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Quantification of Aerosol Hydrofluoroalkane HFA-134a Elimination in the Exhaled Human Breath Following Inhaled Corticosteroids Administration

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Abstract

Inhaled corticosteroids (ICS) and β_2 -agonists are the primary pharmacotherapies of asthma management. However, suboptimal medication compliance is common in asthmatics and is associated with increased morbidity. We hypothesized that exhaled breath measurements of the aerosol used in the inhaled medications might prove useful as surrogate marker for asthma medication compliance. To explore this, 10 healthy controls were recruited and randomly assigned to ICS (Flovent HFA) or short acting bronchodilators (Proventil HFA). Both inhalers contain HFA-134a as aerosol propellant. Exhaled breath sampling and pulmonary function tests were performed prior to the inhaler medication dispersion, immediately after inhalation, then at 2, 4, 6, 8, 24, and 48 hours postadministration. At baseline, mean (SD) levels of HFA-134a in the breath were 252 (156) pptv. Immediately after inhalation, HFA-134a breath levels increased to 300×10^6 pptv and were still well above ambient levels 24 hours postadministration. The calculated ratio of forced expiratory volume in 1 second over forced vital capacity did not change over time following inhaler administration. This study demonstrates, for the first time, that breath HFA-134a levels can be used to assess inhaler medication compliance. It may also be used to evaluate how effectively the medicine is delivered. Clin Trans Sci 2015; Volume #: 1–6

Keywords: 1,1,1,2-Tetrafluoroethane, hydrofluoroalkane, HFA-134a, gas chromatography, inhaled corticosteroids (ICS), Albuterol inhaler, bronchodilator, aerosol, propellant, asthma, compliance

Introduction

Nearly 26 million Americans, including 7 million children currently suffer from asthma.^{1,2} The clinical and economic burden of asthma is tremendous, accounting for about 1.8 million emergency department visits and 439,000 hospitalizations in 2010.^{3,4} Inhaled β_2 agonists and corticosteroids (ICS) are the fundamental first-line therapy in ongoing asthma management.^{5,6} However, in the current literature, compliance is suboptimal ranging between 30% and 70%.^{7–9} Poor compliance can result from patients' fears about the long-term side effects of chronic medication use, particularly, of corticosteroids. In addition, it is well recognized that for many adults and children, the delivery of a medication that requires a full exhalation, followed by a rapid inhalation, simultaneous depression of the canister delivery system while ensuring a tight seal of the lips and the canister mouthpiece is nonphysiologic and difficult for many adults and children. Poor compliance remains a challenging problem for both patients and physicians because it is associated with adverse clinical outcomes.^{10–12} Williams et al.¹³ estimated that approximately 24% of asthma exacerbations were attributable to ICS medication noncompliance. Finally, when faced with patients who suffer from asthma exacerbations, treatment decisions must be made on some estimate of recent medication use, and, as noted, measuring recent inhaler use is challenging at best.

Reliable methods to determine asthma inhaler compliance do not currently exist. Compliance assessment approaches which include patient self-report, canister weighing, or counting inhaler actuations are not accurate, either overreporting or underreporting ICS use, for both adults and children.¹⁴ Other methods, such as electronic monitoring of ICS compliance,¹⁵ are

limited because they are not able to detect whether the medication is actually inhaled.

The hydrofluoroalkane 1,1,1,2-tetrafluoroethane, or HFA-134a, was introduced in the 1990s to chlorofluoroalkane CFC-12 a volatile organic compound (VOC) banned since 1996 in developed countries because of its impact on the stratospheric ozone layer. HFA-134a is mostly used as a refrigerant in automobile air conditioning systems but it is also the most commonly used volatile aerosol propellant in metered dose inhalers to effectively deliver medication to the lungs. The goal of this study is to take the first steps in exploring the possibility that detection of the aerosolized propellant HFA-134a in the exhaled human breath following asthma medication inhalation can be useful in determining compliance. We examine the washout pattern of HFA-134a in the exhaled human breath after a typical single asthma inhaler administration in otherwise healthy people. We demonstrated that HFA-134a can be detected in the exhaled human breath following asthma inhaler administration for at least 24 hours following inhalation.

