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RESEARCH PAPER

The Acoustic Features of Inhalation can be Used to Quantify Aerosol Delivery from a Diskus™ Dry Powder Inhaler

Jansen N. Seheult • Peter O'Connell • Kee Chun Tee • Tariq Bholah • Hasan Al Bannai • Imran Sulaiman • Elaine MacHale • Shona D'Arcy • Martin S. Holmes • David Bergin • Emer Reeves • Richard B. Reilly • Gloria Crispino-O'Connell • Carsten Ehrhardt • Anne Marie Healy • Richard W. Costello

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ABSTRACT

Purpose Some patients are unable to generate the peak inspiratory flow rate (PIFR) necessary to de-agglomerate drug particles from dry powder inhalers (DPIs). In this study we tested the hypothesis that the acoustic parameters of an inhalation are related to the PIFR and hence reflect drug delivery.

Methods A sensitivity analysis of the relationship of the acoustics of inhalation to simultaneously recorded airflow, in a cohort of volunteers ($n = 92$) was performed. The Next Generation Impactor (NGI) was used to assess *in vitro* drug delivery from salmeterol/fluticasone and salbutamol Diskus™ DPIs. Fine particle fraction, FPF, (<5 μm) was measured at 30–90 l/min for 2–6 s and correlated with acoustically determined flow rate (IFRc). In pharmacokinetic studies using a salbutamol (200 μg) Diskus™, volunteers inhaled either at maximal or minimal effort on separate days.

Results PIFRc was correlated with spirometrically determined values ($R^2 = 0.88$). In *in vitro* studies, FPF increased as both flow rate and inhalation duration increased for the salmeterol/fluticasone Diskus™ (Adjusted $R^2 = 0.95$) and was proportional

Jansen N. Seheult and Peter O'Connell: These authors contributed equally to this work.

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J. N. Seheult (✉) • K. C. Tee • T. Bholah • H. Al Bannai • I. Sulaiman • E. MacHale • D. Bergin • E. Reeves • R. W. Costello
Department of Medicine Respiratory Research Division, Royal College of Surgeons in Ireland, Dublin, Ireland
e-mail: jansenseheult@rcsi.ie

K. C. Tee
e-mail: keetee@rcsi.ie

T. Bholah
e-mail: tariqbholah@rcsi.ie

H. Al Bannai
e-mail: hasanalbannai@rcsi.ie

I. Sulaiman
e-mail: imransulaiman@rcsi.ie

E. MacHale
e-mail: elainemachale@rcsi.ie

D. Bergin
e-mail: dbergin@rcsi.ie

E. Reeves
e-mail: emerreeves@rcsi.ie

R. W. Costello
e-mail: rcostello@rcsi.ie

P. O'Connell • C. Ehrhardt • A. M. Healy
School of Pharmacy and Pharmaceutical Sciences, Panoz Institute, Trinity College Dublin, Dublin, Ireland

P. O'Connell
e-mail: peoconne@tcd.ie

C. Ehrhardt
e-mail: ehrhardc@tcd.ie

A. M. Healy
e-mail: healyam@tcd.ie

S. D'Arcy • M. S. Holmes • R. B. Reilly
Trinity Centre for Bioengineering, Trinity College Dublin, Dublin, Ireland

S. D'Arcy
e-mail: shona.darcy@gmail.com

M. S. Holmes
e-mail: holmesms@tcd.ie

R. B. Reilly
e-mail: reillyri@tcd.ie

G. Crispino-O'Connell
Statistica Medica, Dublin, Ireland
e-mail: gloria.crispino@statisticamedica.com

to flow rate only for the salbutamol Diskus™ (Adjusted $R^2 = 0.71$). In pharmacokinetic studies, blood salbutamol levels measured at 20 min were significantly lower when PIFRc was less than 60 l/min, $p < 0.0001$.

Conclusion Acoustically-determined PIFR is a suitable method for estimating drug delivery and for monitoring inhalation technique over time.

KEY WORDS aerosol delivery · asthma · cascade impactor · COPD · inhaler technique

BACKGROUND

The best route of administration of drugs in the treatment of COPD and asthma remains inhaled therapy (1). For greatest benefit, the maximum amount of drug needs to reach the site of action, that is, the airways. This depends on the patient's inspiratory flow, inhaled volume, ramp rate of inhalation and degree of airways obstruction (2,3). Findings from previous studies using the Electronic Lung Model showed that in a large subgroup of patients, only 15–30% of the inhaler dose was deposited in the small airways and alveoli of the lung (4,5).

For patients using a dry powder inhaler (DPI), de-agglomeration of the active drug from its carrier (typically lactose monohydrate) depends on a combination of factors: turbulence, mechanical impaction, particle uptake and mechanical vibration (6,7). One study using a Ventolin Diskhaler™ showed that mechanical impaction was not an effective mechanism for powder de-agglomeration, whereas turbulence was found to have a definite effect (8). Turbulence leads to aerodynamic lift, drag and shear, as well as separation forces. The turbulent energy generated depends on the intrinsic resistance of the inhaler and the flow rate generated by the patient. Some DPIs have high internal resistance, for example the Turbuhaler™, while some have relatively low resistance, like the Diskus™ (9). There is a direct relationship between the intrinsic resistance of a DPI and the peak inspiratory flow rate (PIFR)-dependence for drug delivery. Regardless, it is recommended that optimal drug delivery is achieved with a flow rate of greater than 30 l/min and ideally, greater than 60 l/min (10).

For traditional DPIs, insufficient PIFR can lead to ineffective drug delivery resulting in unintentional non-adherence and poor clinical outcomes. Conversely, some authors have advised that very high inhalation flow rates can lead to increased throat deposition and exhalation of particles that are less than 1 μm in aerodynamic particle size (11,12). While modern, sophistically engineered powders and inhaler devices are less flow-rate dependent, or even flow-rate independent (13), it is our experience that the majority of patients with obstructive airways disease are currently prescribed traditional DPIs like the Seretide Diskus™ or Symbicort Turbuhaler™. Hence, a method of measuring inhaled flow rate, as part of assessing inhaler adherence and technique is required.

Currently, the methods of assessing inhaler technique are limited and problematic. Among these are subjective checklist methods (14). Subjective checklist methods have high inter-operator and intra-operator variability. Apart from this, they do not provide a way to gauge a patient's inhalational flow or duration, which are vital for effective drug delivery. The Clement-Clarke In-Check Dial ™ is a marketed method which simulates the resistance of the main types of inhalers in order to estimate the patient's PIFR (15). However, this method is likely to have poor correlation with the *in vivo* PIFR generated by the patient while using the actual inhaler device. Given the high healthcare burden of respiratory diseases and the cumulative costs of inhaled medications, there is an urgent need for a real-time system for tracking drug delivery. In this study, we propose a novel method to monitor a critical aspect of inhaler technique, namely PIFR determination using acoustics.

We have devised a monitoring device, the INCA™ device, which records the acoustics of an inhalation while a subject uses the Diskus™ DPI (Figs. 1 and 2) [D'Arcy S, *et al.* Design and assessment of an adherence monitoring device for inhalers. Trinity Centre for Bioengineering, Trinity College Dublin, Ireland. Unpublished]. The INCA™ device comprises a high fidelity microphone and on-board storage, which logs the date and time the inhaler is used and stores a recording of the inhalation acoustics. This device can be used for at least 60 recordings and hence can give an indication of inhalation technique over a period of a month. We have previously reported on a relationship between inhalation acoustic parameters and PIFR in a group of 15 healthy volunteers (16). One drawback of this study was that it was a repeated measures design in which volunteers subjectively varied their inhalation for up to eight recordings. Also, it is possible that obstructive airways disease might alter the inhalation acoustics while using a DPI.

