INHALED HYPERTONIC SALINE (7%) IMPROVES THE LUNG CLEARANCE INDEX IN CF PAEDIATRIC PATIENTS WITH FEV_1% PREDICTED ≥ 80%

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A thesis submitted in conformity with the requirements for the degree of Master of Science (Clinical Epidemiology)
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University of Toronto

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Abstract

Objective: To determine if inhaled Hypertonic Saline (7%) improves the Lung Clearance Index in paediatric Cystic Fibrosis patients with FEV_1 ≥80% predicted.

Methods: In a blinded crossover trial, twenty CF patients received 4 weeks of hypertonic saline (7%) (HS) and 4 weeks of isotonic saline (0.9%) (IS) separated by a 4 week washout period. The primary endpoint was the change in LCI in the HS versus the IS treatment periods.

Results: Four weeks of twice daily inhalation of HS significantly improved the LCI as compared to IS by 1.16, 95% CI [0.26, 2.05]; p=0.016. Baseline LCI before IS, 8.71+/-2.10, was not significantly different from baseline LCI before HS inhalation, 8.84+/-1.95 (p=0.73). Randomization order had no significant impact on the treatment effect (p=0.61).

Conclusions: Four weeks of twice daily Hypertonic Saline (7%) inhalations improved the LCI and may be a suitable early intervention therapy for CF patients with mild disease.

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1. Introduction and Background

1.1 Cystic Fibrosis and the Burden of Lung Disease

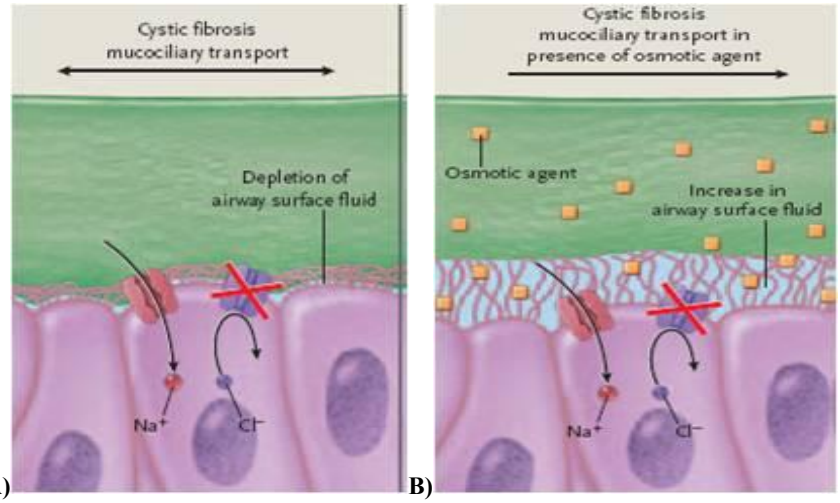
Cystic Fibrosis (CF) is one of the most prevalent fatal, autosomal recessive diseases in Caucasians that occurs in 1 in 2500 live births (1). Despite clinical advances in the past few decades, CF remains a life shortening disease with a median survival of 37 years (2).

CF is the result of a defect in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene which is expressed in the epithelium of several organs in the body including the lungs, pancreas, gastrointestinal tract, reproductive tract, skin and nasal mucosa. The lack of functional CFTR protein results in disease manifestations in organs where CFTR has relevant physiological findings. However, the pulmonary manifestations account for over 90% of the morbidity and mortality in CF patients. In the lung, a lack of CFTR activity leads to decreased chloride secretion as well as sodium hyperabsorption in airway epithelial cells (1). This results in a decreased airway surface liquid (ASL) layer which leads to collapse of respiratory cilia, impaired mucociliary clearance (MCC) and mucus retention in the lower airways (3). As a result, inhaled microorganisms cannot be efficiently cleared from the CF airway which predisposes patients to chronic bacterial infection and inflammation. Bronchiectasis develops which eventually leads to respiratory failure and premature death (1).

1.2 Hypertonic Saline

Several years ago CF physicians in Australia noted that their patients reported an improvement in pulmonary symptoms after surfing. This led researchers to study Hypertonic Saline (HS), an expectorant therapy administered by nebulisation, in CF patients. There are three hypothesized mechanisms of action for Hypertonic Saline: 1) HS induces cough which predisposes mucus to more favorable clearance; 2) HS breaks the ionic bonds within the mucus gel which lowers the viscoelastic properties of the mucus and improves MCC and 3) HS acts as an osmotic agent, restores ASL and subsequently improves MCC; (4,5,6, Figure 1). Most recent evidence would support the latter hypothesis as outlined below in Figure 1 (7).

Figure 1: A) CF airway; and B) CF airway after Hypertonic Saline inhalation



A) **B)**

Prior to 2006, smaller studies using Hypertonic Saline demonstrated promising short term benefits including improvements in MCC and lung function in CF patients (8-11). The

improvement in MCC was concentration dependent and continued to increase up to a concentration of 7% (11). Higher concentrations did not stimulate MCC further and were poorly tolerated as many of the patients complained of oropharyngeal irritation; therefore, 7% was the concentration that was used in subsequent clinical trials (11). *Donaldson et al* was the first to demonstrate the sustained effect of HS on the CF ASL volume (5). The ASL volume increased four-fold after inhaled HS (7%) in normal airways and returned to baseline within 10 minutes. In contrast, the effect lasted up to 8 hours in the CF airway (5).

In 2006, a large multi-center trial for the first time demonstrated the long term benefit of inhaled HS in CF patients with mild, moderate and severe baseline pulmonary function (6). This trial was forty-eight weeks in duration and this duration was chosen to assess the long term effects of HS as trials prior to this had not exceeded 28 days (6). Hypertonic saline was administered twice daily based on previous data from Donaldson et al that suggested that HS has effects on the CF airway that last up to eight hours, as well as an appreciation for the treatment burden associated with doing the inhalation three times a day (5). Table 1 demonstrates the demographic characteristics of the patients. The patients were clinically stable with FEV_1% predicted at the time of screening being within 10% of their baseline values and greater than forty percent predicted (6). The sample size calculation was based on a 10% change in FEV_1 % predicted which the authors assumed to be a clinically significant change. The standard deviation was based on a previous publication that recruited a similar population of CF patients for an interventional study (12).

Table 1: Demographic Characteristics of Patients Enrolled in the *Elkins et al* trial

Characteristic	**Control (n=81)**	**Hypertonic Saline (n=83)**
Age (years)	18.7 ± 9.2	18.4 ± 9.3
Female sex (%)	42	46
FEV_1% Predicted	76 ± 21, (40-127)	73 ± 21, (40-132)
FVC% Predicted	88 ± 18, (44-137)	85 ± 18, (45-127)
FEF_{25-75}% Predicted	61± 35, (10-151)	56 ±34, (11-155)

Adapted from Table 1 (6)
Mean± Standard Deviation, (Range)

Forty-eight weeks of inhaled HS (7%), improved FEV_1 as compared to control patients (6). FEV_1 increased during the first four weeks of treatment with HS but remained unchanged in the control group (6). Therefore, a treatment benefit from HS inhalation was seen after only four weeks. The improvement in lung function with HS remained unchanged after the first four weeks of therapy (6). There were fewer pulmonary exacerbations requiring intravenous antibiotic therapy in the HS group as compared to the control group; the mean number of exacerbations per participant in the control group was 0.89, as compared with 0.39 in the hypertonic saline group (difference, 0.5; 95 percent confidence interval, 0.14 to 0.86; p=0.02) (6). The role, emotional and health domains of the Cystic Fibrosis Questionnaire revised significantly improved with HS inhalation as compared to placebo for participants greater than 14 years of age. Based on the results of this study as well as the previous short term studies, Hypertonic Saline has become licensed by Health Canada for use by CF patients. At present, clinicians consider Hypertonic Saline as an intervention for their patients on a case by case basis. At present, there are no long term studies that demonstrate the effects on lung function, Quality of Life and pulmonary exacerbations once HS therapy has been discontinued.

There are some potential harmful effects of HS therapy. Given that HS is deposited in the CF airway, it could potentially alter airways microbiology and/or induce a pro-inflammatory effect. The above two hypotheses have been studied in a large multi-center trial

(6). HS therapy did not significantly alter the airways concentrations of *Pseudomonas aeruginosa*, *Staphylococcus aureus* nor the acquisition incidence of new respiratory organisms (6). In addition, pro-inflammatory cytokine levels did not appreciably change after HS therapy. However, several short term adverse effects have been reported in the literature from the inhalation of HS; to date, there have been no reported mortalities nor any serious adverse events or long term adverse events (4, 8-11, 13-21).