Methods

Subjects

Ten healthy subjects (five males and five females, 25–48 years) participated in this study. Any subject with a history of any chronic medical conditions such as asthma, subjects with ongoing respiratory infection, food allergy and eczema, current smokers, users of other medications that contain HFA-134, or users of any prescription medication were excluded from the study. Female

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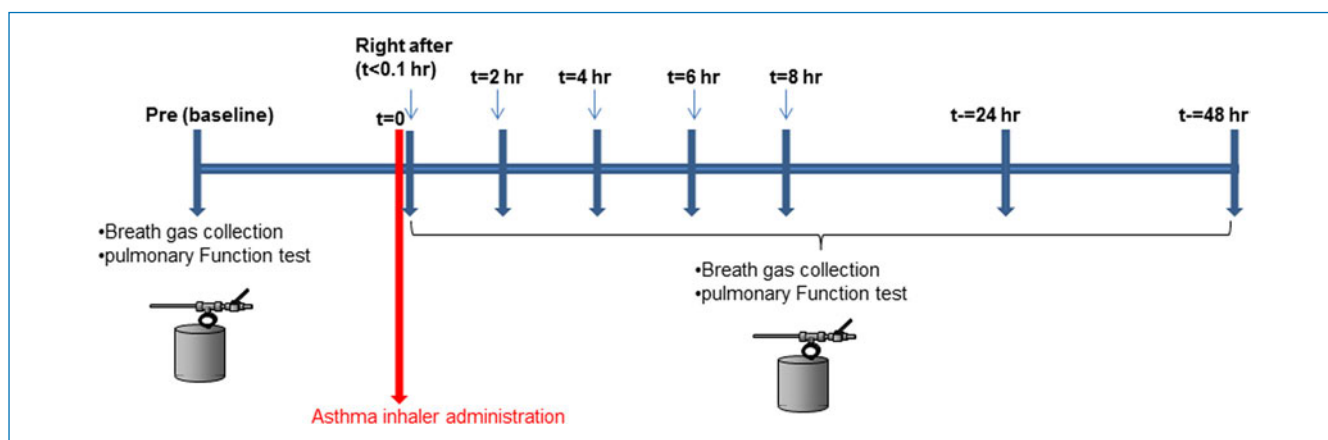


Figure 1. Timeline of the study.

subjects with pregnancy/childbearing potential were excluded because there may be risks associated with asthma inhalers.^{16,17} Pregnancy or child bearing potential status was requested from subjects via self-report at the time of enrollment. The Institutional Review Board at the University of California, Irvine approved the study, and informed consent was obtained from all participants.

Protocol

Study participants were randomly assigned to inhaled corticosteroid (ICS, Flovent HFA, 220 mcg, $n = 5$) or short acting bronchodilator (Albuterol, Proventil HFA, $n = 5$). Baseline exhaled breath sampling and standard lung function test were performed prior to the medication dispersion. After baseline measurements, the participants inhaled two puffs of either Flovent HFA (Glaxo Smith Kline, Research Triangle Park, NC, USA) or Proventil HFA (Merck & Co., Inc., Whitehouse Station, NJ, USA) using a spacer (AeroChamberPlus Flow-Vu, Monaghan, Plattsburgh, NY, USA). Breath sampling and lung function test were performed immediately after inhalation (<5 minutes), and at 2, 4, 6, 8, 24, and 48 hours postadministration (see Figure 1).

Breath sample collection and analysis

Exhaled breath samples were collected in evacuated electropolished stainless steel canisters (volume = 1.9 L). The analytic system used in this study is similar to the system described in Colman et al. (2001).¹⁸ Briefly, 790 mL of the collected air sample is preconcentrated in a stainless steel loop filled with glass beads and submerged in liquid nitrogen to remove the nitrogen, oxygen, and argon present in the sample. The sample is revaporized using hot water (at approximately 80°C) and split into five different column/detector combinations housed in three gas chromatographs (GCs) using UHP helium as the carrier gas: (1) DB-1 column (Agilent J&W, Santa Clara, CA, USA; 60 m, 0.32 mm I.D., 1 μ m film thickness) output to a flame

ionization detector (FID); (2) DB-5 column (Agilent J&W; 30 m, 0.25 mm I.D., 1 μ m film thickness) connected in series to a RESTEK 1701 column (5 m, 0.25 mm I.D., 0.5 μ m film thickness) and output to an electron capture detector (ECD); (3) RESTEK 1701 column (60 m, 0.25 mm I.D., 0.50 μ m film thickness) output to an ECD; (4) PLOT column (Agilent J&W GS-Alumina; 30 m, 0.53 mm I.D.) connected in series to a DB-1 column (Agilent J&W; 5 m, 0.53 mm I.D., 1.5 μ m film thickness) and output to an FID; (5) DB-5ms column (Agilent J&W; 60 m, 0.25 mm I.D., 0.5 μ m film thickness) output to a quadrupole mass spectrometer detector (MSD, HP 5973). The MSD is set to operate in selected ion monitoring mode with one ion chosen to quantify each compound in order to achieve the maximum selectivity and to avoid potential interferences. All GCs and detectors used in this study are manufactured by Hewlett Packard. The analytical system allows for the identification and quantification of different classes of VOCs. HFA-134a levels in the breath samples were detected and quantified using the MSD (5% precision, 10% accuracy). Simultaneously, room air samples were also collected in the same location to quantify corresponding ambient levels of HFA-134a. HFA-134a levels are expressed as mixing ratio. The mixing ratio of HFA-134a is the ratio of the number density of HFA-134a to the total number density of air. Or, in other words, is the ratio of the number of molecule of HFA-134a in a unit volume to the number of molecule of air in a unit volume.