There is, to date, no universally accepted method of assessing airway drug deposition. Three commonly used methods include *in vitro* particle size and deposition characterisation using Cascade Impactors, pharmacokinetic studies and scintigraphic studies.

Fig. 1 INCA™ device and functional position on Diskus™ inhaler.

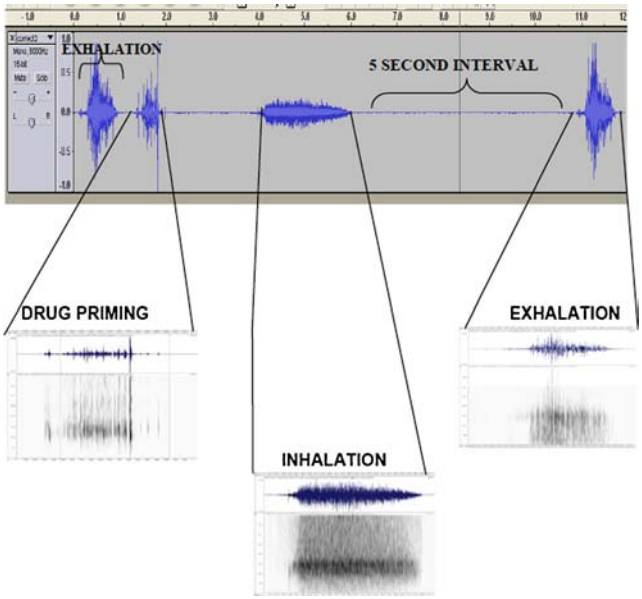

Fig. 2 A sample of the acoustic profile obtained from INCA™ device. The amplitude of the inhalation signal varies proportionately with inhalation flow rate.

Each of these has been applied to the Diskus™ inhaler and results have consistently shown that while Total Emitted Dose may be flow independent, Fine Particle Dose is significantly dependent on inhalation flow rate. Fuller showed that Fine Particle Mass obtained from a 250 μg fluticasone Diskus™ was almost halved by decreasing the flow rate from 60 to 28 l/min (17). Mahler *et al.* also concluded that bronchodilator therapy via nebulization should be considered in patients with COPD who have a suboptimal PIFR (<60 l/min) with a Diskus™ DPI (18).

Modern signal processing techniques mean that it is possible to relate the features of sound from inhalations to other measured values such as inspiratory flow rate. Hence, we hypothesized that by analysing the acoustics of inhalation in a group of patients with a variety of respiratory and non-respiratory diseases we could determine the sensitivity and specificity of our method in classifying inhalation flow rate. Furthermore, we hypothesized that we could estimate the Fine Particle Dose emitted from the Diskus™ inhaler into the Next Generation Impactor (NGI) by using calculated values of flow rate and acoustically determined duration. Finally, we hypothesized that this was clinically relevant *in vivo* by studying the peak concentrations of drug achieved in healthy subjects as a function of PIFR and duration.

METHODS

Study 1: The Relationship Between Acoustics and Physiological Measures of Lung Function

One hundred and ten subjects from a population of patients with asthma, COPD, lung cancer, neuromuscular disease, other respiratory disorders and non-respiratory disorders were recruited by clustered and stratified sampling. All participants were either on inhaled medications as part of their treatment regimens or received training on how to use a Diskus™ inhaler. Patients were recruited from different clinics in Beaumont Hospital in Dublin, Ireland. There were no specific exclusion criteria for this study apart from capacity to comply with instructions. Informed consent was obtained for the study with explanations of the study protocol. Demographics and baseline lung function by spirometry were recorded (Table I). The study was approved by the local Hospital Ethics Committee (ERC/IRB 13/36).

The construction of the airtight container with the associated Diskus™ inhaler, INCA™ device and spirometer connection used in these studies has been described previously (16). A graphical representation of the overall test set up can be seen in Fig. 3. Patients were instructed to exhale gently to functional residual capacity and then inhale at maximal flow rate and duration. Each patient repeated this manoeuvre until two consecutive PIFR readings were within 20% of each other.

The audio files recorded from the subjects were subsequently analysed using Audacity v2 and MATLAB v9 software packages to determine the value of amplitude and duration of each inhalation. In this case, mean absolute deviation (MAD) amplitude was calculated by applying the equation shown below:

$$\text{MAD Amplitude}, A_{MAD} = \text{mean}(\text{abs}(\text{amplitude}, A - \text{mean}(\text{amplitude}, A)) \quad (1)$$

PIFRc was calculated using equations derived from our previous dataset of 15 healthy volunteers: (16)

$$PIFRc = (194.7 * A_{MAD} + 0.1716)/(A_{MAD} + 0.02621) \quad (2)$$

Statistical analysis was done using MATLAB v9 and STATA v13. Creating binary dependent variables using threshold values for measured PIFR, sensitivity and specificity analysis was done comparing acoustically-determined PIFRc with spirometrically-determined PIFRm. Receiver Operating Characteristic Curves were constructed and the value of acoustically-determined PIFRc at which the maximum number of inhalations was correctly classified was determined and presented in tabular form.

Study 2: Correlation of Inhalation Acoustics from a Diskus™ Dry Powder Inhaler with *In Vitro* Drug Delivery

In vitro deposition and aerodynamic particle size of the delivered dose from the Diskus™ DPI was characterized using the Next Generation Impactor (US Pharmacopoeia 601, Apparatus 5) (19). The NGI was used with a pre-separator and cups 1–8. A high capacity vacuum pump (HCP4, Copley Scientific, UK) and Critical Flow Controller (TPK 2000,

Table I Details of High Performance Liquid Chromatographic Techniques Used for Quantification of Salbutamol Sulphate, Fluticasone Propionate and Salmeterol Xinafoate

Active ingredient	Mobile phase (per 1 L)	Flow rate (mL/min)	Column details	Injection volume	Detection wavelength
Salbutamol sulphate	600 mL–methanol 400 mL–deionised water 1 g–sodium dodecyl sulphate	1.5	Waters Nova-Pak® C18 5 μm 3.9 × 150 mm,	100 μL	276 nm
Fluticasone propionate/ salmeterol xinafoate	500 mL–50 mM ammonium phosphate pH2.4 1 mL–triethylamine 250 mL–methanol 250 mL–acetonitrile	1.2	Varian Pursuit XRs C18 3 μm 4.6 × 150 mm,	200 μL	252 nm

Copley Scientific, UK) were attached to the air intake port. Impaction cups 1–5 were lined with filter papers wetted with 2 mls of a mixture of methanol: acetonitrile: water (25:25:50) and cups 6–8 were coated with 2 mls of solvent only to prevent particle bounce and re-entrainment (20).

Two Diskus™ [GlaxoSmithKline, UK] inhalers were used in this study: salmeterol 50 μg/fluticasone 250 μg and salbutamol 200 μg. An audio recording device was attached to each inhaler so that acoustic recordings of each inhalation were obtained.

The study variables were Flow Rate (IFR) and Duration of Inhalation. The Critical Flow Controller was adjusted to achieve flow rates of 30, 60 and 90 l/min at 2, 4 and 6 s durations. Testing was performed in duplicate at each study condition for both inhalers. For each determination, five individual doses were aerosolized into the induction port via a mouthpiece adaptor. The active ingredients were quantitatively recovered from the induction port (throat), pre-separator, and cups 1–8.