The short term harms include bronchospasm resulting in a transient drop in FEV_1, salty taste, cough, hemoptysis, chest tightness, pharyngitis, nausea and vomiting, sinusitis and sneezing and voice changes (4, 8-11, 13-21). Regarding pulmonary function, all acute drops in FEV_1 secondary to inhalation of HS reversed with bronchodilator treatment. Hemoptysis was reported as an adverse event in 2 trials and the development of hemoptysis did not preclude study completion in any of the patients (6, 13). Throat irritation or pharyngitis was reported in three trials; four of the seven patients withdrew from the studies because of pharyngitis (6, 13, 18). *Elkins et al* reported that 2% of the patients developed chest tightness during the inhaled Hypertonic Saline treatment period; two of the three patients with chest tightness completed the trial (6). Only two out of 419 patients have been withdrawn from clinical trial because of nausea and vomiting (6, 20). Sinusitis, sneezing and voice changes did not result in attrition (6).

HS would be an ideal early intervention strategy for CF patients as it addresses the underlying abnormality in CF, airway dehydration.Given that HS is administered by inhalation, it will be more effectively delivered to the peripheral airways in patients with milder lung disease. As CF pulmonary disease progresses, mucous plugging worsens and structural lung damage occurs thus impeding potential delivery of HS to the smaller airways. Additionally, it improves lung function in CF patients in a short period of time, has been well tolerated in clinical trials and is relatively inexpensive at a cost of $140.00 per month (5, 6, 8, 10, 11).

However, there has not yet been a clinical trial assessing the effect of inhaled HS on CF patients where all participants had a baseline $FEV_1 \geq 80\%$ predicted.

1.3 Spirometry

Spirometry provides physiological information about lung function through a series of breathing maneuvers. In general, Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC) and Forced Expiratory Flow from 25 to 75% of the vital capacity (FEF_{25-75}) all provide important functional information but FEV_1 is most commonly used in research trials as well as for the clinical management of CF patients. This is due to a combination of FEV_1 being more reproducible (coefficient of variation of less than 5% as compared to 25% for FEF_{25-75} in adults) and a better predictor of morbidity and mortality for CF patients (22, 23). Although FEV_1 had long been known to be associated with mortality, *Corey et al* published a landmark paper that confirmed this association (23). The authors reviewed the largest existing database of CF patients over a twenty year period and found that of several clinical variables, FEV_1 was the strongest predictor of mortality (23). As such, FEV_1 is the main outcome measure used in therapeutic intervention studies as well as in the daily clinical management of CF patients.

1.4 Limitations of FEV_1

In the last decade, it has been established that CF lung disease begins shortly after birth, progresses despite a lack of clinical signs and symptoms (24-27). As a result, there is an increasing emphasis on early intervention for CF lung disease (28). FEV_1 is of limited use in CF patients with mild lung disease because of its relative stability and insensitivity to

peripheral airways disease; it is also not useful in young children (less than 6 years of age) since active cooperation is required to perform the maneuver (29, 30-32). Therefore, more sensitive measures of lung function are needed for clinical studies in CF patients with early, mild disease. However, at present FEV_1 is the main measure used to guide the clinical management of CF patients with very mild lung disease.

1.5 The Lung Clearance Index

The Lung Clearance Index (LCI), a measure of ventilation inhomogeneity, is a calculated parameter determined during Multiple Breath Washout (MBW) of an inhaled inert gas mixture. The LCI is sensitive to mild peripheral airways disease, the originating site of CF lung injury (1, 29, 33-39). Therefore, mucus obstruction in the lower airways results in gas retention, prolonged gas washout and an increased LCI. A simple tidal breathing technique is used for the multiple breath washout technique; from which LCI is calculated. Given the simplicity of the maneuver it can be used in children less than six years of age. In addition unlike FEV_1, the upper limits of normal are consistent across all ages; published reference ranges are available for healthy control patients of all ages (29, 33-34, 36, 37).

FEV_1 is an established clinical surrogate of mortality for CF patients (23). Although, the LCI has yet to be linked to mortality in longitudinal studies, it has been shown to be closely linked to FEV_1 in its ability to measure airways disease. The LCI has repeatedly been shown to detect lung disease at an earlier stage than spirometry across a wide range of ages (29, 33-34, 36, 37). Further evidence of the LCI's validity comes from a study comparing the LCI and spirometry with high-resolution CT (HRCT) scanning thus bridging structural and physiological measures (38, 39). LCI was found to be the most sensitive measure as one third

of patients with a normal HRCT had an elevated LCI; a normal LCI almost precluded an abnormal HRCT (38).

The reliability of the LCI has also been studied across a wide age range. The intra-visit coefficient of variation ranges from 7.8% for preschool children with CF to 3.2% for healthy adults (34, 37). The inter-visit reproducibility is 0.6 for healthy adults (37).

The LCI is responsive to both disease progression over time as well as to an intervention (35, 40). A Swiss cohort of 142 CF children between the ages of 6 and 20 years were followed and all had a minimum of 4 spirometric and LCI measurements per year (35). The LCI became abnormal first and was more sensitive than all spirometric measures to disease progression (35). *Robinson et al* demonstrated a significant improvement in the LCI from intravenous antibiotic treatment for a pulmonary exacerbation on the LCI in CF patients with moderate to severe lung disease (40).

Therefore, the LCI is an especially promising endpoint for clinical trials in patients with mild lung disease given its superior sensitivity over conventional spirometric measures. However, data from interventional studies in patients with mild disease is currently lacking. At present, the FEV_1% predicted is used to monitor CF patients with mild lung disease in clinical practice as the LCI remains an exclusive research tool at present.

1.6 Quality of Life

Quality of Life (QOL) as defined by the World Health Organization is "a state of physical, mental and social well-being not merely the absence of disease (41). It has been previously shown in CF as well as other chronic diseases that QOL cannot be explained by clinical variables alone thus adding credence to including QOL measures in clinical trials (42).

QOL has been linked to pulmonary function and more recently there is some evidence that it may also be linked to survival (42, 43). Additionally, QOL is responsive. The CF specific QOL measure, the Cystic Fibrosis Questionnaire- Revised (CFQ-R) has demonstrated a treatment effect in three large interventional trials (5, 6, 44). The Minimum Clinically Important Difference (MCID) corresponds to the smallest clinically relevant change a patient can detect. Recently, the MCID has been determined for the Respiratory Domain of the CFQ-R using data from two clinical interventional trials (45). The MCID for a population of stable CF patients was 4.0 and was 8.5 for patients undergoing pulmonary exacerbations (45).Using the MCID for the CFQ-R provides a systematic way to identify CF treatments that improve both symptoms and physiologic variables, potentially leading to better treatment adherence and clinical outcomes (45). There has yet to be an interventional trial that has based the sample size calculation on the MCID at present, but this will likely change in the future.

2. Rationale and Relevance

CF lung disease begins shortly after birth and progresses in the absence of clinical signs and symptoms (24-27). Therefore, even in CF patients with $FEV_1 \geq 80\%$ predicted, 43% of the CF patients in our institution, the lung disease inevitably progresses. As a result, there is an increasing emphasis on early intervention strategies to prevent structural lung damage and thus improve both short and long term outcomes (28). The LCI is an ideal endpoint for early intervention trials because of its superior sensitivity to spirometry, ability to be performed by children of all ages and established measurement properties.

Hypertonic saline is a suitable early intervention therapy but has never been studied in a clinical trial in CF patients with very mild lung disease ($FEV_1 \geq 80\%$ predicted). Therefore, we

designed a study to determine if the inhalation of HS is beneficial to pediatric CF patients with $FEV_1\% \geq 80\%$ predicted.

3. Objectives

3.1 Primary Objective:

1. To determine if the inhalation of Hypertonic Saline (7%) has a beneficial effect on the Lung Clearance Index in pediatric CF patients with very mild lung disease ($FEV_1\% \geq 80\%$ predicted).

3.2 Secondary Objectives:

1. To determine if the inhalation of Hypertonic Saline (7%) has a beneficial effect on spirometry (FEV_1 % predicted, FVC % predicted and FEF_{25-75} % predicted) in pediatric CF patients with very mild lung disease ($FEV_1\% \geq 80\%$ predicted).