Pulmonary function test

A standardized pulmonary function test was performed in order to assess lung function in all subjects at baseline and postmedication administration. This test includes forced expiratory volume in 1 second (FEV_1), calculated ratio of FEV_1 to forced vital capacity (FEV_1/FVC), and forced expiratory flow 25–75% (FEF_{25-75}).

Results

Ten subjects completed the study procedure without any complications, and no serious adverse events associated to the study were reported. The physical characteristics of the subjects are presented in Table 1.

We successfully detected and quantified HFA-134a in all 10 participants' exhaled breath at baseline (before applying asthma inhalers), right after inhaler administration,

| Characteristic | Flovent HFA-134A | Proventil HFA-134A |
|---------------------------|------------------|--------------------|
| Assigned asthma inhaler | Flovent HFA-134A | Proventil HFA-134A |
| Age (years), mean (range) | 29 (26–37) | 41 (32–48) |
| Male/female, No. | 2/3 | 3/2 |
| BMI, mean (range) | 23.8 (21.1–26.6) | 21.8 (18.7–25.7) |

Table 1. Physical characteristic of the study participants (BMI = body mass index).

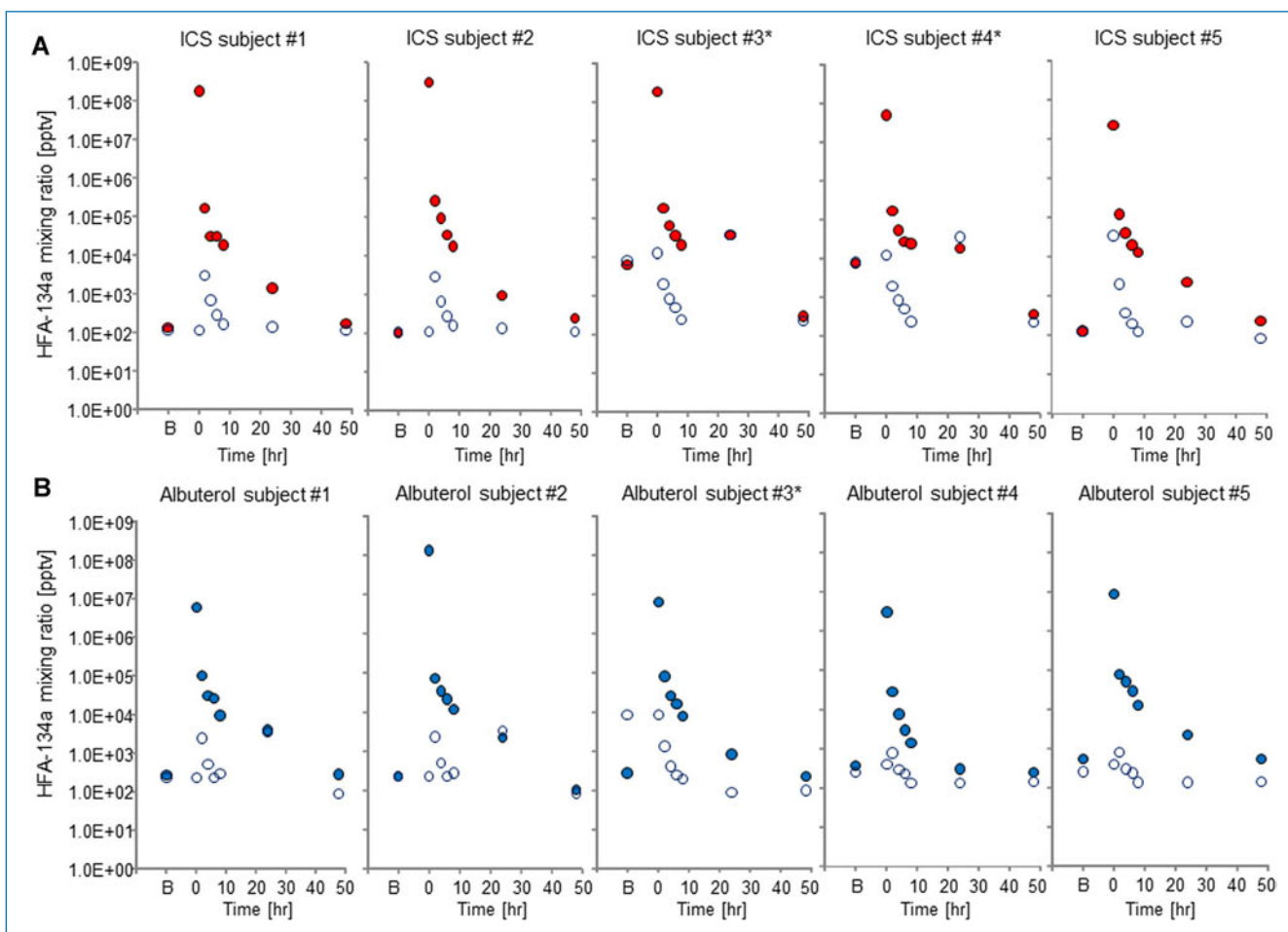


Figure 2. HFA-134a levels (mixing ratios on log scale) measured over a 48 hours period in the exhaled breath of healthy subjects who took (A) Flovent HFA (in red) and (B) Proventil HFA (in blue). Closed circles represent exhaled breath HFA-134 levels and open circles represent corresponding room HFA-134a levels. *The mixing ratio of HFA-134a is the ratio of the number density of HFA-134a to the total number density of air. Or, in other words, is the ratio of the number of molecule of HFA-134a in a unit volume to the number of molecule of air in a unit volume.

