Hypertonic saline has a prolonged effect on mucociliary clearance in adults with cystic fibrosis

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Abstract

Background: Inhaled hypertonic saline (HS) has been shown to increase mucociliary clearance (MCC) and improve clinical outcomes in adults and adolescents with cystic fibrosis (CF). However, in younger children with CF, a large study failed to demonstrate clinical benefits. This discrepancy could reflect pharmacodynamic differences in the MCC response to HS in different populations. We previously demonstrated the absence of a sustained effect of HS on MCC in healthy adults and in this study sought to characterize the durability of the MCC response to HS in adults with CF.
Methods: At two study sites, MCC was measured in CF adults using gamma scintigraphy during three separate visits: at baseline, 15 min, and 4 h after a single dose of HS (7% NaCl, 4 mL). Particle clearance rates at these visits were used to assess the durability of the MCC response to HS.
Results: The average 90-minute clearance rate measured 4 h after HS was significantly increased (21.81% ± 12.8) when compared to baseline (13.77% ± 8.7, $p = .048$) and showed no apparent slowing relative to the rate measured 15 min after HS. While not all subjects responded to HS, the acute response strongly predicted the sustained effect in these subjects ($r = 0.896$, $p < .0001$).
Conclusions: These results suggest that, in contrast to healthy adults, a single dose of HS has a prolonged effect on MCC in adults with CF, which lasts at least 4 h. This may explain its clinical efficacy in this population.

Keywords: Hypertonic saline; Mucociliary clearance; Airway hydration; Pharmacokinetics

1. Introduction

Dehydration of airway secretions is believed to be a critical step in the pathogenesis of cystic fibrosis (CF) lung disease [1]. Impaired anion conductance through the cystic fibrosis transmembrane conductance regulator (CFTR) and accelerated sodium transport through the epithelial sodium channel (ENaC) cause dehydration of airway secretions, alter mucus rheological properties and impair mucus clearance [2–5]. Poor mucus clearance, in turn, promotes the development of chronic infection, inflammation, and progressive airways destruction [6]. Therapies such as hypertonic saline (HS) that increase the hydration of airway secretions are expected to improve mucociliary

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clearance (MCC) and, thereby, yield clinical benefits. Numerous studies have indeed demonstrated that HS acutely accelerates MCC in healthy and diseased individuals, including those with CF [7–10]. A year-long, placebo-controlled clinical trial of HS in CF patients by Elkins et al. demonstrated improved lung function, a marked reduction in exacerbation frequency and reduced requirement for antibiotic interventions [11]. As a result, approximately 70% of patients over 6 years of age currently use this therapy [12].

The efficacy of HS in older CF patients has also driven usage in younger populations. One third of patients under six years of age were prescribed HS in 2015 [12]. This practice has evolved despite a large, placebo controlled trial by Rosenfeld et al. that showed no improvement in clinical outcomes after one year of treatment in young CF children [13]. Interestingly, Laube et al. also found, on average, no significant improvement in MCC immediately after HS inhalation in young CF subjects (7–14 years) who had normal lung function [14]. Together, these data suggest that the physiologic response to treatment with HS may vary with the stage of disease and impact its resulting clinical efficacy.

Unfortunately, our understanding of HS effects on airway physiology is incomplete. In vitro studies using normal, human bronchial epithelial cultures suggested that HS has only a transient effect on airway surface liquid volume [7, 15]. Studies using CF epithelia yielded mixed results which depended upon the experimental conditions [7, 16, 17]. Previous in vivo measurements of MCC in adult CF subjects using gamma scintigraphy demonstrated that HS acutely accelerated clearance [7, 9, 10] and led to a sustained improvement (>8 h) in MCC after 2 weeks of repetitive treatment [7]. In contrast, in vivo measurement of MCC after HS in healthy individuals revealed that a single dose of HS only transiently increased mucus clearance, and in fact led to a significant, paradoxical slowing when measured 4 h post-dose [18]. These studies leave unanswered whether a single dose of HS may have a prolonged effect on MCC in adults with CF, in contrast to the data obtained in healthy individuals, or whether repeated doses are required to achieve a sustained improvement in MCC. A durable MCC response after a single dose of HS in adults with CF could explain the sustained effect seen with repetitive HS treatment, and might also provide a mechanistic framework to understand the differential clinical effects of HS in other disease populations, including young children with CF.