2. To determine if the inhalation of Hypertonic Saline (7%) has a beneficial effect on Quality of Life as measured by the Cystic Fibrosis Quality of Life Questionnaire-Revised (CFQ-R) in pediatric CF patients with very mild lung disease ($FEV_1\% \geq 80\%$ predicted).

4. Research Hypothesis

Amongst paediatric Cystic Fibrosis patients followed at the Hospital for Sick Children with very mild lung disease (i.e. FEV_1 % predicted ≥80%), four weeks of twice daily

Hypertonic Saline (HS) (7%) inhalation as compared to four weeks of twice daily Isotonic Saline (IS) (0.9%) inhalation will have a beneficial effect on the Lung Clearance Index.

5. Research Design

A single center, randomized, cross-over, placebo-controlled treatment trial was performed to determine if HS inhalation would have a beneficial effect on the LCI. The study was conducted over a 12 week period; there were two treatment periods of 4 weeks separated by a 4 week washout period.

6. Setting

All tests were performed in the infant or pediatric pulmonary function laboratory at the Hospital for Sick Children, Toronto, Canada between March 2008 and December 2008.

7. Study Participants

7.1 Inclusion Criteria:

Eligible patients for the study had a confirmed diagnosis of CF (52)**;** were between six and eighteen years of age; were able to perform reproducible pulmonary function tests; at the screening visit had a FEV_1% predicted greater than or equal to 80% (Wang reference equations) and an oxyhemoglobin saturation of greater than or equal to 90 percent in room air.

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7.2 ***Exclusion Criteria:***

Patients were excluded from the study for any of the following reasons: airway cultures yielded *Burkholderia cepacia* complex in the previous 2 years or non tuberculous mycobacteria (NTM) in the past year; current oral corticosteroid use; oxygen supplementation; lung transplantation; intravenous antibiotics or oral fluoroquinolone within 14 days of enrolment; or investigational drugs within 30 days of enrolment.

7.3 Methods of Recruitment and Consent:

The study was introduced to patients in the CF clinic by either the responsible physician or by the CF clinic nurse. Patients were then approached by study personnel if interested. Patients were informed that enrolment was voluntary, they could withdraw from the study at any time and that the decision to participate had no bearing on the medical care they received. The study was explained verbally, according to the information included in the Consent Forms. Consent was recorded with a signature. If the patient was eligible for the study and under 16 years of age, approval of a parent or legal guardian was obtained. Patients less than 16 years of age were asked to give consent if they were thought to have sufficient understanding and capacity to make that decision. This decision was made by the individual obtaining consent on a case by case basis. Verbal assent from the study participants was obtained in addition, where appropriate. One copy of the patient consent form went into the Health Records Chart, one copy went to the patient and one copy was kept by the investigators. Study participants were recruited from the Cystic Fibrosis clinic at the Hospital for Sick Children, Toronto, Canada.

8. Allocation of Interventions

Participants were assigned to a treatment intervention order (ie HS then IS versus IS then HS) by means of a concealed, computer generated randomization performed by a research pharmacist not otherwise involved in the study. All of the treatment boxes were packaged and supplied by the Central Research Pharmacy at the Hospital for Sick Children. Each study participant was allocated two treatment boxes. Each treatment box contained a four week treatment supply of the study solutions packaged in ampoules (see ***9.1 Treatment Intervention***). One box was provided at each of the first and third study visits. Participants, clinicians, study investigators and research assistants were unaware of the treatment assignments throughout the study.

9. Research Procedure

9.1 Treatment Intervention:

The active treatment was Hypertonic Saline (7%) and the placebo was Isotonic Saline (0.9%). The solutions were indistinguishable from each other in appearance and packaging but not taste. Both solutions were packaged in ampoules containing 8mL of solution (1 ampoule per day). The solutions were administered in 4mL aliquots twice daily for 28 days. The solutions were inhaled using the PARI LC® Star reusable nebulizer (Pari,Midlothian, VA, USA) (Health Canada Licence No.: 6135). Two, 100ug puffs of salbutamol (Ventolin) was administered before each inhalation of study solution using an

aerochamber (Aerochamber Max, Trudell, London Canada) as HS inhalation can lead to bronchospasm if patients are not pretreated with a bronchodilator as per the Elkins et al trial study protocol (5, 6).

9.2 Study Schedule

At a screening visit, demographic characteristics, clinical data and spirometric values were recorded. Complete physical examinations were performed prior to study enrolment. Eligible participants were enrolled and began the study the same day or within 7 days (Table 2). At each study visit, participants completed the Multiple Breath Washout (MBW) to determine the LCI, Spirometry, and the Cystic Fibrosis Questionnaire-Revised (CFQ-R). The CFQ-R, Parent was completed if the study participants were between the ages of 6 and 13 years of age. A clinical assessment and medication review was also performed at each visit.

Table 2: Summary of Study Visits

	VISIT 1 (V1) DAY 0	**VISIT 2 (V2) DAY 28 (28 ±7 DAYS AFTER V1)**	**VISIT 3 (V3) DAY 56 (28 ± 14 DAYS AFTER V2)**	**VISIT 4 (V4) DAY 84 (28 ± 7 DAYS AFTER V3)**
Informed consent	X			
Medical/medication history	X	X	X	X
Physical exam	X	X	X	X
Vital signs, weight, height	X	X	X	X
Eligibility	X	X	X	X
Randomization to treatment group	X			
MBW	X	X	X	X
Spirometry	X	X	X	X
CFQ-R and CFQ-R parent	X	X	X	X
Current medication review	X	X	X	X
Adverse event update	X	X	X	X
Drug compliance check		X		X
Dispense study drug or placebo	X		X	

9.3 Assessment of Trial Outcomes

9.31 Primary Outcome

Lung Clearance Index

The technique for performing MBW in children has been described previously (29, 33, 34). The MBW setup was identical to the one used in previous publications by *Gustafsson et al* and *Aurora et al* with the exception of the pneumotachometer (29, 33, 34). All tests were performed in the sitting position. The study participants wore noseclips and were required to breathe through a mouthpiece (VacuMed mouthpieces # 1000 and 1004, Ventura, CA, USA) . Flow was measured using a pneumotachometer (Rudolph Linear Pneumotach, Hans Rudolph**,** Shawnee, KS, USA) and gas concentrations were measured by mass spectrometer (AMIS 2000; Innovision A/S, Odense, Denmark)**.** During the test, the participants were encouraged to watch a video, listen to a portable music device or read a book with the intention of distracting the subjects, thus encouraging regular tidal breathing.

Briefly, each MBW test consists of two phases: a wash-in phase and a washout phase. Patients were asked to tidal breathe a dry gas mixture containing 4% SF_6, 4% He, 21% O_2 and balance N_2 via a flow past system connected to the pneumotachometer until the inspiratory and expiratory SF_6 concentration were equilibrated and stable at 4% (see Figure 2). Subsequently, during expiration the flow past system was disconnected and the subject was asked to tidal breathe room air; the washout phase was completed when the end tidal SF_6 concentration was <0.1% (1/40 of the initial SF_6 concentration) for 3 consecutive tidal breaths (see Figure 3). The Functional Residual Volume determined by MBW is the Cumulative Exhaled Volume divided by the difference in the starting and end concentrations of SF_6. The LCI was calculated as the

number of lung volume turnovers (cumulative expired volume divided by the Functional Residual Capacity) required to reduce end-tidal SF_6 concentration to $1/40^{th}$ of the starting value (29, 33, 34). Therefore, the LCI is the ratio of the cumulative exhaled volume divided by the FRC. Each MBW was performed in triplicate. The final LCI is the average of three LCI maneuvers calculated from three technically acceptable washout trials during each test occasion. Each MBW takes five minutes to complete, for a total of 15 minutes for each patient. None of the study participants had previously performed a MBW.