at 2, 4, 6, 8, and at 24- and 48-hour posttypical asthma inhaler administration, a useful interval in the clinical setting (see Figures 2 and 3). We monitored breath carbon dioxide (CO₂) as

a reference breath gas to ensure that the gas sampled was alveolar gas. The mean (standard deviation) CO₂ level was 4.1 (0.6)% of all breath samples.

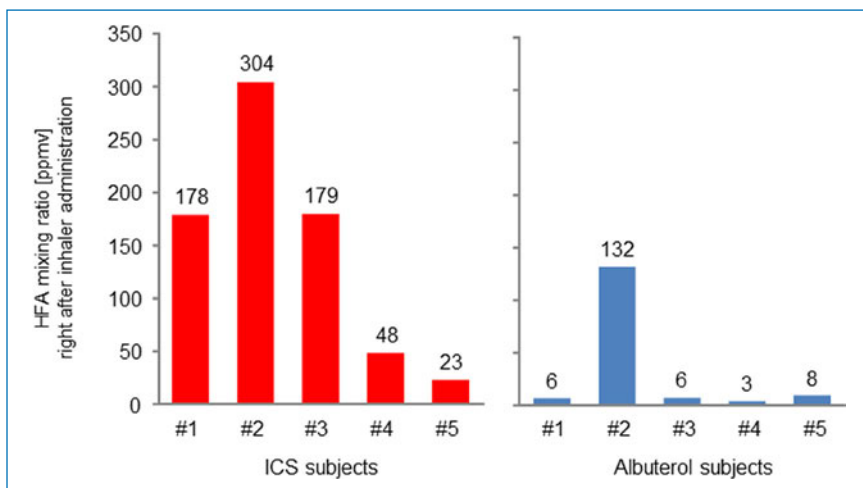


Figure 3. Individual difference of breath HFA-134a levels (or mixing ratio) measured right after a typical single administration of Flovent HFA (red bar) or Proventil HFA (blue bar).

Seven out of ten participants (ICS subjects #1, #2, and #5; Albuterol subjects #1, #2, #4, and #5 in Figure 2) showed the following trend: trace levels of exhaled breath HFA-134a at baseline in the parts per trillion by volume (pptv) range, and then significantly higher levels, approximately 10⁶ orders of magnitude (in the 3–300 part per million by volume, ppmv, range) after a typical single inhalation followed by a bi-exponential decaying pattern for at least 48 hours postinhalation. At baseline, mean (SD) levels of HFA-134a in the breath of these seven participants were 252 (156) pptv and the corresponding mean ambient HFA-134a levels in the room air were 178 (72) pptv. Exhaled breath HFA-134a levels went up to 3–300 ppmv right after inhalation (see Figure 3) and then gradually decreased back to baseline levels. At 48-hour postinhaler