2. Methods

2.1. Study design & subjects

We conducted a two-center, randomized, open-label, cross-over study to measure the durability of the MCC response to a single dose of HS in individuals with CF at the University of North Carolina at Chapel Hill (UNC) and at Johns Hopkins University (JHU). Subjects were eligible for enrollment if they were ≥18 years of age and had confirmed, mild-to-moderate CF-related lung disease, defined by forced expiratory volume in one second (FEV_1) > 50% of predicted ($ppFEV_1$). Subjects were excluded if they were pregnant, had unstable disease as defined by an absolute decline in $ppFEV_1$ of >15% over the prior six months, demonstrated radiographic findings not considered part of the usual progression of CF-related lung disease, or had a change in symptoms or medical regimen in the preceding two weeks.

All subjects completed three separate MCC studies performed at baseline, 15 min after a dose of nebulized HS (7% NaCl, 4 mL, via PARI LC Star® nebulizer with PRONEB® Ultra Compressor), and 4 h after HS. Following enrollment, subjects underwent baseline MCC study and then were randomized to an order for which to complete the 15-minute and 4-hour studies. Each study was performed at least three but not >21 days apart. Prior to each study visit, subjects withheld HS and dornase alfa for at least 3 days. Long and short-acting bronchodilators were withheld before each study for 12 and 6 h, respectively. Subjects underwent spirometry testing following 2 puffs of albuterol from a metered dose inhaler on each study day prior to administration of HS (prior to MCC measurement on baseline study days), which served as a verification of the subject's clinical stability as well as pre-treatment prior to HS administration.

Informed consent was obtained from each subject prior to enrollment. The study was reviewed and approved by the institutional review boards at UNC and JHU. The study was registered on ClinicalTrials.gov (NCT01094704).

2.2. Measurement of MCC rate

During each study visit, subjects inhaled radiolabeled particles (Tc^{99m}-sulfur colloid) using a standard operating procedure at the two centers, followed by gamma scintigraphy, with only minor procedural variations [19]. In procedures performed at UNC, two fiducial markers containing Am^{241} (0.9 μCi each) were placed over the subject's spine at approximately C7 and L1 to facilitate alignment of the serial images obtained during the MCC scan, and a Co^{57} planar source was used to obtain a transmission image to define lung borders and regions of interest (ROI). At Johns Hopkins University, a low-dose external source of Tc99m was briefly held at the sternal notch of the patient before the start of each imaging procedure to assist with alignment of the subject with a reference mark on the computer screen. In place of a transmission scan, a Xe^{133} gas equilibrium scan, obtained by rebreathing 1–2 mCi of Xe^{133} through a Pulmonex system (Atomic Products, Shirley, NY) until equilibrium was achieved, was used to identify lung borders and define ROIs.

Radiolabeled sulfur colloid particles suspended in 0.9% saline were aerosolized with a jet nebulizer (DeVilbiss® Model 646; Mass Median Aerodynamic Diameter: 5 μm). In order to standardize particle deposition, subjects breathed in time to a metronome (1 beat/s) to maintain a 1-second inspiratory time, and used visual feedback to maintain an inspiratory flow rate of 0.5 L/s, as described previously [19]. Approximately 40 μCi was deposited in the lung over 2–3 min using real time monitoring. Immediately following isotope inhalation, subjects gargled and swallowed water to clear retained isotope from the oropharynx. Gamma emission images from the lungs were recorded serially over the ensuing 94 min using two-minute acquisitions. After an initial 64 min of imaging, subjects were asked to perform a series of 20 coughs every 10 min (60 coughs

total). Subjects returned the following day for a 30-minute image to assess particle retention after 24 h.

