 in normal subjects [11].

The exact mechanism mediating the EXU-associated changes in FEV1 and $D_{L,CO}$ is currently unknown; however, the lack of acute changes in FEV1 10 and 60 min after EXU dosing in patients with type 1 diabetes suggests that acute smooth muscle contraction with resulting bronchoconstriction is not the mechanism mediating these changes [6]. Furthermore, the present data provide direct evidence that inflammation is also not the causative mechanism. One hypothesis is that the repeated osmotic load of the pre-meal dry powder EXU formulation could result in physiological adaptation and in subtle physiological fluid shifts within the airways and alveoli, giving rise to the observed small, nonprogressive and reversible changes in lung function.

Since EXU therapy is associated with small changes in lung function, EXU should not be used in patients with lung disease such as asthma or chronic obstructive pulmonary disease (COPD), and EXU is contraindicated in patients with severe (Global Initiative for Chronic Obstructive Lung Disease stage III or IV) COPD or poorly controlled, unstable or severe asthma [29]. Ongoing studies will assess the safety and efficacy of EXU therapy in patients with mild-to-moderate asthma or COPD.

It is important to note that the findings and interpretation of the present study are constrained to the actual formulation of the EXU powder. The EXU formulation consists of human insulin in a homogeneous powder formulation containing sodium citrate (dihydrate), mannitol, glycine and sodium hydroxide. Other inhaled insulin formulations containing alternative excipients may engender a different response within the lung.

One potential limitation to the interpretation of the present study is the open-label design. All phase 2/3 studies in the EXU development programme were open-label design. Subjects with diabetes mellitus are required to carefully titrate their pre-meal insulin doses (both SC and EXU) based on pre-meal blood glucose levels, meal size and expected activity levels, in order to adequately control blood glucose while avoiding hypoglycaemia. Based on these considerations, an open-label design was deemed necessary to assure patient safety. This may result in bias to some study end-points, especially concerning patient- and physician-reported adverse events, although bias in BALF total cell count and differentials are less likely to occur.

A second potential limitation is the duration of the treatments, particularly with regard to longer-term EXU effects. Previous studies show that the EXU-associated changes in pulmonary function occur as early as 1–2 weeks following initiation of therapy and are fully manifest by the 3-month time-point [3, 4, 6]. The primary goal of the present study was to evaluate whether changes in BALF cell count and differentials consistent with inflammation occur within the same time period as the changes in pulmonary function. Therefore, 12 weeks was chosen as the optimal treatment duration for the detection of these changes. This duration of treatment may not be sufficient to detect potential long-term EXU effects. One theoretical effect of inhaled

TABLE 3 Statistical analysis for primary end-points:[#] type 1 and 2 diabetes primary analysis set

	Type 1 diabetes[¶]		Type 2 diabetes	
	Within-subject difference[+]	95% CI of within-subject difference	Within-subject difference[+]	95% CI of within-subject difference
Total cell count $\times 10^6$ cells·mL^{-1}	0.041±0.029	-0.022–0.104	0.039±0.033	-0.030–0.108
Lymphocytes	0.150±2.167	-4.498–4.798	-2.475±1.935	-6.510–1.560
Neutrophils	-2.070±1.449	-5.178–1.038	-1.788±0.834	-3.527– -0.048[§]
Macrophages	1.840±3.095	-4.798–8.478	5.125±2.113	0.718–9.532[§]
Eosinophils			-0.846±0.606	-2.109–0.417

Data are presented as per cent of total cell count, unless otherwise stated. CI: confidence interval. [#]: ANOVA with week and subject as fixed effects and an unstructured within-subject covariance matrix and the following covariates: centre, sex, atopic disease status and smoking history (never- *versus* ever-smoker). [¶]: statistical analysis of eosinophils (percentage of total cells) was not possible because the data consisted mainly of values of 0.0. [+]: adjusted mean difference±SEM between successive differences: (EXU-SC)-(SC-baseline). These differences were obtained through contrasts on the weekly values. [§]: statistically significant treatment group difference with an increase in percentage of neutrophils associated with SC insulin administration and an increase in percentage of macrophages associated with EXU therapy. SC: subcutaneous; EXU: Exubera®.