Figure 2: Phase 1 of the MBW: Wash-in

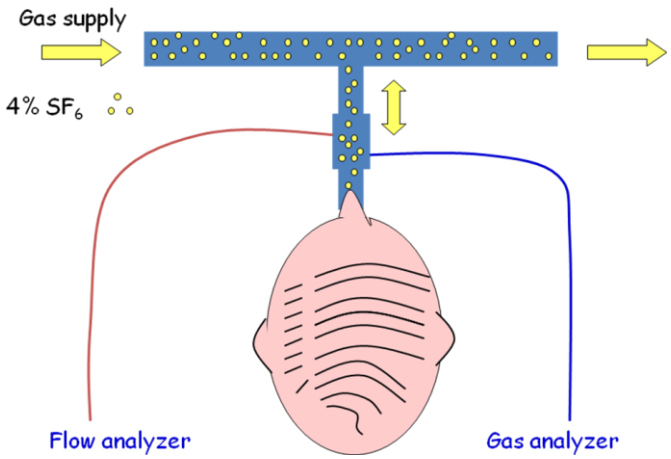


Figure 3: Phase 2 of the MBW: Washout

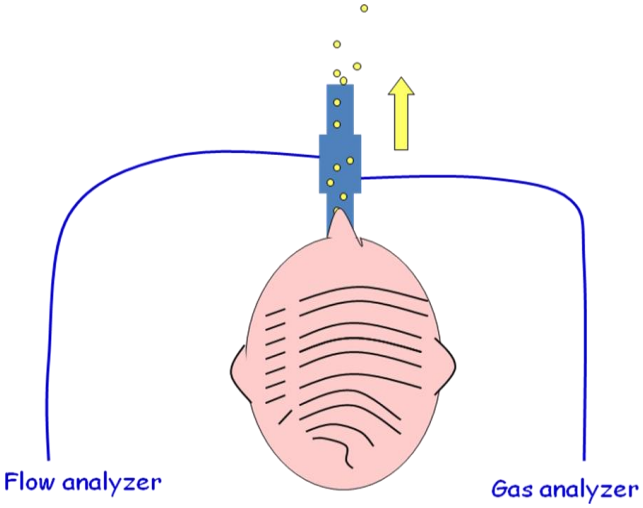
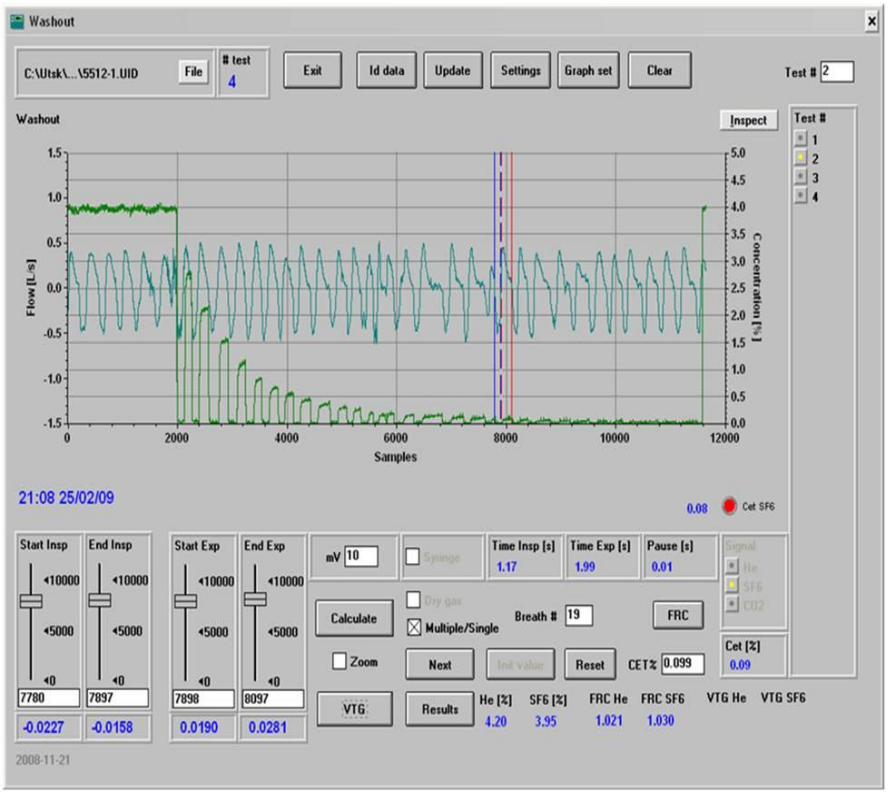


Figure 4: An example of a MBW tracing



x axis: number of gas samples taken
y axis (left): Flow (Liters/second)
y axis (right): Concentration (%)
Turquoise tracing represents the tidal volume of the patient
Green tracing represents the SF_6 concentration for the patient

9.32 Secondary Outcomes

a) Pulmonary Function Tests

Spirometry was performed according to the American Thoracic Society and European Respiratory Society guidelines using the Vmax systems (VIASYS, Cardinal Health, Dublin, USA) (46).

b) Quality of Life

Quality of life was assessed using the CF specific Cystic Fibrosis Questionnaire-Revised (CFQ-R) (47). One of the three participant formats was used depending on the age of the participant: Adolescent and Adults (patients 14 years old and older), Children Ages 12 and 13 and Children Ages 6-11. A parent questionnaire was completed in addition where appropriate as per the CFQ-R administration guidelines: Parents/Caregivers (Children Ages 6 to 13) (47, 48). For children between the ages of 6 and 12 years of age, the questionnaire is administered as an oral interview. Each questionnaire yielded a score of 0 to 100 for each domain with higher numbers indicating better function (47, 48). The Cystic Fibrosis Questionnaire-Revised was administered prior to lung function testing.

9.4 Safety

At the first and third visit, the first dose of the study solution for the treatment period was administered in the hospital. Spirometry and pulse oximetry were performed at baseline, fifteen minutes after salbutamol administration and fifteen minutes after inhalation of the study drug. Participants whose oxyhemoglobin saturation exceeded 90 percent and whose FEV_1%

predicted exceeded 80% of the post-bronchodilator value 15 minutes after inhalation completion were eligible to proceed in the trial. Post-bronchodilator values were considered as per the Elkins et al trial, as a bronchodilator is known to transiently improve the FEV_1% predicted (6). Patients were withdrawn from the study if they required hospital admission for a pulmonary exacerbation (49); corticosteroids for a new diagnosis of Allergic Bronchopulmonary Aspergillosis or oral fluoroquinolones during either of the two study periods. Worsening CF symptoms and/ or the prescription of antibiotics were treated as adverse events. The prescription of antibiotics was at the discretion of the responsible physician in accordance with current CF practice guidelines at our hospital. If participants required outpatient fluoroquinolone antibiotics during the washout period, the washout period was then extended to allow fourteen days after antibiotic completion before the second study period was started.

9.5 Compliance

Adherence was based on returned used and unused ampoules to study personnel at study visits 2 and 4. Compliance was quantified based on the number of returned ampoules for each study period.

9.6 Statistical Analysis

9.61 Statistical Analysis Software

Statistical Analysis Systems software version 9.2 (SAS Institute, Inc., Cary, NC) was used to conduct all analyses. P values of less than 0.05 were considered to be of statistical significance.

9.62 Descriptive Statistics

To describe the study population characteristics, means and proportions were used to summarize continuous and dichotomous variables respectively. Baseline Lung Clearance Indices were compared to the published estimates from Sweden and the United Kingdom (29, 33). The proportion of Lung Clearance Indices that were greater than the upper limits of normal for the Swedish and United Kingdom populations were calculated as proportions and expressed as z scores.

9.63 Repeated Measures Analysis of Variance and Mixed Model Analysis

A two-way Repeated Measures Analysis of Variance (ANOVA) was used to test the treatment effects of HS and IS on the LCI. This method was chosen because all study participants were measured under two different conditions (HS and IS treatments). A mixed effects model was used to perform the repeated measures ANOVA. The predictor variables for the model included treatment type (HS or IS), randomization order (HS then IS or vice-versa) and the treatment by randomization order interaction. Similar models as above were generated for each secondary outcome measure.

A second mixed model was generated to compare baseline LCI values before each of the two treatment periods. Similar models were generated to compare the baselines for each secondary outcome.

Cross-over trials are at risk of three sources of bias: period effect, sequence effect and carryover (50). The mixed models were evaluated for these potential sources of error as outlined above. Baseline values that were not significantly different were consistent with no carry-over effect. Randomization orders that were not significantly different were consistent with no sequence effect. A non significant treatment by randomization order interaction was consistent with the absence of a period effect.

9.64 Correlations

Two group correlations (LCI and each secondary outcome measure) were determined using Pearson correlation coefficients for normally distributed data and Spearman correlation coefficients for all non-normative data. Normality was determined using the Kolmogorov-Smirnov test.