2.3. Image analysis and clearance quantification

Images obtained from both sites were centrally analyzed at UNC. The Cobalt transmission (UNC) and Xenon gas equilibrium (JHU) scans were used to identify and outline the lung fields, as previously described [18, 19]. Because swallowed particles in the stomach overlying the lower left lung field confound analysis of clearance from this lung region, only the right lung field was analyzed. A whole lung rectangular region of interest (ROI) was created for the entire right lung field using either the transmission or gas equilibrium scan from each patient [20]. The total gamma activity in the ROI was measured, correcting for isotope decay and background activity, and plotted as a fraction of the initial activity measured between 0–4 min post-inhalation. Percent clearance of isotope, defined by 100 × (1 − retention), was calculated at 10-minute intervals. The total percent clearance through 24 h was measured by acquiring a single 30-minute image at this time point and performing appropriate decay- and background-corrections. Average isotope clearance through 90 min (Ave90Clr), which incorporates both cilia- and cough-driven clearance, was the pre-defined primary outcome measure. Ave90Clr was calculated by averaging the nine clearance measurements made at 10-minute intervals. This single parameter, therefore, utilizes all data points to describe the entire clearance curve, and is directly proportional to the area under the clearance curve. Secondary outcome measures included average isotope clearance through 60 min (Ave60Clr, representing predominantly cilia-driven clearance), isotope clearance between 60 to 90 min while voluntary coughs were being performed (Ave60-90Clr, representing cough and cilia-driven clearance), and 24-hour clearance.

The heterogeneity of particle deposition was described by the statistical measure of skew, a unit-less measure of the asymmetry of a probability distribution. The skew of the deposition histogram in the whole lung ROI was obtained from the initial gamma scintigraphic image and was calculated in order to compare particle deposition at different visits as a potential covariate that might explain changes in MCC rates. Skew was used in favor of the more commonly used central to peripheral ROI (C/P) activity ratio as the primary description of deposition because the methods used to normalize C/P to lung thickness at the two centers (Cobalt transmission vs Xenon equilibrium scans) yields different results and cannot be directly compared [20]. Higher skew values typically indicate foci of greater signal intensity in large airways, and, like C/P, have been shown to correlate with accelerated particle clearance [21–23].

2.4. Statistical analyses

The predefined primary analysis was a comparison of whole-lung Ave90Clr at baseline and 4 h post-HS treatment using a two-tailed Wilcoxon matched-pairs signed rank test to determine whether HS had a prolonged effect on MCC. Secondary analyses included similar comparisons of skew, Ave60Clr, Ave60-90Clr, and 24-hour clearance at the different visits (baseline, 15 min, and 4 h after HS). The correlation (using Spearman's method) between the acute ($Ave90Clr_{15min} - Ave90Clr_{baseline}$) and sustained ($Ave90Clr_{4h} - Ave90Clr_{baseline}$) effect of HS on MCC within each subject was calculated to investigate this relationship. The correlation between mucociliary clearance (Ave90Clr) and particle deposition pattern (skew) at each visit was calculated in a similar fashion to examine deposition-clearance relationships. Finally, to determine whether any observed changes in mucociliary clearance could be explained by alterations in deposition, the correlation between the change in clearance versus the change in deposition skew was also calculated.

3. Results

3.1. Subjects

Fourteen subjects were recruited from the two centers: four from JHU and ten from UNC. One subject was excluded from data analysis as an outlier after being found to have an extreme difference in isotope deposition at both post-HS studies (skew > 4 standard deviations from the overall mean value), which precluded meaningful interpretation of this subject's MCC data. Importantly, inclusion of this subject would have created a bias that favored our hypothesis and final conclusions, and exclusion of these data did not change the statistical significance of our data. Subject characteristics of the remaining 13 subjects are described in Table 1.

Of note, ten subjects (77%) were being treated with HS at enrollment, of which seven (54%) were also being treated with dornase alfa. One subject was being treated with ivacaftor/lumacaftor at the time of enrollment and was continued throughout the study.

3.2. Mucociliary clearance and cough clearance

Fig. 1 shows the mean particle clearance curves of inhaled radiotracer particles through 90 min at each study visit. As shown, particle clearance 4 h after HS is faster than baseline, with no indication of slowing relative to the clearance curve obtained 15 min after HS inhalation. In the primary analysis, Ave90Clr was significantly increased 4 h after HS treatment (21.81% ± 12.8), compared to baseline (13.77% ± 8.7) ($p = .048$, Fig. 2). Other MCC parameters, including Ave60Clr and 24-h clearance were also increased 4 h after HS inhalation, further supporting the primary outcome (Table 2). Importantly, there were no differences in the number spontaneous coughs (average ranging from 6.2 at baseline to 2.9 at the 4-h visit) observed across the treatment periods that would explain these effects, and clearance during the cough period was not different from baseline. Detailed MCC results for individual subjects are included in Supplementary Table 1.