High performance liquid chromatography (HPLC) analysis was performed using a Waters Alliance Separations module equipped with a temperature programmable autosampler and Waters 2996 PDA detector. Chromatographic data was recorded and integrated using Waters Empower chromatography software and quantified using external standards. HPLC conditions for salbutamol sulphate (21), and fluticasone propionate/ salmeterol xinafoate are detailed in Table I. Analytical method validation was demonstrated for both methods with regard to accuracy, precision, specificity and linearity as per ICH guidelines (22). The limits of detection for salbutamol, fluticasone and salmeterol peaks were 0.045, 0.032 and 0.014 μg/mL, respectively, while the LOQ values for the same three peaks were 0.136, 0.101 and 0.042 μg/mL, respectively

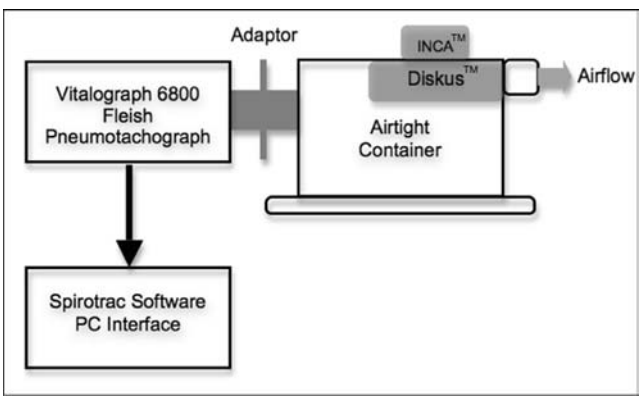

Fig. 3 Apparatus used for Study 1 showing spirometer with PC connection, airtight container and INCA™ Device.

The Total Emitted Dose (TED) was determined as the sum of the total drug recovered from the Throat, PS, and cups 1–8. This was averaged for each study condition. The Fine Particle Dose (FPD), i.e. cumulative dose less than particle size 5 μm, was calculated by interpolation on a log-probit plot using pre-specified stage cutpoints at each flow rate. Fine Particle Fraction (FPF) was calculated by expressing the FPD as a percentage of the label claim dose. The Upper Airway Dose (UAD) corresponded to the cumulative dose above an aerodynamic particle size of 5 μm. Flow Rate (IFRc) was calculated from the acoustic parameters using Eq. 1. Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) were also calculated at each study condition for both formulations using published methods (23,24).

Statistical Analysis was performed using STATA v13 and MATLAB v9. Multivariate regression analysis was performed using TED, FPF and UAD as dependent variables and IFR, Duration, IFRc and Acoustic Duration as independent variables. Bar graphs of TED, FPF, and Upper Airway Dose (UAD) for both formulations were generated, grouping by IFR and duration. The regression effect size (η^2) was calculated for IFR and duration in each model. Coefficients of Variation (CVs) were determined for IFRc at different levels of measured IFR and for acoustic duration at different levels of preset inhalation duration to analyse our method precision.

Study 3: Pharmacokinetic Study Comparing Inhalation Acoustics with Drug Delivery

This study was approved by the local Hospital Ethics Committee (ERC/IRB 13/53). Ten healthy volunteers were recruited. An INCA™ acoustic recording device was attached

to a 200 μg salbutamol Diskus™ with a hot-wire anemometer (FS5, IST, Switzerland) inserted into an air intake port of the Diskus™. The hot-wire anemometer gave a voltage output which was calibrated against flow rate using a vacuum pump.

Blood samples were collected in 7.5 ml serum separator tubes and allowed to coagulate for 20 min. Tubes were then centrifuged at 5,000*g* for 15 min and 2–3 ml of serum pipetted into vials for storage at −20°C.

Serum concentration of salbutamol was determined using a competitive Enzyme Linked Immunosorbent Assay [MaxSignal® Salbutamol ELISA Test Kit (Reference 1022-01) from New Market Scientific, UK]. Limit of detection for serum/plasma was 0.25 ng/ml and the assay was linear in the range of 0.05 to 10.0 ng/ml. Total assay imprecision was determined to be 14% with recoveries between 85 and 115%. To account for interference between protein components in the serum and the assay, the baseline sample concentration was subtracted from timed samples.

Preliminary pharmacokinetic profiling showed serum peaks at 20 min and at 2–3 h post-inhalation (Fig. 4). The sampling time of 20 min was used for the comparative study below because this has been reported to represent pulmonary absorption (25).

Due to the wide inter-subject variation in metabolism of salbutamol and other similar compounds, we used each subject as his/her own control to determine the effect of flow rate and duration of inhalation on peak concentration. Each subject was asked to perform a single inhalation at maximal effort [PIFR >60 l/min] and duration from the study apparatus. This was followed by a 10 s breath hold and then a mouth rinse to reduce gastro-intestinal absorption of salbutamol. A previous study has shown this to be an effective method (25). Blood samples were collected at time zero and at 20 min. This was followed by at least a 24 h washout period. The procedure was repeated at a low flow rate [PIFR <60 l/min] and duration (≤50% of maximal duration) after this washout period.

Statistical analysis was done in STATA v13. PIFR and inhalation duration were determined both from the hot-wire anemometer and from the INCA device and correlated for each inhalation. A line graph was done for each subject and an overall regression model was developed using peak concentration as the dependent variable and measured PIFR, duration, calculated PIFR and acoustic duration as independent variables.

RESULTS

Study 1: The Relationship Between Acoustics and Physiological Measures of Lung Function

Eighteen of the 110 patients recruited had corrupted audio recordings. Table II shows the baseline demographics and lung function for the remaining 92 patients. The majority of the patients had obstructive airways disease, either asthma or COPD. Asthmatics, obese patients and patients with non-respiratory conditions had a significantly higher PIFR than the other patient groups.

Figure 5 shows a scatterplot of Test, i.e. Acoustically Determined PIFR *versus* Reference, i.e. Spirometrically Determined PIFR. Difference and Relative Difference

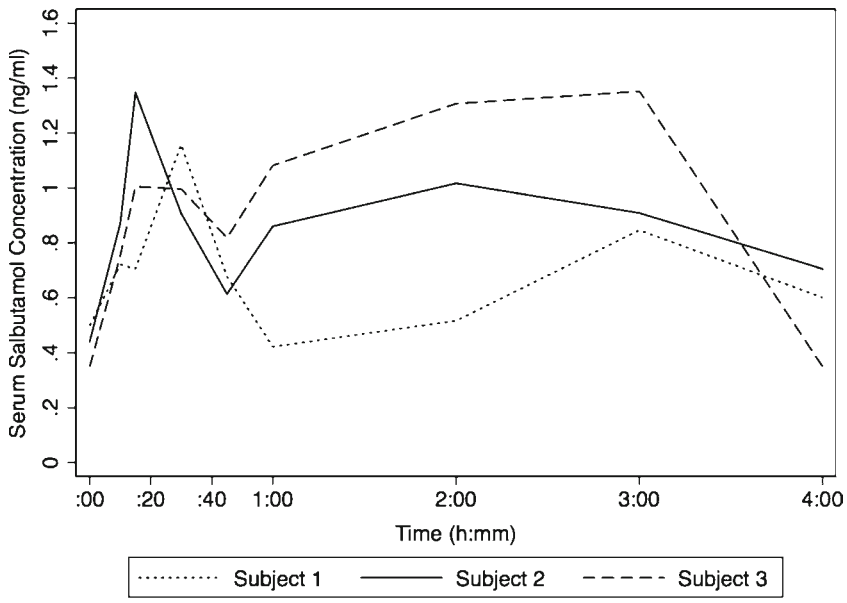

Fig. 4 *Line* graph showing serum drug concentration *versus* time post-inhalation of a 200 microgram dose of Salbutamol via Diskus™ inhaler for three healthy individuals. Note the two distinct peaks in drug concentration at 20–25 min and at 2–3 h.

plots are shown in Fig. 6. Limits for Absolute Difference (+/− 1.96SD) were −11.9 to 19.4. There is a high degree of correlation between the values, with an R^2 of 0.884. There is a statistically significant mean bias of 3.78 and mean relative bias of 6.6% from the Reference Method.

$$PIFRc(l/min) = 1.01 * PIFRm(l/min) + 3.18 \quad (3)$$

The results were partitioned by PIFR values of 45, 90 and 120 l/min. There was a mean bias of 3.4 between 0–45 l/min and 3.8 between 45–90 l/min. The bias above 90 l/min was not significant.