9.65 Sample Size Calculation

We calculated the sample size required for testing Hypothesis 1 using Hypertonic Saline as the main exposure variable and the LCI as the primary outcome variable. Our estimate was based on published Mean +/- Standard Deviation LCI of 11.54 +/- 2.86 for a population of CF children between the ages of 6 and 16 years which was similar to our study population (33). We estimated a treatment effect of 3.00 +/-2.86 in the LCI from HS versus IS at the five percent

level. We calculated that 17 participants would be needed to complete this crossover study to provide 80 percent power (50, 51). However, based on an attrition rate of 20% from similar trials from our center, we estimated that we would need to recruit a total of 20 patients.

9.66 Post Hoc Sample Size Calculations

Post hoc sample size calculations were performed for all secondary outcome measures. The treatment effect and standard deviation for each measure were obtained from the study's results. Assuming 80 percent power and a significance level of five percent, sample sizes were determined for each of the secondary outcome measures (51). This was performed to illustrate the sensitivity of the LCI as compared to the other secondary outcome measures.

10. Ethics Approval

This study was approved by the Research Ethics Boards at the Hospital for Sick Children and the University of Toronto. The study was also approved by Health Canada.

11. Trial Registration

This trial was registered with ClinicalTrials.gov, number NCT00635141.

12. Results

12.1 Study Sample

Fifty-five patients were approached and refused participation in the study (Figure 5). Patients refused participation because of an inability to comply with the study procedure (91%) or because of a dislike of HS inhalation (9%) based on a previous sputum induction study performed at our institution using HS. Twenty patients entered the study and underwent randomization (Figure 5). The baseline characteristics of the study participants are shown in Table 3. The baseline characteristics of the patients that refused participation were reviewed and were not significantly different from the study participants (Table 4). The refusal rate for our study was 52% as compared to an average of 75% for CF interventional studies at our CF center. The LCI results of one patient failed to meet the quality control criteria for all four study visits and were therefore excluded from the analysis. One patient receiving IS withdrew from the study after completion of the initial 4 week study period because of difficulties to complying with the study protocol. Another participant had uninterpretable LCI data at study visit 2 due to irregular breathing. The above two patients are represented graphically in Graphs 1A) and B) but were excluded from the repeated measures ANOVA mixed model statistical analysis of the LCI because the missing data points prevented inclusion. Nineteen patients were included in the analysis but complete crossover data was available for 17 patients.

Figure 5: Randomization and Enrolment of Study Participants

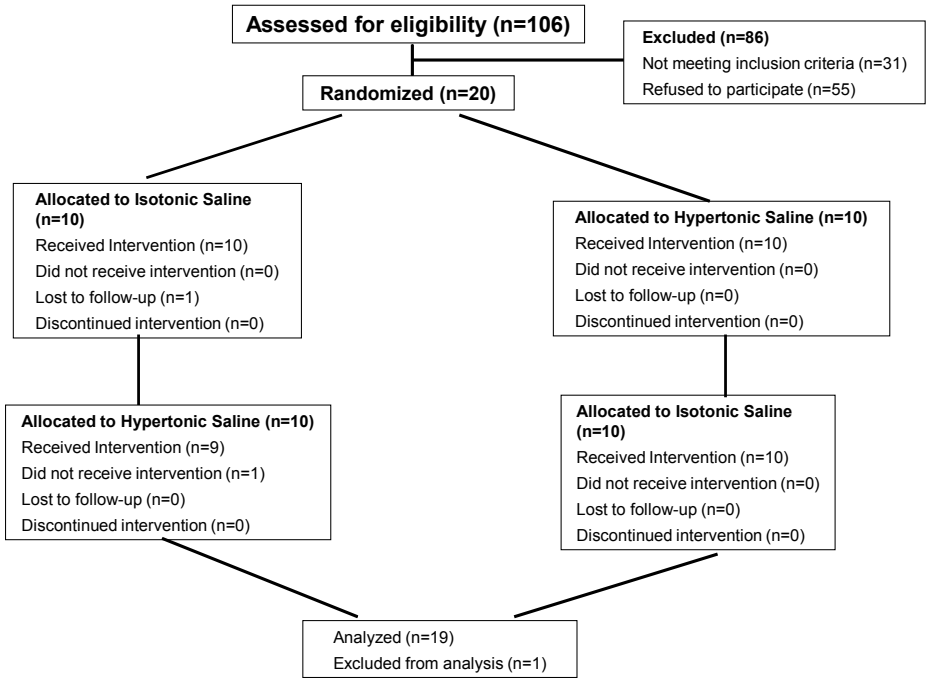


Table 3: Baseline characteristics of study participants

	Study Participants (n=19)
Age (years) Mean ± SD	10.5 ± 3.1
Female/male	12/7
***Pseudomonas aeruginosa* +ve**	7
Pancreatic Insufficient %	84
ΔF508/ ΔF508 %	42
ΔF508 compound Heterozygous %	21
FVC % predicted Mean ± SD (range)	101 ± 11.3 (81-121)
FEV_1 % predicted Mean ± SD (range)	96 ± 12 (79-118)
FEF_{25-75}% predicted Mean ± SD (range)	84 ± 24 (53-120)

Mean ± standard deviation
Range presented in brackets
FEV_1 denotes forced expiratory volume in one second; FVC forced vital capacity; $FEF_{25–75}$ forced expiratory flow at 25 to 75 percent of the forced vital capacity
Pseudomonas aeruginosa + defined as two or more positive cultures in the previous year and/or currently on inhaled anti-pseudomonal therapy.

Table 4: Baseline characteristics of study participants versus those patients that refused participation

	Study Participants (n=19)	Refused Participation (n=55)	P value
Age (years) Mean ± SD	10.5 ± 3.1	11.0 ± 3.7	0.57
Female/male	12/7	28/27	0.60
***Pseudomonas aeruginosa* +ve**	7	42	0.71
Pancreatic Insufficient %	84	95	0.16
ΔF508/ ΔF508 %	42	53	0.43
ΔF508 compound Heterozygous %	21	35	0.28
FVC % predicted Mean ± SD (range)	101 ± 11.3 (81-121)	100 ± 11 (71-128)	0.63
FEV_1 % predicted Mean ± SD (range)	96 ± 12 (79-118)	97 ± 12 (80-128)	0.78
FEF_{25-75}% predicted Mean ± SD (range)	84 ± 24 (53-120)	85 ± 29 (38-164)	0.95

Mean ± standard deviation
Range presented in brackets
FEV_1 denotes forced expiratory volume in one second; FVC forced vital capacity; $FEF_{25–75}$ forced expiratory flow at 25 to 75 percent of the forced vital capacity
Pseudomonas aeruginosa + defined as two or more positive cultures in the previous year and/or currently on inhaled anti-pseudomonal therapy.

12.2. LCI

Baseline LCI before IS, 8.71 ± 2.10 was not significantly different from baseline LCI before HS inhalation, 8.84 ± 1.95 (p=0.73). Therefore, there was no carryover for LCI. Two previously published series of normative data in children obtained with the same equipment developed by one of the investigators (Dr Per Gustafsson) have been published. The mean ± SD in the Swedish series was 6.33 ± 0.43 and 6.45 ± 0.49 in the UK series (29, 33). Based on these publications, either 16/19 (84%), z-score = 0.62 or 12/19 (63%), z-score of 0.45 of the CF patients in our study had LCIs above the normal range for healthy children (29, 33).

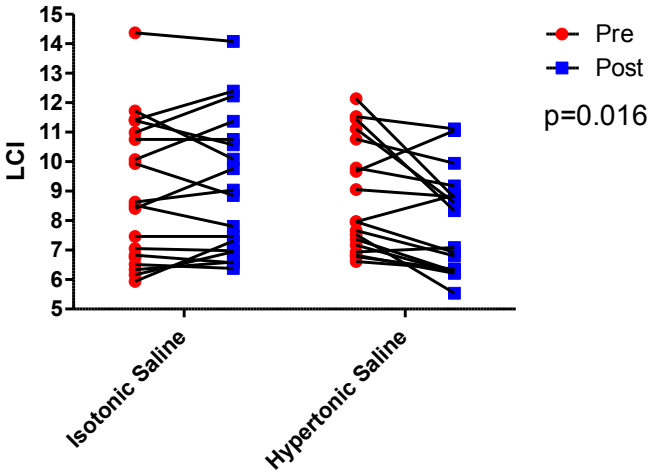
LCI values for each participant pre and post four weeks of HS and IS inhalation are shown in Graphs 1A and B. The LCI was significantly lower after four weeks of HS inhalation, 7.86 ± 1.76, as compared to IS, 8.89 ± 2.10 (p=0.016) (Table 5). There was no sequence effect (p=0.91). The significant treatment effect was then tested for a treatment by time interaction. The randomization order was found to have no significant impact on the treatment effect. (p=0.61) Therefore, there was no period effect.