Although the pattern of MCC acceleration was quantitatively similar when measured 15 min after baseline, the change from baseline was not statistically significant. However, most striking was the very strong correlation between the acute and sustained effect of HS (post-treatment minus baseline) in individual subjects

Table 1
Subject characteristics.

	Subject	Age	Sex	Genotype	Baseline FEV_1	CFRD	Prescribed HS	Prescribed dornase	Chronic pseudomonas
JHU	1	38	F	F508del/3849 + 10 kb C > T	75%	No	Yes	Yes	Yes
	2	24	M	F508del/F508del	70%	No	Yes	Yes	Yes
	3	18	M	F508del/H939R	94%	No	No	No	No
UNC	4	36	F	F508del/F508del	64%	Yes	Yes	Yes	Yes
	5	37	F	F508del/2789 + 5G > A	85%	No	No	No	No
	6	22	F	F508del/F508del	107%	No	No	No	Yes
	7	28	M	F508del/F508del	70%	No	Yes	No	Yes
	8	28	F	F508del/F508del	79%	Yes	Yes	Yes	Yes
	9	22	M	F508del/F508del[a]	77%	No	Yes	Yes	Yes
	10	32	M	F508del/F508del	72%	No	Yes	No	No
	11	38	M	F508del/R347P	71%	No	Yes	Yes	No
	12	30	F	F508del/Y913X	72%	No	Yes	Yes	Yes
	13	55	F	2789 + 5G > A/2789 + 5G > A	74%	No	Yes	No	Yes

[a] Indicates patient being treated with lumacaftor/ivacaftor.

($r = 0.896$, $p < .0001$, Fig. 3A and B). This tight correlation demonstrates the repeatability of the MCC assay and reveals that the acute response to HS accurately predicted a prolonged pharmacodynamic benefit. Finally, this suggests that the trend toward more skewed particle deposition seen only at the 4-hour post-HS scan did not drive the sustained acceleration of MCC.

3.3. Particle deposition

Across all subjects, skew significantly correlated with Ave90Clr at baseline and 15 min post-HS, but did not reach significance at 4 h post-HS ($r = -0.72$ at baseline [$p = .007$], -0.63 at 15 min [$p = .025$], and -0.54 at 4 h [$p = .058$]). These data confirm the relationship between initial particle deposition and MCC rates. Importantly, no significant difference in mean skew values was observed between the three study visits (Table 2). Nevertheless, we also explored whether within subject changes in particle deposition skew could explain faster MCC rates 4 h after HS inhalation. The absence of a significant correlation between changes in skew and AveClr90 ($r = 0.264$, $p = .384$), suggests that the observed prolonged improvement in MCC was not attributable to changes in the heterogeneity of particle deposition. Although skew was used as the primary measure of initial particle deposition, for reasons described above, C/P values were calculated and are reported in Supplementary Table 1. A significant increase in C/P was observed at 4 h compared to baseline ($p = .004$), although no difference was observed at 15 min ($p = .376$).

4. Discussion

Our data suggest that, in contrast to healthy individuals [18], a single dose of HS in adults with CF improves mucus clearance with an effect that, on average, lasts at least 4 h. Although the observed acute response to HS did not reach statistical significance in this small study, and the size of the acute response to HS is quantitatively consistent with that reported in a prior study (Ave60Clr in prior study vs current study: Baseline 9.3 ± 1.1% vs 10.5 ± 2.1%, acute HS 17.2 ± 2.9% vs 16.2 ± 3.1%) [7]. Furthermore, the prolonged effect of a single dose of HS we observed here may explain the >8 h acceleration in MCC that was previously observed after repeated HS use (Ave60Clr measured