Receiver Operating Characteristic (ROC) Curves for various thresholds of measured PIFR are shown in Fig. 7. AUCs are close to 1 for classification of PIFR as > = 30, 45, 60 and 90 l/min. We were able to correctly classify 95% of inhalations >30 l/min, 91% >45 l/min, 93% >60 l/min and 92% >90 l/min. Both sensitivity and specificity were greater than 90% for any threshold of measured PIFR (Table III).

Study 2: Correlation of Inhalation Acoustics from a Diskus™ Dry Powder Inhaler with *In Vitro* Drug Delivery

There was a high correlation between calculated flow rate (IFRc) and the flow rate at which the impactor was operated (IFR); overall imprecision was less than 10% at all three flow rates (Fig. 8). Imprecision of acoustically-determined duration was approximately 3% (Fig. 9).

When regressions through the origin were performed for our data, plots of studentized residuals *versus* the independent variables highlighted non-horizontal linear trends indicating that a nonzero intercept should be suspected. Hence, all our regression models below included a nonzero intercept, since it is statistically significant.

Fine Particle Fraction (FPF) was directly proportional to inhalation flow rate and duration of inhalation for the salmeterol/fluticasone preparation but FPF was proportional to only IFR for the salbutamol Diskus™. The relationships between FPF, IFRc and duration of inhalation for salmeterol (adjusted R^2=0.9509), fluticasone (adjusted R^2=0.9509) and salbutamol (adjusted R^2=0.7104) are given by the following equations:

$$\textbf{Salmeterol } \boldsymbol{FPF}(\%) = 0.1755314 * IFRc(l/min) + 0.6265714 * Duration(s) + 5.915075$$
$$IFRc\left(\mathrm{p} = 0.000, \eta^2 = 0.90115106\right), Duration\left(\mathrm{p} = 0.029, \eta^2 = 0.05008581\right) \quad (4)$$

$$\textbf{Fluticasone } \boldsymbol{FPF}(\%) = 0.1778574 * IFRc(l/\mathrm{min}) + 0.6396681 * Duration(s) + 5.538353$$
$$IFRc\left(\mathrm{p} = 0.000, \eta^2 = 0.90115106\right), Duration\left(\mathrm{p} = 0.029, \eta^2 = 0.05008581\right) \quad (5)$$

$$\textbf{Salbutamol } \boldsymbol{FPF}(\%) = 0.1796275 * IFRc(l/min) + 29.73383$$
$$IFRc\left(\mathrm{p} = 0.001, \eta^2 = 0.74660896\right), Duration(\mathrm{p} = 0.147 : \text{excluded}) \quad (6)$$

While both calculated flow rate and acoustic duration are statistically significant in the regression models for FPF from the salmeterol/fluticasone inhaler, inhalation duration has a minimal effect compared to IFR as estimated by the η^2. Duration was not a significant variable in the FPF model for salbutamol and all of the models for TED and UAD (S1 and S2 in Online Supplement). The trends for TED were similar to those seen with FPF (Figs. 10 and 11).

A significant proportion of active drug is of a diameter greater than 5 μm and hence, likely to be deposited in the upper airways and throat (Fig. 12). IFRc is only moderately correlated with UAD, with an adjusted R^2 of 0.7076 for salmeterol, 0.2951 for fluticasone and 0.5270 for salbutamol. Inhalation duration has no effect on Upper Airway Deposition. Bar graphs of TED, FPF and UAD for both formulations grouped by IFR and duration are displayed in Figs. 10, 11, and 12.

Tables 1, 2 and 3 of the Supplementary Appendix present the Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) for both salmeterol and fluticasone according to flow rate and duration of inhalation. There is a clear trend to a lower MMAD at higher flow rates for both Diskus™ formulations. However, the GSD or spread of particle diameters increases as flow rate increases from 30 to 90 l/min. The MMAD is also consistently lower for the salbutamol formulation under all study conditions.

Study 3: Pharmacokinetic Study Comparing Inhalation Acoustics with Drug Delivery

Baseline demographics for the ten subjects recruited in this study are shown in Table 4 of the Supplementary Appendix.

Figure 13 shows that there was a significant difference between peak salbutamol concentration (measured at

Table II Demographics and Baseline Lung Function Tests for Patients by Disease Category

	All	Asthma	COPD	Neuro-muscular disease	Obesity	Other respiratory condition	Non-respiratory condition
Number	92	27	25	9	7	10	14
Age (years)	53.1 ± 18.0 (18–84)	53.1 ± 16.6 (18–79)	65.8 ± 6.7 (52–80)	39.1 ± 19.0 (17–78)	46.4 ± 14.8 (23–62)	59.2 ± 23.8 (23–84)	38.4 ± 17.4 (21–77)
Gender (M:F%)	42:58	30:70	44:56	78:22	86:14	30:70	29:71
BMI (kg/m^2)	27.24 ± 6.35 (16.65–49.20)	27.26 ± 6.03 (16.65–37.80)	26.51 ± 5.24 (19.00–38.02)	26.01 ± 3.80 (21.3–33.6)	39.87 ± 6.80 (30.0–49.2)	24.70 ± 3.00 (20.1–28.7)	24.32 ± 3.96 (18–31.7)
FIVC (L)	2.49 ± 1.11 (0.40–5.42)	2.38 ± 0.74 (1.17–3.78)	2.22 ± 0.81 (0.71–3.59)	2.00 ± 1.84 (0.40–5.42)	3.49 ± 1.18 (1.41–4.74)	2.23 ± 0.93 (0.87–3.85)	3.19 ± 1.25 (1.02–5.25)
PIFR (l/min)	187.3 ± 93.6 (28–456)	205.7 ± 85.4 (59–415)	155.5 ± 66.0 (55–275)	138.1 ± 105.9 (28–323)	233.4 ± 98.0 (104–389)	147.0 ± 73.3 (35–292)	245.8 ± 114.9 (59–456)
FEV_1 (L)	2.17 ± 1.12 (0.24–5.07)	1.82 ± 0.92 (0.82–4.59)	1.75 ± 0.94 (0.24–3.80)	2.65 ± 1.65 (0.33–5.07)	2.98 ± 0.84 (1.58–3.97)	2.07 ± 1.03 (0.84–3.97)	2.93 ± 0.95 (1.02–5.07)
FVC (L)	2.88 ± 1.19 (0.38–5.66)	2.51 ± 0.96 (1.23–5.40)	2.58 ± 0.98 (0.38–4.30)	3.09 ± 1.89 (0.38–5.66)	3.71 ± 1.05 (1.89–4.96)	2.74 ± 1.15 (1.08–4.96)	3.65 ± 1.11 (1.37–5.66)
FEV_1/FVC	0.74 ± 0.15 (0.35–0.99)	0.71 ± 0.16 (0.44–0.94)	0.65 ± 0.18 (0.35–0.89)	0.87 ± 0.10 (0.71–0.99)	0.82 ± 0.06 (0.71–0.89)	0.74 ± 0.11 (0.50–0.87)	0.80 ± 0.08 (0.70–0.92)