| Participants taking Flovent (n = 5) | Baseline | Mean percentage change from baseline | | | | | | |
|-------------------------------------|----------|--------------------------------------|---------|---------|---------|---------|----------|----------|
| | | Immediately after | 2 hours | 4 hours | 6 hours | 8 hours | 24 hours | 48 hours |
| FEV ₁ (liter) | 3.40 | -0.56 | 1.32 | 1.96 | 1.86 | 0.96 | 0.62 | 0.78 |
| FEV ₁ /FVC, ratio | 0.84 | -1.68 | 0.66 | 0.96 | 1.78 | 1.08 | 0.24 | -0.26 |
| FEF ₂₅₋₇₅ liter/second | 3.68 | -5.68 | 1.3 | 3.1 | 6.14 | 5.62 | -3.5 | -4.82 |

| Participants taking Proventil (n = 5) | Baseline | Mean percentage change from baseline | | | | | | |
|---------------------------------------|----------|--------------------------------------|---------|---------|---------|---------|----------|----------|
| | | Immediately after | 2 hours | 4 hours | 6 hours | 8 hours | 24 hours | 48 hours |
| FEV ₁ , liter (%) | 3.46 | 3.18 | 3.74 | 2.42 | 0.44 | 0.36 | -0.4 | -0.78 |
| FEV ₁ /FVC, ratio (%) | 0.79 | 4.72 | 4.16 | 3.72 | 2.42 | 0.62 | 1.34 | 1.38 |
| FEF ₂₅₋₇₅ liter/second (%) | 3.15 | 19.94 | 13.82 | 11.74 | 5.994 | 3.62 | 3.3 | 2.9 |

Table 2. Summary of pulmonary function test.

administration, mean (SD) levels of HFA-134a in the exhaled breath were 260 (138) pptv. Corresponding HFA-134a levels in the ambient room air at 48-hour postinhaler administration were 111 (23) pptv.

Three participants presented higher exhaled breath and/or room HFA-134a levels either at baseline or during the 24-hour time point (ICS subjects #3 and #4; Albuterol subject #3 in *Figure 2*). Precisely, we observed that the ambient room sample corresponding to the baseline time point for Albuterol subject #3 was particularly elevated (8,320 pptv; *Figure 2B*). However, breath HFA-134a time trend for this subject followed the same pattern observed for the previous seven subjects with a baseline breath HFA-134a value of 274 pptv, a right after medication level of 6.2 ppmv and a 48-hour postmedication level of 224 pptv. The remaining two subjects (ICS subjects #3 and #4 in *Figure 2*) showed particularly elevated levels of HFA-134a in the breath at baseline with 6350 and 7360 pptv, respectively (the corresponding room samples were elevated as well). However, for these two subjects the breath HFA-134a value reached right after drug administration was consistent with the general trend of the other subjects (179 and 48 ppmv, respectively) as well as the 48-hour time point (287 and 351 pptv).

As shown in *Table 2*, at baseline, mean (SD) of FEV₁ (liter), FEV₁/FVC (ratio), and FEF₂₅₋₇₅ (liter/s) were 3.40 (0.89) and 3.46 (1.02); 0.84 (0.05) and 0.79 (0.05); 3.68 (1.05) and 3.15 (0.42) for Flovent HFA group and for Proventil HFA group, respectively. Mean (SD) of FEV₁ and FEF₂₅₋₇₅ percent predicted at baseline were 95 (4)% and 93 (15)%; 92(12) %, and 89(9) % for Flovent HFA group and for Proventil HFA group, respectively. FEV₁ and FEV₁/FVC did not change significantly over time for both Flovent HFA group and Proventil HFA group while FEF₂₅₋₇₅, an index of small airways obstruction changed more than 10% for both Flovent HFA group and Proventil HFA group. The percent change of FEF₂₅₋₇₅ from baseline is presented in *Figure 4*.

Discussion

To our knowledge, this study is the first that explores a novel, noninvasive way to evaluate inhaler use by measuring in the exhaled breath the biologically inactive aerosol propellant HFA-134a present in asthma medications. Our study demonstrated that we can successfully measure propellant HFA-134a levels in

the human breath as low as parts per trillion for at least 48 hours after a typical single asthma inhaler administration.