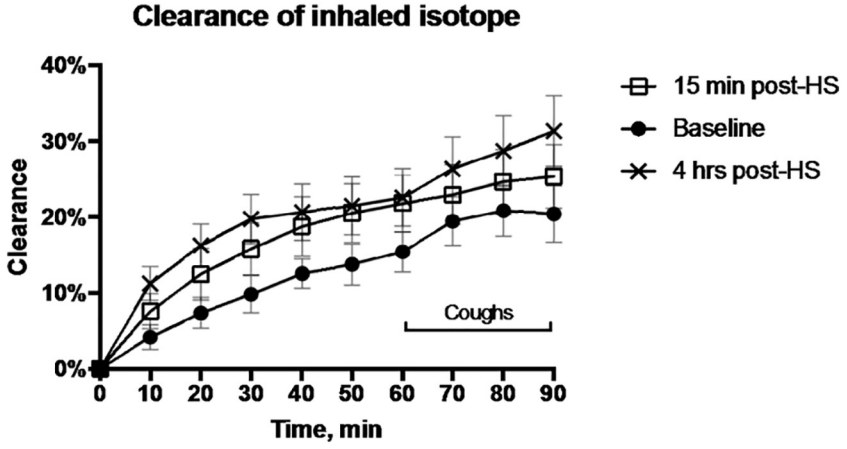

Fig. 1. Curves demonstrating average isotope clearance across all subjects versus time (±SEM) from the whole lung compartment at each visit. The first 60 min represents cilia-driven clearance, whereas cough-assisted clearance is assessed between 60 and 90 min after isotope inhalation.

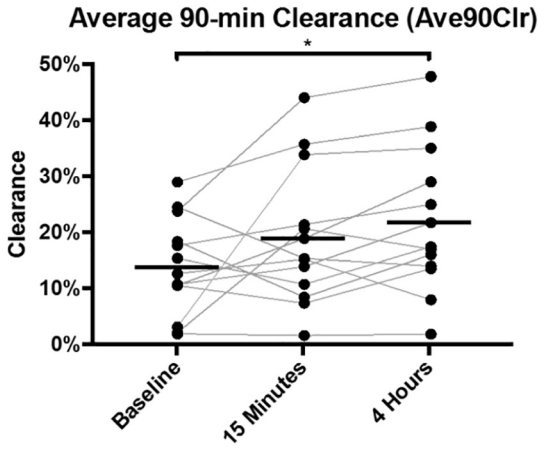

Fig. 2. A plot showing individual average 90-minute clearance (Ave90Clr) in the whole right lung compartment at baseline, 15 min, and 4 h after inhaling HS. Individual responses are connected with grey lines. In a paired analysis, Ave90Clr was significantly faster at 4 h compared to baseline (p = .048).

8–12 h after HS in prior study: 14.1 ± 1.1%, vs. 18.7 ± 3.2% 4 h after HS in current study). These data support the hypothesis that the clinical benefits of HS in individuals with established CF-related lung disease results from a prolonged effect on mucus transport, rather than repeated short-duration effects. Interestingly, the substantial impact of pulmonary exacerbations observed by Elkins et al. occurred despite adherence to twice daily dosing that ranged from only 60–72% (Supplementary appendix) [11]. This, again, supports the concept that fairly infrequent HS dosing is beneficial because of its durable effect on MCC.

In light of these data, the lack of apparent clinical benefit in young children with CF raises the question whether the pharmacodynamic response to HS in this population is more similar to short duration effect we previously observed in healthy individuals [18]. This conjecture is supported by findings from Laube et al. [14], where acute HS effects were not robust in relatively healthy CF children with normal lung function, although sustained effects were not assessed. The link between a prolonged physiologic effect of HS in CF adults and clinical improvements also suggests that novel hydrator agents in development should strive to achieve similarly prolonged effects on MCC. Our data provide a reference comparison for these investigational therapies, while emphasizing that selection of the study population, such as age or severity of disease, may have profound effects on study outcomes. The need for long-acting non-osmotic hydrators, including CFTR modulators and ENaC inhibitors, is supported by the observation that individual patients and different disease phenotypes may have less robust responses to an osmotic hydrator such as HS.