[a] *BMI* body mass index

[b] FEV_1 forced expiratory volume in 1 s

[c] *FVC* forced vital capacity

[d] *PEFR* peak expiratory flow rate

[e] *FIVC* forced inspiratory vital capacity

[f] *PIFR* peak inspiratory flow rate

20 min) achieved when PIFR was above 60 l/min compared to when PIFR was below 60 l/min for each individual. A *t*-test for difference in means of groups above and below 60 l/min gave a p value < 0.0001 with a mean difference of 0.786 (95% CI: 0.472–1.100).

Duration of inhalation, and by extension, inspiratory volume did not significantly contribute to the multi-level regression model. The R^2 for the clustered regression model was 0.5631 ($p < 0.00001$), with standard error adjusted for 10 clusters of subjects. A large proportion of the variance in peak salbutamol concentration could not be explained by inhalation flow rate and duration.

DISCUSSION

In this study we extended our prior observations which showed that analysis of the acoustics of inhalation from a Diskus™ Dry Powder Inhaler could be used to calculate PIFR. Firstly, using a large sample of patients with widely varying PIFR rates, there was a very strong relationship between measured PIFR and calculated PIFR. To confirm that drug delivery to the lungs is dependent on flow rate, and hence can be estimated from the acoustic sounds of inhalation, we performed *in vitro* and *in vivo* studies. *In vitro*, we showed that Fine Particle Dose was dependent on both the inhalation flow rate and the duration of inhalation for salmeterol and fluticasone; duration was not significant for salbutamol FPF. Using the acoustic parameters to determine IFRc and the duration of inhalation, we were able to explain more than 95% of the variance in FPF for salmeterol/fluticasone but only 70% of the variance for salbutamol. In contrast, the Upper Airway Deposition was relatively constant regardless of flow rate and duration. The implications of this is that patients with poor inhalational technique may have all the side effects of thrush and GI absorption with very few beneficial effects of the medication. We also tested the relationship between PIFR and duration of inhalation on drug delivery, *in vivo*, in ten healthy subjects and showed that there was a significant difference in the serum concentrations of salbutamol when PIFR was low (≤60 l/min) compared to when the PIFR was >60 l/min. Together these data suggest that the acoustics of inhalation from a Diskus™ DPI can be used to objectively quantify pulmonary drug delivery.

We undertook this study in order to test the hypothesis that there is a relationship between the acoustic energy an individual generates when they inhale and the resulting peak inspiratory flow rates. Some authors have described the Diskus™ DPI as flow-independent (26). However, on careful review of their results, FPF from the Diskus™ is flow-dependent,

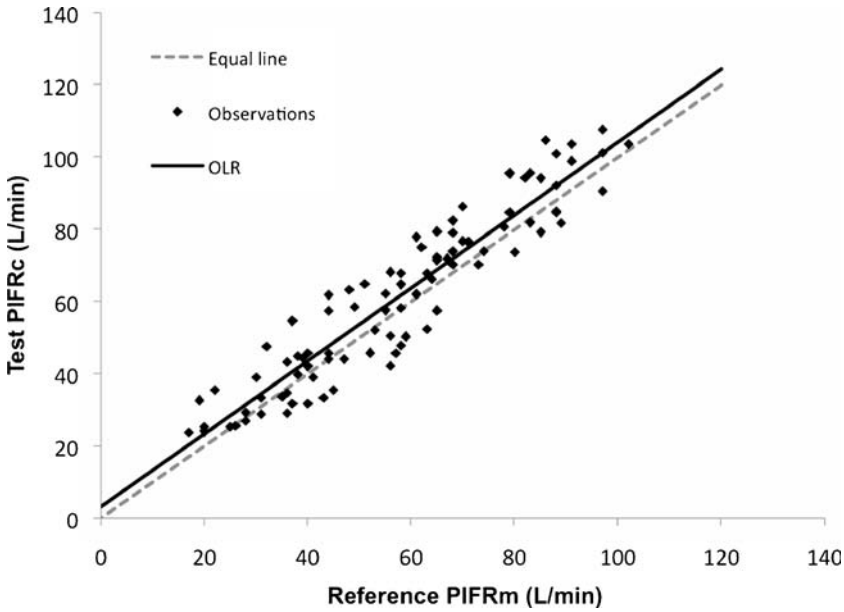

Fig. 5 Scatter plot of test (acoustically-determined) PIFRc *versus* reference (spirometrically-determined) PIFRm. The *equal line* represents no difference between methods (y = x). The ordinary least squares regression line is also shown ($R^2 = 0.884$, Test PIFR = 1.01*Reference PIFR + 3.18, Mean bias = 3.78, Mean relative bias = 6.6%).

although not to the same degree as that from the Turbuhaler™. There is little published data on the effect of duration or inhaled volume on drug delivery. Our data suggest the effect of inhalation duration to be minimal. However, duration is a significant variable in our regression models for salmeterol and fluticasone FPF and it is likely that at borderline flow rates between 30 and 45 l/min, inspiratory duration plays a more important role in inhaler efficacy. Further studies at inhalation durations less than or equal to 1 s are required to further evaluate any possible relationship.

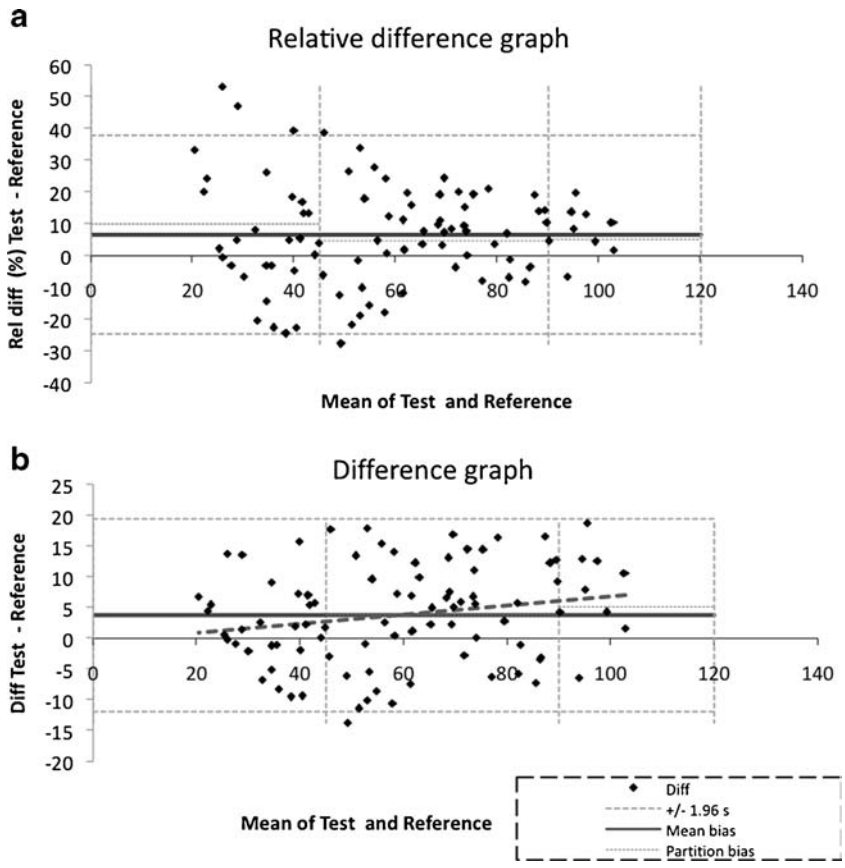

Fig. 6 Difference (**a**) and relative difference (**b**) plots for test (acoustically-determined) PIFRc versus reference (spirometrically-determined) PIFRm.