The fate of propellant HFA-134a after the sustained exposure had previously been studied and it has been reported that HFA-134a is mainly eliminated by exhalation within the first few minutes after administration.^{19,20} Gunnare et al.,^{19,20} followed both plasma and breath HFA-134a levels from participants who were exposed in 500 ppm of HFA-134a for 2 hours. His study revealed that plasma HFA-134a concentration rises rapidly right after the 500 ppm of HFA-134a exposure, and maintained high levels during 2 hours of continued exposure period. The exhaled breath HFA-134a decreased almost an order of magnitude faster than HFA-134a in plasma when exposure stopped, and was not-detectable the day after the exposure. The rapid elimination of HFA-134a in the breath was also proved by measuring body retention rate using ¹⁸F-labeled HFA-134a.^{21,22} Pike et al.²¹ demonstrated that the ¹⁸F labeled HFA-134a was rapidly eliminated by ventilation during the first few minutes, and the body retention of remaining ¹⁸F-HFA-134a was below 10–15% at 5–10 minute from exposure from both healthy control subjects and patients with chronic airflow limitation. Furthermore, these studies revealed that ¹⁸F-HFA-134a was distributed throughout the body with no obvious accumulation in any specific region; and was not metabolized even after repeated dosing.^{21,22}

HFA-134a is an inert gas, and the only metabolite originating from HFA-134a was trifluoroacetic acid. However, trifluoroacetic acid was only detected in some human urine samples at trace levels (i.e., less than 0.0005% of the administered dose).²³ Harrison et al., also reported that HFA-134a levels in blood samples decreased to below 10% of the initially administered concentration, and thus demonstrated that the removal of HFA-134a from the blood was rapid.²⁴

This study is not only consistent with the previous findings but also demonstrates the ability of detecting HFA-134a levels in the human breath in concentrations as low as parts per trillion. At baseline (before inhaler use) and at 48 hours postinhaler administration, breath HFA-134a levels were similar to the room HFA-134a levels (approximately 200 pptv). After two puffs of asthma inhaler administration, the breath HFA-134a levels varied between 3 and 300 ppmv (see *Figure 3*). This wide range of breath HFA-134a concentrations measured right after

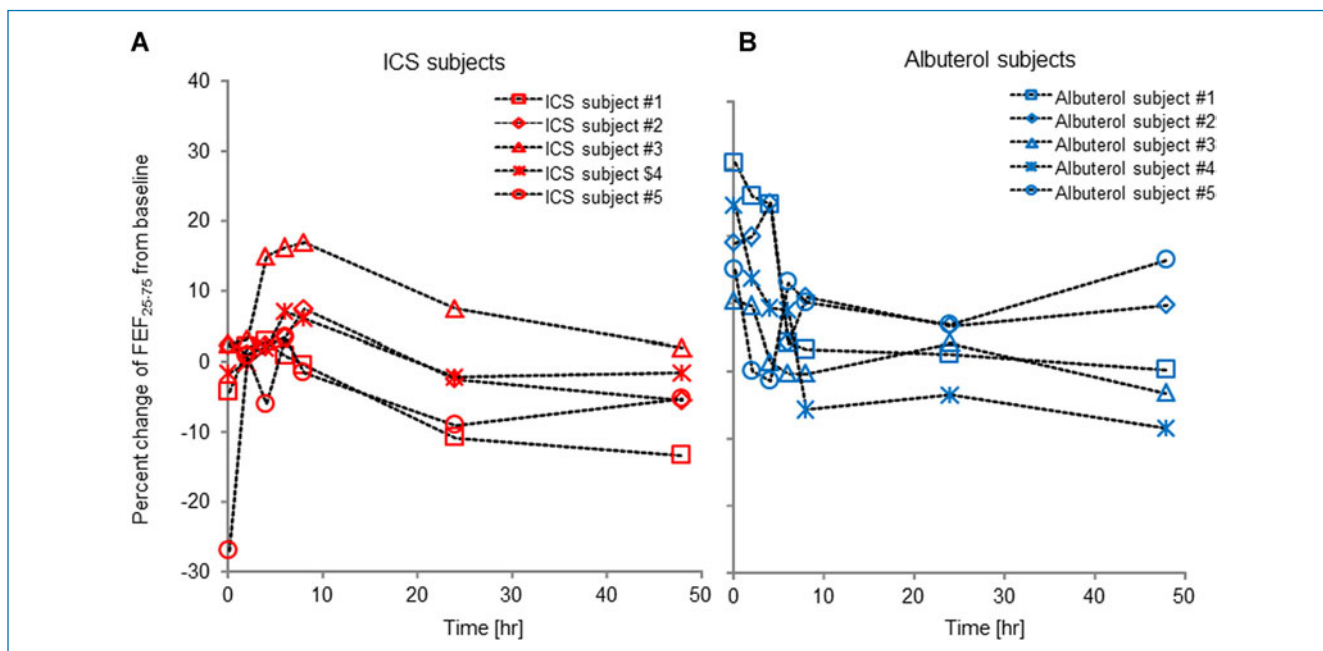


Figure 4. The percent change of FEF₂₅₋₇₅ from baseline after (A) Flovent HFA and (B) Proventil HFA administration.

inhalation may reflect individual differences in the ability of human beings to coordinate the complex maneuver required for successful inhalation. Additionally, there may be individual subject differences in HFA-134a transport and elimination in the airway mucosa, and/or dynamic changes of HFA-134a elimination that occur relatively in the early phases of HFA washout.