The finding that a single dose of HS accelerates clearance in CF adults for more than 4 h raises questions about the underlying mechanism. It was previously proposed that the absence of the CFTR conductive pathway could potentially slow absorption of a hyperosmotic salt solution, leading to prolonged MCC effects [7]. However, the absence of clinical benefits in younger CF populations suggests that the prolonged effect of HS in CF adults might be attributable to other factors, and not as a direct result of CFTR dysfunction.

The 2-gel model of the airway surface liquid layer that has been proposed posits that the overlying, secreted mucus gel layer may absorb excess fluid [5]. In established CF lung disease, the volume of secreted airway mucus is often markedly expanded and significantly dehydrated [24]. This dehydrated mucus load may, in turn, provide a large capacity to retain water drawn into the luminal compartment by HS, while reducing its solids content and improving its transportability. In the healthy lung, and perhaps the mildly affected CF lung, a relatively sparse, well-hydrated mucus layer could eliminate the capacity to store volume driven into the airway lumen by HS, resulting in its rapid transepithelial reabsorption and a short duration effect on MCC. In this study, no correlation was found between sustained HS response and disease severity as represented by baseline FEV_1 ($r = -0.325$, $p = .276$) or baseline Ave90Clr ($r = -0.231$, $p = .448$).

Our study data also demonstrates that the response to HS in CF adults is variable, and likely reflects local differences in the airway milieu between patients. The fraction of subjects with a sustained increase in MCC after HS (9/13 with a positive response to HS; 7/13 with >5% absolute increase in Ave90Clr at the 4 h time point) is similar to what was observed in our prior study of HS in CF [7], and is also consistent with the variability in responses observed by Laube et al. [14]. Unfortunately, our study was too small to identify clinical features (such as those listed in Table 1 and referenced in Fig. 3A) that might predict the MCC response to HS, and we cannot directly link this physiologic response to clinical benefits. The identification of clinical features or biomarkers that predict a HS response (such as percent solids content of sputum or mucin concentration) could potentially

Table 2
Average whole right lung MCC parameters. The average value across all subjects is shown, ±the standard deviation. For values 15 min and 4 h post-HS treatment, the p-value of a Wilcoxon matched-pairs signed rank test is shown for within-subject comparisons to baseline values.

	Baseline	15 min post-HS	4 h post-HS
Average 90 min clearance	13.77% ± 8.7	18.90% ± 12.2 (p = .305)	21.81% ± 12.8 (p = .048)[a]
Average 60 min clearance	10.52% ± 7.6	16.17% ± 11.3 (p = .244)	18.66% ± 11.5 (p = .017)[a]
Average 60–90 min clearance	6.01% ± 7.2	3.60% ± 3.6 (p = .376)	8.65% ± 6.6 (p = .191)
24-hour clearance	26.56% ± 15.4	33.26% ± 17.4 (p = .244)	38.19% ± 19.3 (p = .048)[a]
Skew	2.21 ± 1.22	2.06 ± 1.38 (p = .588)	2.63 ± 1.28 (p = .244)

[a] Denotes a comparison with a p-value <.05.