Table 5: Summary of LCI for the Isotonic Saline and Hypertonic Saline Treatment Periods

	Isotonic Saline		**Hypertonic Saline**		**Treatment Effect***	**P value****
	Pre	**Post**	**Pre**	**Post**		
LCI	8.71±2.10	8.89±2.00	8.84±1.95	7.86±1.71	-1.16 ±0.94	0.016

Expressed as Means +/- SD
* Treatment Effect for Hypertonic Saline versus Isotonic Saline
** P Value for the Treatment Effect

Graph 1: Pre and Post LCI for Isotonic Saline and Hypertonic Saline



12.3. Spirometry

Baseline values for the two study periods for all spirometric measures were not significantly different (Appendix 2). Therefore, there was no carryover effect. FVC % predicted, FEV_1% predicted and FEF_{25-75}% predicted were not significantly different after HS inhalation compared to IS for any of the three parameters (Table 6, Graph 2).

Table 6: Summary of Spirometric Outcome Measures for the Isotonic Saline and Hypertonic Saline Treatment Periods

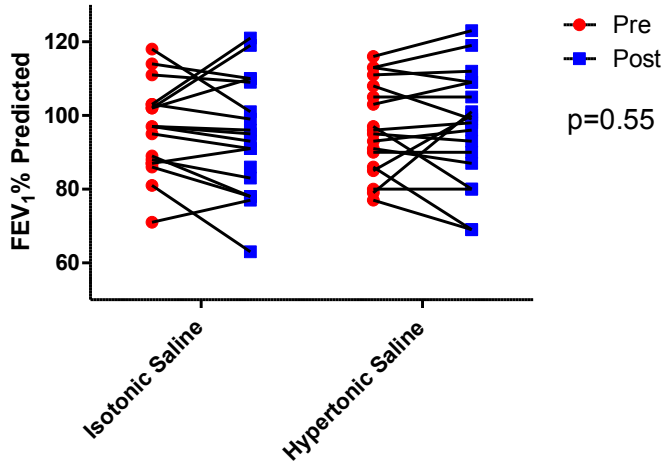
	Isotonic Saline		Hypertonic Saline		Treatment Effect*	P value**
	Pre	Post	Pre	Post		
FVC % predicted	98.50±11.61	99.78±13.27	101.2±11.00	99.33±11.83	-2.37 ± 9.77	0.37
FEV_1% predicted	96.00±11.96	96.29±13.89	96.28±12.67	97.00±14.29	1.78 ± 11.95	0.55
FEF_{25-75} %predicted	84.94±24.12	86.78±24.95	82.94±21.74	85.39±26.27	5.26 ± 22.26	0.53

Expressed as means +/- SD

* Treatment effect for Hypertonic Saline versus Isotonic Saline

** P Value for the treatment effect

Graph 2: Pre and Post FEV_1% Predicted for Isotonic Saline and Hypertonic Saline



12.4. CFQ-R

All study participants completed one of three versions of the CFQ-R at each study visit depending upon their age. Each of the three versions of the CFQ-R included differing domains and the ones presented in this manuscript are in common to the three versions of the questionnaires. CFQ-R scores were not significantly different from HS versus IS inhalations for any of the domains (Table 7). The Respiratory, Physical and Eat domains improved with HS therapy as compared to IS inhalation but not significantly. A carryover effect was present for the Digestion domain (Appendix 2).

Table 7: Summary of CFQ-R for the Isotonic Saline and Hypertonic Saline Treatment Periods

CFQ-R	Isotonic Saline		Hypertonic Saline		Treatment Effect*	P value **
	Pre	Post	Pre	Post		
Respiratory	77.77±13.94	80.09±18.72	76.85±12.99	83.95±16.0	5.16 ± 23.12	0.33
Physical	83.74±23.08	87.83 ±17.66	80.07±18.67	85.62±17.49	0.37±22.02	0.95
Emotion	76.91 ±8.14	79.41±10.23	77.21±14.57	78.63±10.56	-2.42±20.94	0.64
Eat	80.39±26.51	77.78±23.90	76.47± 24.81	79.08±22.87	5.20 ± 21.89	0.34
Treatment Burden	66.01±22.04	73.20± 23.59	65.36±24.81	67.97±26.02	-3.67± 22.80	0.52
Social	69.00±20.48	71.90±19.19	75.49 ±17.09	77.50±12.70	-0.37 ±14.35	0.92
Body	77.12±27.35	77.76±32.39	77.78±34.25	79.74±31.24	-8.37± 22.92	0.15
Digestion	63.40±24.14	70.59±23.22	79.74±22.99	75.82±23.32	-11.04±26.47	0.10

CFQ-R Domains in common between three versions of questionnaire
Expressed as means +/- SD
* Treatment effect for Hypertonic Saline versus Isotonic Saline
** P Value for the treatment effect

12.5. CFQ-R Parent

CFQ-R parent questionnaires were completed by 15 of the 19 study participants as per the CFQ-R administration guidelines. The Digestion domain was significantly higher after four weeks of HS inhalation, 77.78 ± 20.90, as compared to IS, 71.11± 1.74 (p=0.032) (Table 8). The baseline values for the Digestion domain were not different (p=0.88). The significant treatment effect was then tested for a treatment by time interaction. The randomization order was found to have no significant impact on the treatment effect for the Digestion domain (p=0.57).

Table 8: Summary of CFQ-R Parent for the Isotonic Saline and Hypertonic Saline Treatment Periods

CFQ-R Parent	Isotonic Saline		Hypertonic Saline		Treatment Effect*	P value **
	Pre	Post	Pre	Post		
Respiratory	84.07±12.92	81.85±12.32	78.14±14.47	82.59±12.92	5.91±-16.15	0.15
Physical	90.12±8.48	87.90 ±16.59	89.63±15.60	88.89±13.50	-4.56 ±17.59	0.79
Emotion	82.67±12.80	84.00 ±14.43	88.44±15.63	86.19±12.67	-1.08±18.24	0.82
Eat	74.44± 22.60	73.33±22.54	66.67±32.12	73.08±25.94	5.24 ± 16.46	0.25
Treatment Burden	60.00±22.93	57.78±25.61	61.48±20.08	54.07±25.15	-5.54± 23.63	0.38
Body	73.33 ±28.11	70.37±35.30	75.56 ±32.31	73.81±31.00	1.98 ± 25.18	0.77
Digestion	74.81 ±18.53	71.11± 21.74	74.07 ±21.28	77.78±20.90	10.44±16.96	0.032
Health Perceptions	80.00±15.83	80.74±12.22	82.22±20.91	79.26±20.08	-4.00±21.84	0.49
Vitality	68.44±14.79	72.00±13.38	73.78±15.83	70.00±17.10	-1.16 ± 14.74	0.77
Weight	64.44 ±38.76	55.56 ±39.17	60.00 ±38.21	68.89±38.76	13.07 ± 28.85	0.10
School	88.89±11.11	82.22±19.61	83.70 ±19.18	78.52±29.93	-0.57± 20.57	0.92

Expressed as means +/- SD
* Treatment effect for Hypertonic Saline versus Isotonic Saline
** P Value for the treatment effect

12.6. Correlations

Of all the secondary outcome measures, the strongest correlation was found between LCI and FEV_1% predicted (r= -0.61, p<0.0001) (Graph 3). The LCI significantly correlated with the CFQ-R Respiratory, Physical, Emotion and Body Domains of which the strongest correlation was with the Body Domain (r=-0.50, p<0.0001) (Table 9, Graph 6). The LCI correlated with all CFQ-R parent domains except for Treatment Burden and Digestion (Graph 7).