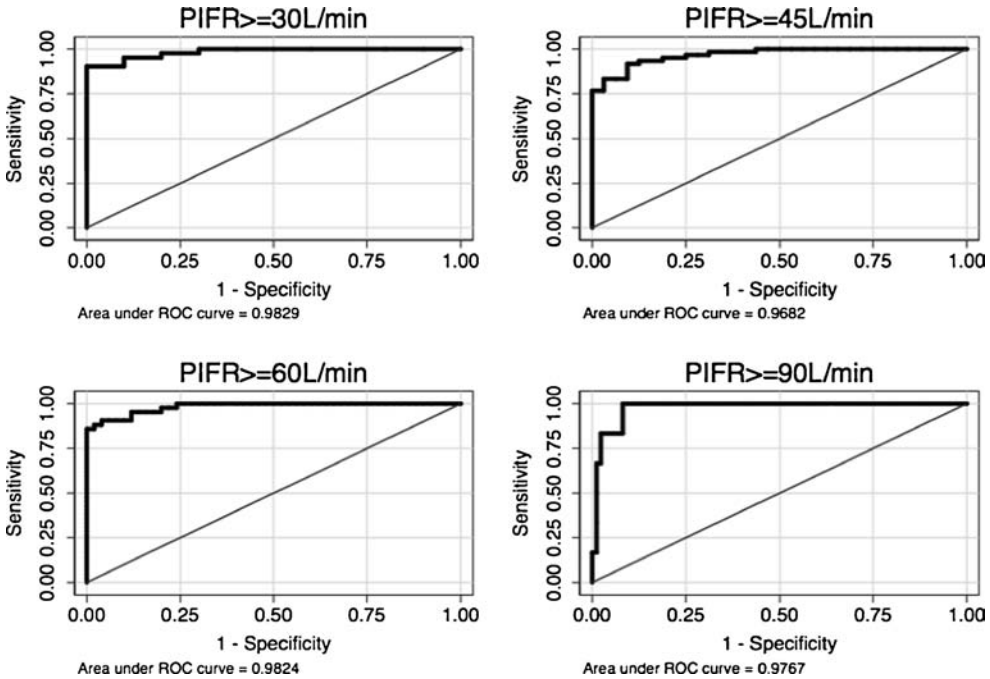

Fig. 7 Receiver operating characteristic (ROC) curves for acoustically-determined PIFR *versus* thresholds of measured PIFRm of 30, 45, 60 and 90 l/min. The *equal line* represents an Area Under the Curve (AUC) of 0.5.

A number of studies have reported that very high inhalational flow rates through the Diskus™ inhaler may be detrimental to airway drug delivery, arguing that throat deposition is increased and that particles less than 1 μm in size are more likely to be exhaled immediately after inhalation (11,12). In contrast, our study found that even though MMAD decreases as flow rate increases, the lowest MMAD achieved for the salmeterol/fluticasone Diskus™ was 3.47 μm with a GSD of 2.22, which means that a significant proportion of particles would still be in the range of 2–5 μm to be active on the small airways. It is worth mentioning that the MMAD values for salbutamol were lower than the salmeterol/fluticasone formulation. Hence, for the salbutamol formulation, PIFRs >60 l/min may lead to lower pulmonary deposition due to exhalation of particles <1 μm.

We also tested the relationship between PIFR and duration on drug delivery *in vivo* in ten healthy subjects. We used a salbutamol Diskus™ because salbutamol has the shortest half-life of the drugs studied and it reaches relatively high concentrations in the blood after inhalation with a short time to maximum concentration. It was straightforward to measure serum plasma concentrations using a commercially available ELISA. In preliminary experiments there was an initial peak at 20 min that was distinct from the peak at 2–3 h, which is likely secondary to GI absorption. The initial peak was therefore most likely related to pulmonary absorption and hence, pulmonary deposition and aerodynamic particle size. Our results were concordant with the *in vitro* studies using the NGI Impactor and confirmed the relationship between PIFR and peak blood concentration. We used each subject as his or her own control since inter-individual drug metabolism is highly variable. We found that each individual achieved a lower C_{max} when his or her inhalation flow rate was less than 60 l/min. Furthermore, our equations to estimate PIFR from acoustics were able to correctly classify all of the inhalations as either above or below 60 l/min and acoustically-determined PIFRc explained more than 50% of the variance in C_{max}. The remainder of the variance is likely due to differences in drug metabolism between individuals. The study was underpowered to detect a relationship between duration of inhalation and peak concentration. The existence of such a relationship is however, questionable since the results of our *in vitro* studies were inconclusive (even though the results for salmeterol and fluticasone FPF were statistically significant, the magnitude of the effect is minimal).

In Study 1, our acoustic method was also shown to be both sensitive and specific for classifying inhalations according to PIFR, being able to correctly classify upwards of 89% of all

Table III Table Showing Threshold Values of Acoustic Method for which Most Inhalations are Correctly Classified, with Corresponding Sensitivity and Specificity

Reference method (l/min)	Test method (l/min)	Sensitivity	Specificity	Correctly classified
≥30	≥33.55	95.12%	90.00%	94.57%
≥45	≥47.91	91.67%	90.62%	91.30%
≥60	≥66.27	90.48%	96.00%	93.48%
≥90	≥90.57	100.00%	91.86%	92.39%

Reference method represents spirometric values and test method represents acoustic method

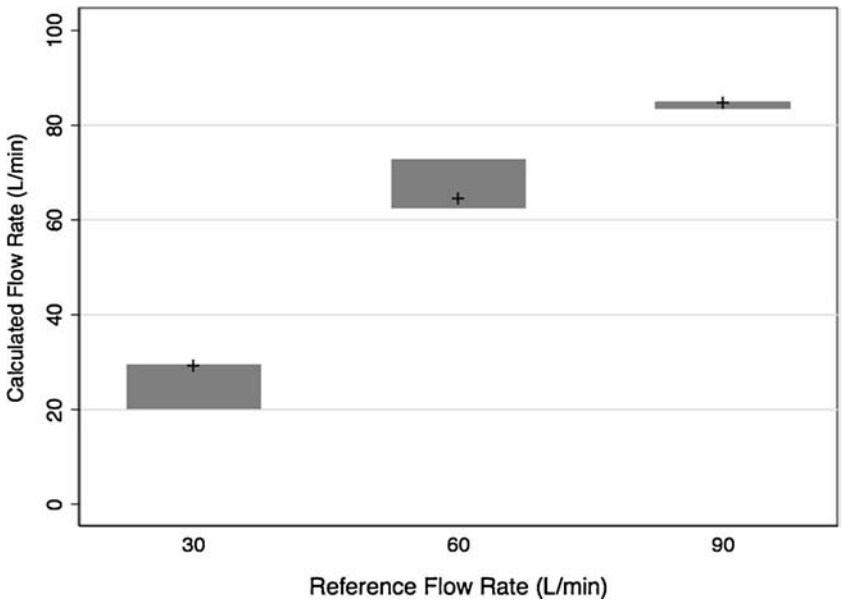

Fig. 8 *Boxplot* of calculated flow rate at each preset flow rate for the NGI impactor.

inhalations according to preset thresholds of spirometrically-determined PIFR. For these analyses the sensitivities and specificities were greater than 90%. Furthermore, we have shown that the relationship between flow rate and sound amplitude is independent of disease state and is therefore applicable to a large subset of the population.