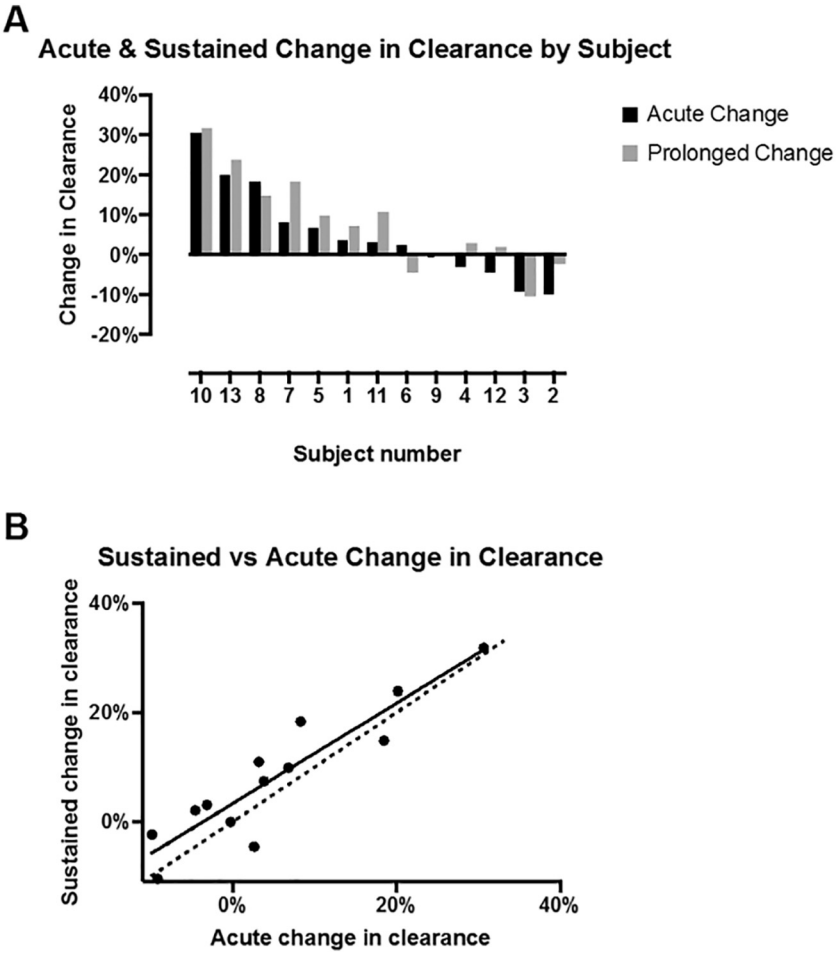

Fig. 3. A: Waterfall plot of the acute (black bars) and sustained (grey bars) change in clearance after HS in individual subjects, ordered by the acute response. B: Plot of sustained versus acute clearance showing a significant correlation ($r = 0.896$, $p < .0001$) approaching the line of identity (dotted line).

assist in the identification of individuals who will benefit from HS.

One limitation of this study was the use of different methods used to identify lung borders (Xenon equilibrium at JHU versus transmission scan at UNC) and normalize lung thickness. As result of this difference, C/P ratios could not be reliably compared across subjects. This difference led us to rely on deposition skew as the primary descriptor of particle deposition. Importantly, skew was in fact strongly correlated to particle clearance at baseline, whereas C/P was not ($r = 0.72$, $p < .01$; vs. 0.25, $p = .40$). Suggesting that it is a more reliable index of deposition with regards to the potential interaction between particle deposition and clearance. However, for the sake of completeness, paired comparisons of C/P ratios were made and revealed a significant difference in C/P between the 4-h and baseline scans. Although this could have contribute to faster clearance at the 4-h scan, we note that the quantitatively similar (but non-significant) increase in clearance 15-minute post-HS was observed without a change in C/P ($p = .38$) or skew, and the intra-subject correlation between acute and sustained HS effects were highly correlated (Fig. 3). Together with the observation that 4-h clearance from the peripheral lung region, which is relatively independent of deposition, was nearly double that at baseline (8.5% vs 4.8%; $p = .203$), adds to the evidence that a true sustained acceleration in MCC occurred. We speculate that the higher C/P value 4 h after HS may be a result of the sustained acceleration of MCC rather than driver of it, as mucus moving from the periphery into the central compartment is expected to increase central deposition due to impaction from turbulent flow.

By further delineating the physiologic effects and pharmacodynamic profiles of HS in different populations, we may be able to achieve more personalized treatment approaches that accentuate the benefits of therapies like HS while reducing the amount of ineffective use and potential side effects. Additional mechanistic studies in mildly affected CF children and other potential target populations are needed to reach this goal. In an era of multiple treatment options and high treatment burden, this would be of significant interest to patients and the CF community. The use of an integrated biomarker, such as MCC, to not only identify effective novel therapeutics, but to also help us understand the populations that are most likely to benefit has the potential to move us toward our goal of more personalized care irrespective of genotype-specific therapies.

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Author contributions

BLL, DW, WDB and SHD contributed to the study design. All authors except DW and ASC contributed to study execution. Data analysis and interpretation was performed by ATT, BLL, KLZ, JW, ASC, WDB and SHD. The manuscript was prepared by ATT, WDB and SHD, and reviewed by all authors.

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