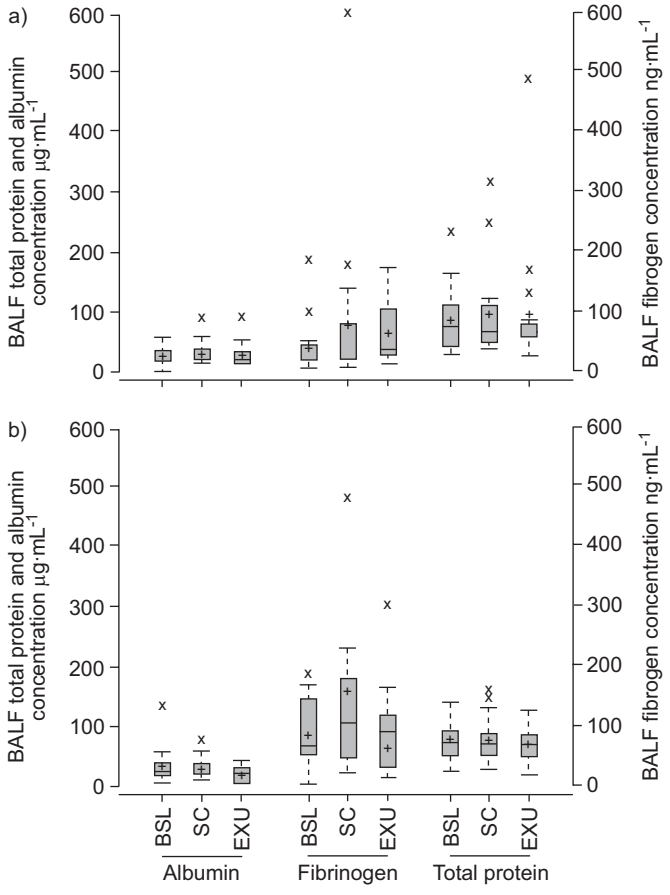

FIGURE 3. Box plot showing the concentration of bronchoalveolar lavage fluid (BALF) proteins at baseline (BSL) and following 12 weeks of treatment with either subcutaneous (SC) insulin or Exubera® (EXU) in subjects with a) type 1 (primary analysis set; n=20) or b) type 2 (primary analysis set; n=24) diabetes. The box plot represents the median and the 25th and 75th percentiles (+: mean). Whiskers extend from the box to the farthest point within 1.5 times the interquartile range; values beyond those points are represented by an X.

insulin would be its mitogenic potential *via* interaction with the insulin-like growth factor (IGF)-1 receptor, although native insulin is a relatively weak ligand for this receptor, requiring 50–100 times the concentration of IGF-1 to have the same effect [30]. Long-term studies are needed in order to address these concerns.

In conclusion, treatment of type 1 or type 2 diabetes with Exubera® does not appear to have any effect on lung lining composition as assessed by visual inspection, bronchoalveolar lavage fluid total cell counts, and leukocyte cell differentials

TABLE 4 Forced expiratory volume in one second (FEV_1) and diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$) measurements

	Type 1 diabetes		Type 2 diabetes	
	Subjects n	Mean±SD	Subjects n	Mean±SD
FEV_1 L				
Baseline (week 0)	20	3.65±0.79	24	3.49±0.59
SC (week 11)	20	3.66±0.78	23	3.63±0.52
Change SC-baseline	20	0.011±0.16	23	0.061±0.16
EXU (week 24)	20	3.60±0.77	21	3.39±0.60
Change EXU-SC	20	-0.067±0.11	20	-0.027±0.14
2-week follow-up	19	3.58±0.82	24	3.45±0.61
$D_{L,CO}$ mL·min^{-1}·mmHg^{-1}				
Baseline (week 0)	20	28.32±6.33	24	28.91±5.43
SC (week 11)	20	28.02±6.36	23	27.76±4.55
Change SC-baseline	20	-0.303±1.86	23	-0.676±1.41
EXU (week 24)	20	27.32±6.16	21	26.95±4.74
Change EXU-SC	20	-0.699±2.11	20	-0.902±1.21
2-week follow-up	19	27.86±6.12	24	27.96±4.53

SC: subcutaneous; EXU: Exubera®.