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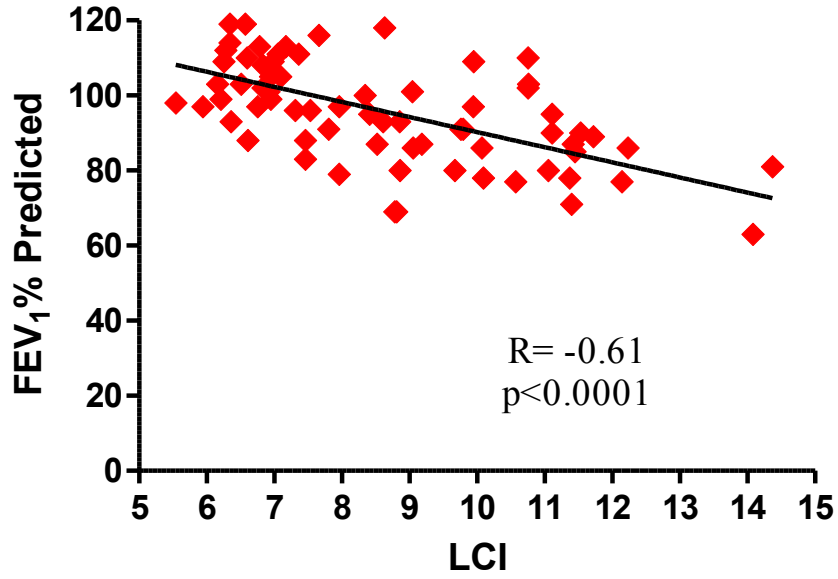
Table 9: Correlations of LCI with Secondary Outcome Parameters

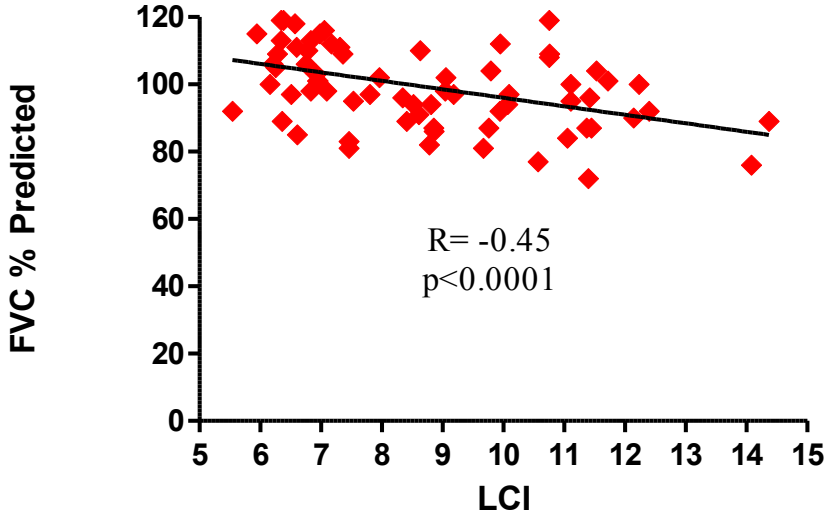
	R	P value
Spirometry		
FEV_1% predicted	-0.61*	<0.0001
FVC % Predicted	-0.45*	<0.0001
FEF_{25-75}% predicted	-0.53*	<0.0001
CFQ-R Domains		
Respiratory	-0.43	<0.0001
Physical	-0.43	0.0002
Emotion	-0.23	0.046
Eat	-0.049	0.68
Treatment Burden	-0.19	0.11
Social	0.013*	0.91
Body	-0.50	<0.0001
Digestion	-0.17	0.16
CFQ-R Parent Domains		
Respiratory	-0.34	0.0082
Physical	-0.50	<0.001
Emotion	-0.42	0.0010
Eat	-0.25	0.061
Treatment Burden	0.12	0.35
Body	-0.29	0.025
Digestion	-0.07	0.60
Health Perceptions	-0.35	0.0059
Vitality	-0.38	0.0027
Weight	-0.33	0.010
School	-0.18	0.16

*Pearson correlation coefficient (all other correlations are Spearman)

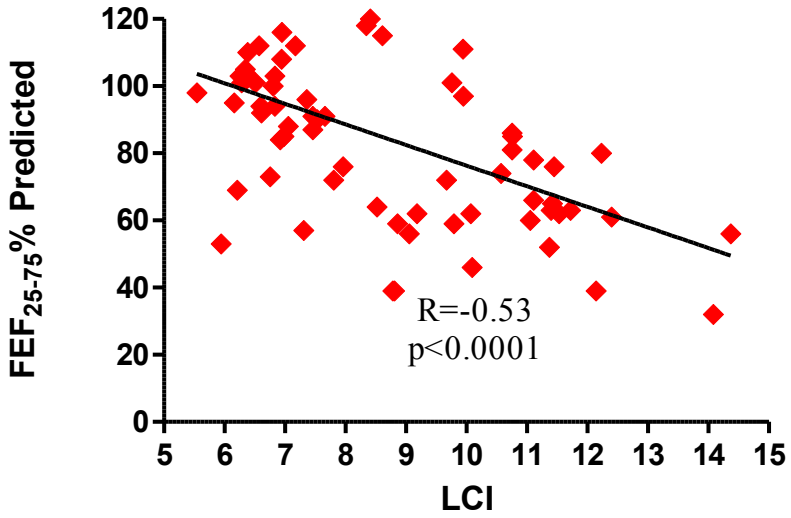
Graph 3: Correlation between LCI and FEV_1% Predicted



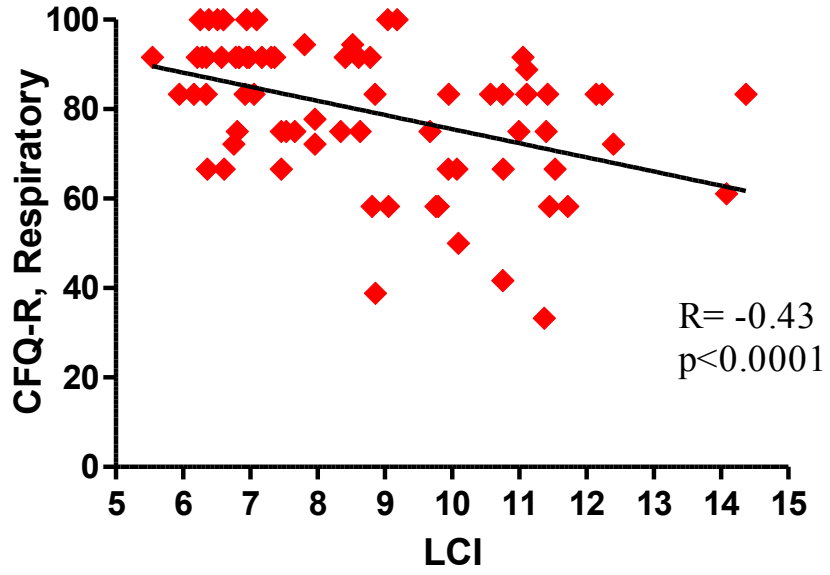
Graph 4: Correlation between LCI and FVC% Predicted



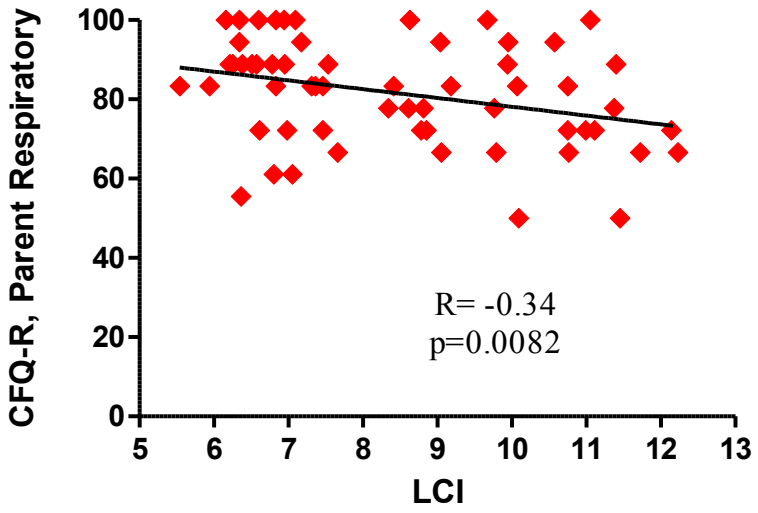
Graph 5: Correlation between LCI and FEF $_{25-75}$ % Predicted



Graph 6: Correlation between LCI and CFQ-R, Respiratory Domain



Graph 7: Correlation between LCI and CFQ-R, Parent Respiratory Domain



12.7. Safety

After administration of the assigned solution, the FEV_1% predicted fell by a mean of 116 ± 140ml (-5.20 ± 6.07 % predicted) after HS inhalation and 41 ± 88ml (-2.81± 4.96 % predicted) after IS inhalation. None of the patients had a drop of FEV_1% predicted $\geq$ 20%; therefore, none of the patients were excluded from the trial because of safety violations.