In many previous studies, investigators fail to measure the ambient levels of the target VOC. The importance of this was illustrated in this study. For all the participants, the 2-hour ambient HFA-134a concentration was always high. The room HFA-134a levels measured at baseline was between 109 and 254 pptv, consistent to the average level observed in the atmosphere. However, the HFA-134a levels in the room at the 2-hour time point ranged between 780 and 2960 pptv. Two different factors could be contributing to this room HFA-134a level enhancement, the first being the involuntary release of traces of HFA-134a inhalers in the room during the patient's drug administration. Additionally, the room air sample for the 2-hour time point could be affected by the extremely elevated levels of HFA-134a that the subject was exhaling in the exam room at this time point (28,000-266,000 pptv), and that have been exhaled when the asthma inhalers was administered 2 hours prior (3-300 ppmv).

The high levels of HFA-134a in the room samples measured for the baseline time point for Albuterol subject #3 could be the result of an unexpected HFA-134a emission in the exam room. Ambient levels decreased gradually for the subsequent time point reaching average ambient levels approximately at the 6-hour time point (Figure 2). Finally, we note that high HFA-134a levels were measured for both breath and room samples for the baseline time point of ICS subjects #3 and #4 (Figure 2A).

As shown in Figure 4, FEV₁ and FEV₁ over forced vital capacity (FEV₁/FVC) did not change significantly over time following inhaler administration for all 10 healthy control participants. However, remarkably, we observed a substantial effect of both Flovent HFA and Proventil HFA on FEF₂₅₋₇₅ in these control

participants. FEF₂₅₋₇₅ is an average forced expiratory flow during the mid (25-75%) portion of the forced vital capacity, and the reduction in FEF₂₅₋₇₅ indicates a possible obstructive defect in small airways. The current study demonstrated that the short-acting bronchodilator (Proventil HFA) usage improves small airway obstruction or FEF₂₅₋₇₅ immediately after administration; while ICS usage (Flovent HFA) shows delayed responses, with an improved small airway obstruction recorded 4-8 hours after the Flovent HFA administration.

Conclusion

This study demonstrated that (1) HFA-134a can be measured in the exhaled breath of healthy participants down to part per trillion levels; and (2) the HFA may be detectable above ambient levels for up to 24 hours following an inhalation. Breath HFA-134a is a promising biomarker that could be used to determine inhaler medication compliance and/or as a tool to teach patients optimal ways to use the inhalers. However, the concept of breath HFA-134a for monitoring asthma compliance is new and thus, most of the pharmacokinetic fundamentals are simply unknown including a link between breath HFA-134a levels to the blood levels of active drug (albuterol or ICS). In future studies, if a link between exhaled breath HFA to actual circulating medication levels is discovered, then it might be possible to work with pharma to use the two different HFA, HFA-134a and HFA-227, to better distinguish ICS from β -2 agonists.