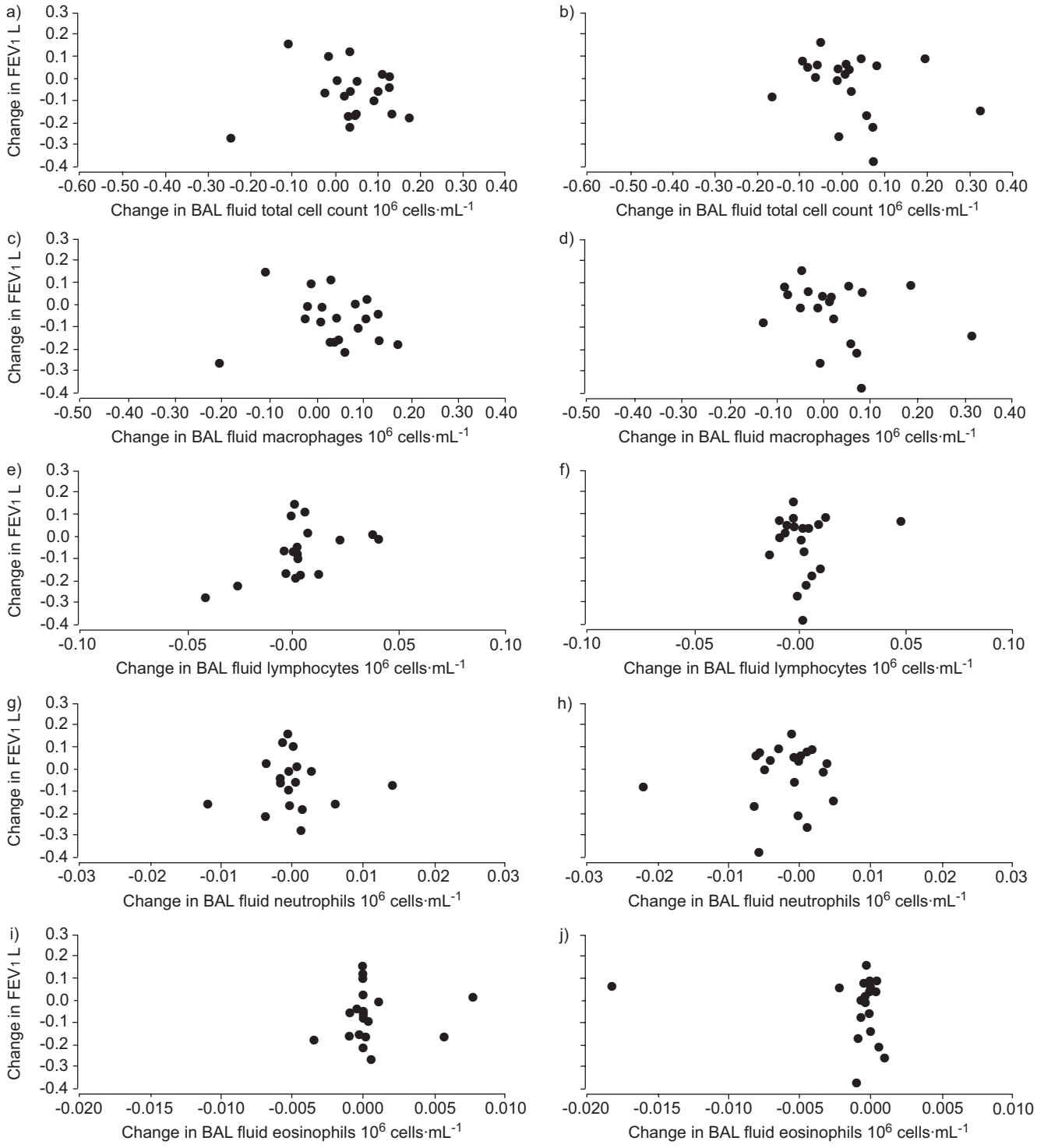

FIGURE 4. Scatter plots correlating the change in forced expiratory volume in one second (FEV_1) during the Exubera® (EXU) treatment phase from week 11 to 24 with changes in total cell counts (a and b) and leukocyte subset counts (c–j) in bronchoalveolar lavage (BAL) fluid during the EXU treatment phase from week 12 to 25 in patients with either type 1 (a, c, e, g and i) or type 2 (b, d, f, h and j) diabetes mellitus. Spearman correlation coefficients are as follows: a) -0.075; b) -0.181; c) -0.187; d) -0.239; e) 0.374; f) -0.067; g) -0.145; h) 0.066; i) 0.085; j) -0.009. Data were obtained from 20 subjects in all cases. BALF: bronchoalveolar lavage fluid.

(lymphocytes, neutrophils, eosinophils or macrophages). Similarly, no effect on protein composition of lung lining fluid was apparent when assessed by bronchoalveolar lavage fluid total protein, albumin and fibrinogen concentrations. Based on the present results, there is no evidence that 12 weeks of treatment with Exubera® causes clinically meaningful cellular changes within the lung that would indicate inflammation or other clinically important lung processes. These results

indicate that pulmonary inflammation is not driving the small, nonprogressive, reversible treatment effect of Exubera® on pulmonary function that has been consistently observed in randomised controlled studies.

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