There are many ways to signal average the inhalation sound; previously, we measured the average power in the frequency band 300–600 Hz, Root Mean Square of Amplitude and Mean Absolute Deviation of the Acoustic Amplitude and found that the first had the best correlation with PIFR (16). In this study, we found that MAD Amplitude had the strongest correlation with PIFR. The most likely explanation for this is that MAD Amplitude is more robust to inter-individual changes and mean power may shift in different frequency bands depending on upper and lower airway anatomy. This is in accordance with previous studies, which showed that the optimum frequency band to calculate average power is different in healthy subjects compared to asthmatics (27).

Furthermore, we found that patients with Neuromuscular Disease and COPD generated lower PIFRs compared to asthmatics, obese patients and those with non-respiratory illnesses. This has important implications in that different sub-populations may be able to use the Diskus™ inhaler with different efficacies. Even though their PIFR may be close to their personal best, they may still not be able to generate sufficient turbulent energy to benefit from the DPI.

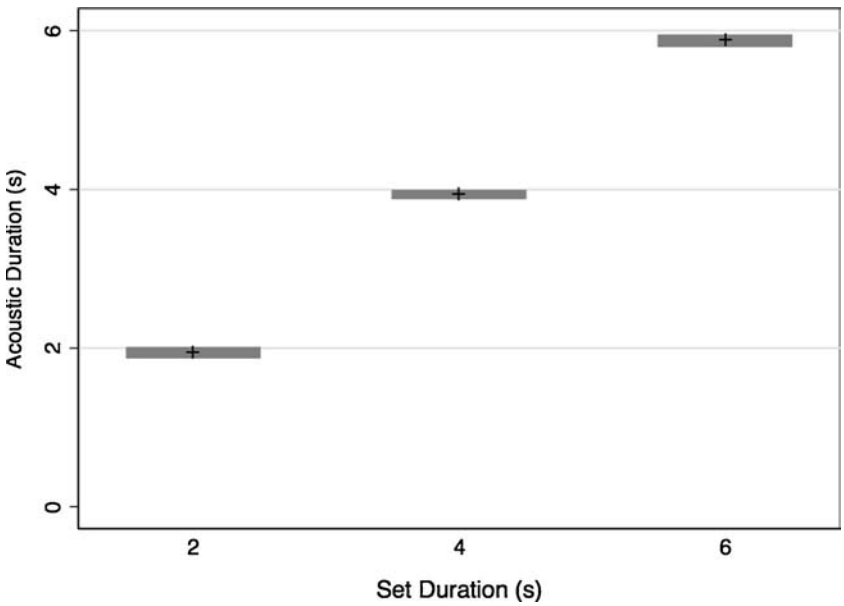

Fig. 9 *Boxplot* of acoustic duration categorized by preset flow controller duration for the NGI impactor.

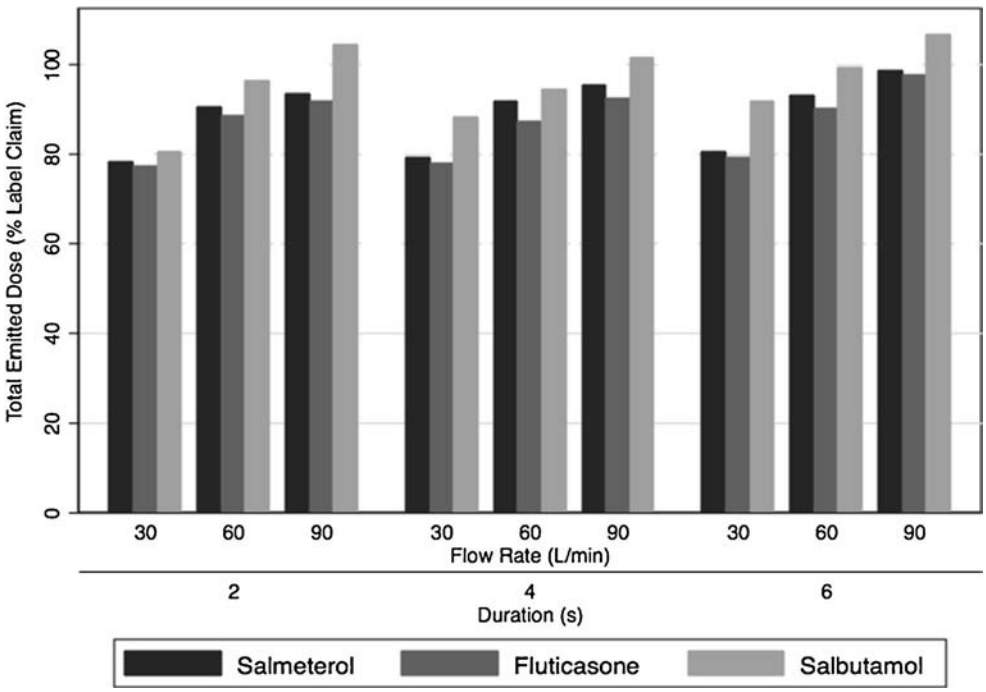

Fig. 10 *Vertical bar* graph of total emitted dose as a % of label claim *versus* calculated flow rate for salmeterol, fluticasone and salbutamol for (**a**) 2 s inhalation, (**b**) 4 s inhalation and (**c**) 6 s inhalation.

Our study does have a number of limitations. The acoustic method is subject to noise interference in everyday life situations. This is likely to affect the calculated values from our models. Noise filtering will allow us to address this problem adequately. We have previously reported the limitations of the apparatus used in Study 1 (16).

One of the limitations of cascade impactor studies is that they require multiple dose actuations in order to enhance detection of very small drug levels in the lower Stages. This increases the chances of particle re-entrainment with each subsequent inhalation and hence, the drug recovered in each stage is likely to be higher than that expected if only one actuation were performed.