Acknowledgment

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References

- Bloom B, Cohen RA, Freeman G. Summary health statistics for U.S. children: National Health Interview Survey, 2011. *Vital Health Stat.* 2012; 254: 1–88.
- Schiller JS, Lucas JW, Peregoy JA. Summary health statistics for U.S. adults: National Health Interview Survey, 2011. *Vital Health Stat.* 2012; 256: 1–218.
- National Hospital Ambulatory Medical Care Survey: 2010 Emergency Department Summary Tables Table 12 Centers for Disease Control and Prevention. 2013.
- National Hospital Discharge Survey: 2010 table, average length of stay and days of care Centers for Disease Control and Prevention. 2013.
- Hawkins G, McMahon AD, Twaddle S, Wood SF, Ford I, Thomson NC. Stepping down inhaled corticosteroids in asthma: randomised controlled trial. *BMJ* 2003; 326(7399): 1115–1120.
- Pedersen S, O'Byrne P. A comparison of the efficacy and safety of inhaled corticosteroids in asthma. *Allergy* 1997; 52(39 Suppl): 1–34.
- Apter AJ, Wang X, Bogen DK, Rand CS, McElligott S, Polsky D, Gonzalez R, Priolo C, Adam B, Geer S, et al. Problem solving to improve adherence and asthma outcomes in urban adults with moderate or severe asthma: a randomized controlled trial. *J Allergy Clin Immunol.* 2011; 128(3): 516–523.
- Bender B, Milgrom H, Rand C. Nonadherence in asthmatic patients: is there a solution to the problem? *Ann Allergy Asthma Immunol.* 1997; 79(3): 177–185.
- Onyirimba F, Apter A, Reisine S, Litt M, McCusker C, Connors M, ZuWallack R. Direct clinician-to-patient feedback discussion of inhaled steroid use: its effect on adherence. *Ann Allergy Asthma Immunol.* 2003; 90(4): 411–415.
- Gamble J, Stevenson M, McClean E, Heaney LG. The prevalence of nonadherence in difficult asthma. *Am J Respir Crit Care Med.* 2009; 180(9): 817–822.
- Murphy AC, Proeschal A, Brightling CE, Wardlaw AJ, Pavord I, Bradding P, Green RH. The relationship between clinical outcomes and medication adherence in difficult-to-control asthma. *Thorax* 2012; 67(8): 751–753.
- Suissa S, Ernst P, Kezouh A. Regular use of inhaled corticosteroids and the long term prevention of hospitalisation for asthma. *Thorax* 2002; 57(10): 880–884.
- Williams LK, Peterson EL, Wells K, Ahmedani BK, Kumar R, Burchard EG, Chowdhry VK, Favro D, Lanfear DE, Pladevall M. Quantifying the proportion of severe asthma exacerbations attributable to inhaled corticosteroid nonadherence. *J Allergy Clin Immunol.* 2011; 128(6): 1185–1191.
- Patel M, Perrin K, Pritchard A, Williams M, Wijesinghe M, Weatherall M, Beasley R. Accuracy of patient self-report as a measure of inhaled asthma medication use. *Respirology.* 2013; 18(3): 546–552.
- Chan AH, Reddel HK, Apter A, Eakin M, Riekert K, Foster JM. Adherence monitoring and e-health: how clinicians and researchers can use technology to promote inhaler adherence for asthma. *J Allergy Clin Immunol Pract.* 2013; 1(5): 446–454.
- George J, Abramson MJ, Walker SP. Asthma in pregnancy: are inhaled corticosteroids safe? *Am J Respir Crit Care Med.* 2012; 185(5): 476–478.
- Tegethoff M, Greene N, Olsen J, Schaffner E, Meinschmidt G. Inhaled glucocorticoids during pregnancy and offspring pediatric diseases: a national cohort study. *Am J Respir Crit Care Med.* 2012; 185(5): 557–563.
- Colman JJ, Swanson AL, Meinardi S, Sive BC, Blake DR, Rowland FS. Description of the analysis of a wide range of volatile organic compounds in whole air samples collected during PEM-tropics A and B. *Anal Chem* 2001; 73(15): 3723–3731.
- Gunnare S, Ernstgard L, Sjogren B, Johanson G. Toxicokinetics of 1,1,1,2-tetrafluoroethane (HFC-134a) in male volunteers after experimental exposure. *Toxicol Lett.* 2006; 167(1): 54–65.
- Gunnare S, Ernstgard L, Sjogren B, Johanson G. Experimental exposure to 1,1,1-trifluoroethane (HFC-143a): uptake, disposition and acute effects in male volunteers. *Toxicol Lett.* 2007; 172(3):120–130.
- Pike VW, Aigbirhio FI, Freemantle CA, Page BC, Rhodes CG, Waters SL, Jones T, Olsson P, Ventresca GP, Tanner RJ, et al. Disposition of inhaled 1,1,1,2-tetrafluoroethane (HFA134A) in healthy subjects and in patients with chronic airflow limitation. Measurement by 18F-labeling and whole-body gamma-counting. *Drug Metab Dispos.* 1995; 23(8): 832–839.
- Ventresca GP. Clinical pharmacology of HFA134a. *J Aerosol Med.* 1995; 8(Suppl 1): S35–S39.
- Monte SY, Ismail I, Mallett DN, Matthews C, Tanner RJ. The minimal metabolism of inhaled 1,1,1,2-tetrafluoroethane to trifluoroacetic acid in man as determined by high sensitivity 19F nuclear magnetic resonance spectroscopy of urine samples. *J Pharm Biomed Anal.* 1994; 12(12): 1489–1493.
- Harrison LI, Donnell D, Simmons JL, Ekholm BP, Cooper KM, Wyld PJ. Twenty-eight-day double-blind safety study of an HFA-134a inhalation aerosol system in healthy subjects. *J Pharm Pharmacol.* 1996; 48(6): 596–600.