There is also limited applicability of our results to new inhaler devices and modern engineered powders, which are not dependent on flow rate for drug delivery. The need to

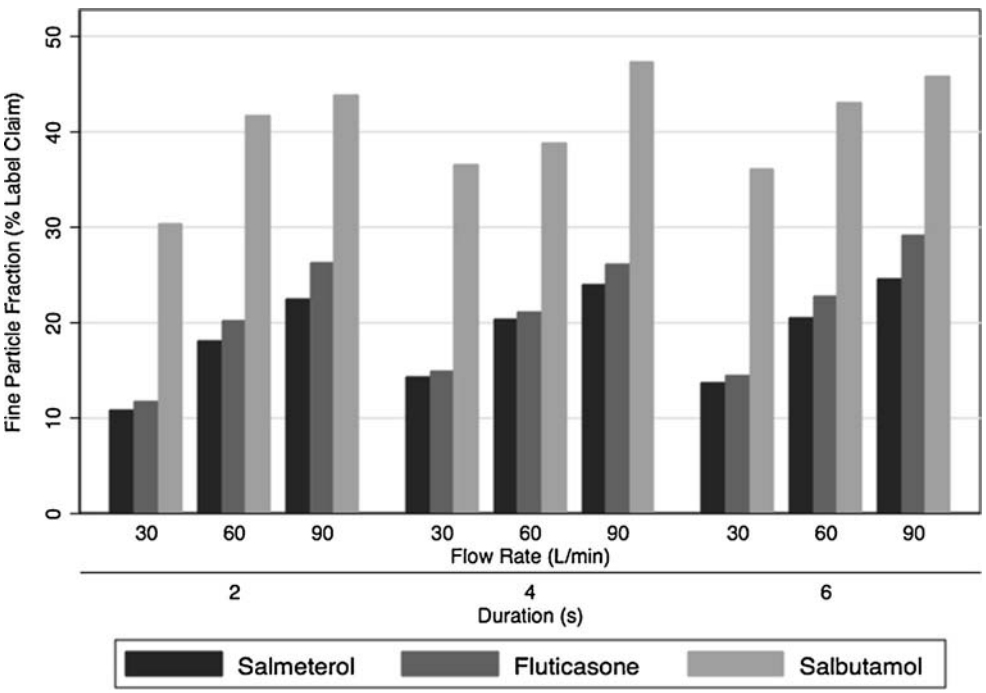

Fig. 11 *Vertical bar* graph of fine particle fraction as a % of label claim *versus* calculated flow rate for salmeterol, fluticasone and salbutamol for (**a**) 2 s inhalation, (**b**) 4 s inhalation and (**c**) 6 s inhalation.

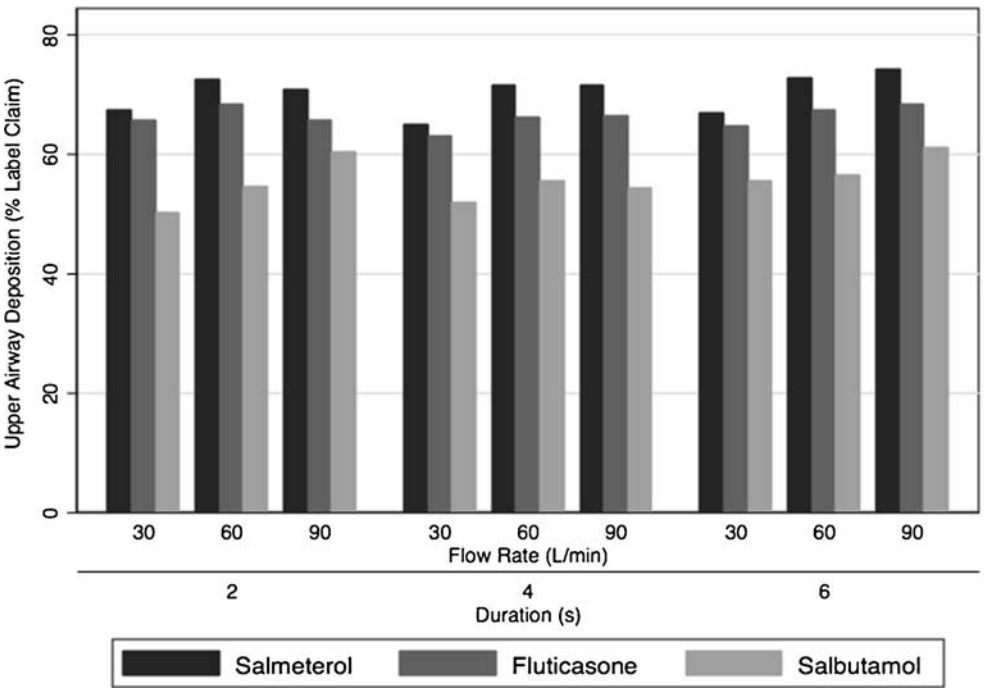

Fig. 12 *Vertical bar* graph of upper airway deposition as a % of label claim *versus* calculated flow rate for salmeterol, fluticasone and salbutamol for (**a**) 2 s inhalation, (**b**) 4 s inhalation and (**c**) 6 s inhalation.

monitor PIFR during inhaler use may be unnecessary in the future when the use of these more novel products becomes widespread.

Finally, in our pharmacokinetic study we used salbutamol without giving charcoal to the subjects to minimize GI absorption. A consensus statement from the British Association for Lung Research recommends the use of an inhaled drug like fluticasone, which has less than 1% oral bioavailability, in pharmacokinetic studies or another drug in combination with activated charcoal (28). However, we based our method on a previous study, which showed that mouth-rinsing effectively eliminates GI absorption (25). Our data from three volunteers also shows that the peak due to GI absorption happens much later than when we collected our blood samples. It would also have been ideal to use an HPLC or LC-MS/MS assay for detection of salbutamol but our method validation of the ELISA showed that is had an acceptable precision and good recovery.

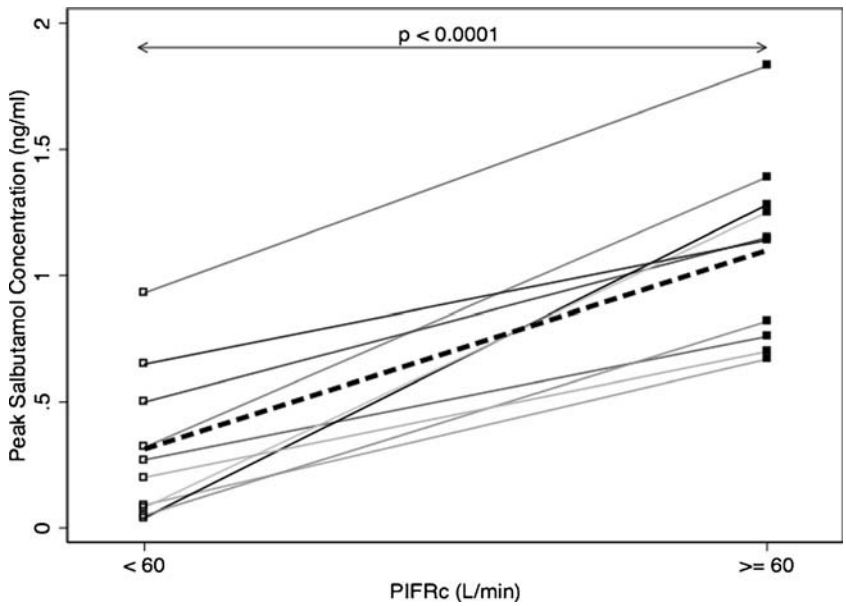

Fig. 13 *Line* and *dot plot* of peak serum concentration of salbutamol *versus* flow rate category (less than or greater than 60 l/min) for ten healthy subjects. Each *line* represents a separate individual and *points* represent actual values of concentration and calculated PIFR. The *dotted line* represents the overall regression line for all the data points. *P*- value for difference in means between high flow rate and low flow rate groups is less than 0.0001.

CONCLUSION

We have shown that our acoustic method for determining drug delivery to the airways is robust and reliable. There is no perfect method of determining pulmonary deposition and our methods are limited to those widely available today. Nonetheless, the INCA™ device provides a novel and more objective way of monitoring a critical aspect of a patient's inhalation technique over a prolonged period of time.

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The patented acoustic device [INCA™] used in this study is manufactured by Vitalograph, Ireland. The first authors of this paper have no affiliation to Vitalograph and are not listed as a holder of the relevant patents. RBR, SD and RWC are listed on the patents.

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