There were significantly more adverse events during the HS treatment period as compared to IS period (p=0.0035). Adverse events included increased sputum production, fever, ear infection, rhinorrhea, malaise and adverse drug reactions. None of the above adverse events required additional treatment. Adverse drug reactions (ie adverse events that in the opinion of the examining investigator were directly and temporally related to the inhalation of

the trial solution) were not significantly different between the HS and IS inhalation treatment periods (p=0.17) (Table 10).

Table 10: Adverse Events

	Isotonic Saline	Hypertonic Saline
Increased Sputum Production	1	0
Fever	0	1
Rhinorrhea	1	3
Malaise	1	3
Ear Infection	0	1
Adverse Drug Reaction	5	7
Cough	3	6
Chest Pain	1	0
Hoarseness	1	1
Total	8	15

P=0.0035 (overall)
P for adverse drug reaction= 0.17

12.8. Compliance

Adherence to treatment, as judged by the number of returned ampoules overall for both study periods was 95.3 ± 31.24 % percent for the HS study period and 84.47 ± 19.28% for the IS study period. Greater than or equal to 80% compliance was seen in 16/17 patients during the HS treatment and 15/19 during IS treatment. This difference was not significant (p=0.20). In addition, there was no significant difference in compliance for HS in treatment period 1 versus treatment period 2 (p=0.26).

Of the 19 study participants, 18 were asked to guess their treatment allocation order. One of the study participants was not asked as she withdrew from the study after the first treatment period. Of the 18 patients, 17 patients (94%) correctly guessed their treatment allocation order.

12.9. Post Hoc Sample Size Calculations

Post hoc sample size calculations were performed for all secondary outcome measures except the CFQ-R, Parent Digestion domain (significant treatment effect, p=0.032) based on the trial results. Sample sizes ranged from 41 for the CFQ-R, Parent Weight domain to 27767 for the CFQ-R Physical domain (Table 11).

Table 11: Post Hoc Analysis of Sample Size Calculations for Secondary Outcome Measures

Outcome Analysis	Treatment Effect*	Required Sample Size
Spirometry		
FEV_1% predicted	1.78 ± 11.95	356
FEF_{25-75} % predicted	5.26 ± 22.26	143
CFQ-R Domains		
Respiratory	2.87 ± 14.22	195
Physical	0.37 ± 22.02	27767
Eat	5.20 ± 21.89	142
CFQ-R Parent Domains		
Respiratory	5.91 ± 16.15	61
Eat	5.24 ± 16.46	80
Body	1.98 ± 25.18	1272
Weight	13.07 ± 28.85	41

*Treatment Effect between isotonic saline and hypertonic saline treatment periods
** Required number of patients for a crossover trial to achieve 80% power at 5% significance level
Outcome measures excluded from post hoc sample size calculations if they worsened with Hypertonic Saline (7%) inhalation or if the treatment effect was significant

13. Discussion

13.1. Hypertonic Saline improves the LCI in Patients with Very Mild Lung Disease

In this twelve week trial, we compared the efficacy and safety of Hypertonic Saline and Isotonic Saline in paediatric CF patients with very mild lung disease. This study is the first to demonstrate that twice daily HS improved the LCI in paediatric CF patients with normal spirometric lung function. This finding is of significant importance due to the paradigm shift in recent years towards early, aggressive treatment of CF lung disease given that the pulmonary

disease begins shortly after birth, progresses in the absence of clinical exacerbations and results in early decrements in lung function (24-27). Therefore, it is becoming increasingly important to demonstrate that therapies are effective in patients with mild disease as the majority of paediatric CF patients now have normal or only mildly reduced lung function when defined by spirometry.

Multiple early interventions strategies are currently under development and previous studies have required large sample sizes or long periods of follow up to detect treatment effects in milder patients – thus precluding the rapid integration of novel therapies into practice. In this study of short duration we were able to show a significant treatment effect in only 17 patients whereas using other established surrogate markers such as FEV_1 or quality of life measures, we would have required a much larger sample size. The ability of the LCI to detect changes in a relatively small number of patients with very mild lung disease makes it an attractive candidate for early studies to evaluate responses to treatment approaches that target the underlying defect in CF which is the future of CF therapeutics.

13.21 Hypertonic Saline as an Early Intervention Strategy: Efficacy

Inhaled Hypertonic Saline (7%) twice daily is an ideal early intervention strategy. HS acts at the cellular level to increase Airway Surface Liquid and therefore is potentially effective right from birth before extensive lung disease occurs. Secondly, HS therapy is effective after twice daily inhalation for only twenty-eight days. This is important given that decrements in lung function begin early on in life (26, 27). Therefore, HS is an opportunity for clinicians to disrupt the pathogenesis of the pulmonary disease early on.

13.22 Hypertonic Saline as an Early Intervention Strategy: Safety

HS therapy has been shown to be safe and well tolerated in CF patients greater than five years of age in previous publications and our study was consistent with previous findings (5, 6). Although, there were more overall adverse drug reactions in the HS group in our study, the number of adverse drug reactions (felt to be related to be directly and temporally related to the inhalation of the trial solution) was not significantly different between the two therapies. However, more patients did experience cough after HS inhalation as compared to IS inhalation; increased cough clearance is in fact one of the proposed mechanisms of action for HS and may explain the increased incidence of cough after HS inhalation. Given that this intervention can be applied to younger children to preserve their lung function, safety in young children is imperative. *Subbarao et al* and *Dellon et al* have demonstrated that a single inhalation of HS is safe and well tolerated in infants and children aged 4 months to 7 years (17, 21). However, if HS is to be used as an early intervention strategy then repeated doses of HS must also be well tolerated. The Infant Study of Inhaled Saline in Cystic Fibrosis (ISIS) that is currently underway will be able to address this question (521).

13.23 Hypertonic Saline as an Early Intervention Strategy: Compliance and Affordability

Patients were very compliant with the therapy suggesting both a high degree of motivation among the patients as well as a high tolerability. Although compliance within clinical trials is usually much higher than in everyday life, one would hypothesize that the compliance among young CF patients with mild disease would be higher than that expected in older CF patients given the parental involvement. Therefore, pragmatic compliance of HS may

actually approximate the compliance seen in our study. Lastly, inhaled HS twice daily is a relatively affordable CF therapy at a cost of $140.00 per month as compared to other CF therapies such as Pulmozyme (Genentech, USA), which retails at $1200.00 per month.

13.3. Effects of Hypertonic Saline on the LCI Versus FEV_1% Predicted

Four weeks of twice daily HS inhalation significantly improved the LCI but not pulmonary function. Our trial and two other previous publications have demonstrated modest improvements in pulmonary function (5, 6). In our study, the improvements in pulmonary function were smaller and failed to reach significance. The smaller treatment effect size in our trial can be potentially explained by the differences in baseline pulmonary function in our study participants as compared to the other two populations; our study participants had milder disease (Table 12). The significant correlation between spirometry and LCI in our trial is consistent with the superior sensitivity of the LCI. Therefore, an explanation for our findings is that the LCI is a more sensitive outcome measure than spirometry. There is both cross-sectional and longitudinal pediatric data in support of this statement (29, 33-36, 38). FEV_1 is a measure of flow and reflects airways resistance. Although, the peripheral airways account for the majority of the lung's total surface area, they account for a small proportion of the lung's airways' flow-resistive pressure losses. Therefore, disease in the peripheral lung can be masked by airflow through the non flow limited airways. In comparison, the LCI is derived from the MBW technique which reflects ventilation inhomogeneity and is thus sensitive to disease in the peripheral airways, thus accounting for the test's superior sensitivity.

Table 12: Baseline and Change in FEV_1 % Predicted for Our trial and 2 Previous Publications

	Baseline Pulmonary Function	**Change in FEV_1% Predicted from HS**
Our Trial	96+/-12	1.78 (-7.92- 4.37)
Elkins et al	73+/-21	3.2 (0.1- 6.2)
Donaldson et al	78 +/-19	4.7 (-1.3- 10.6)

Mean ± Standard Deviation
Numbers in brackets are 95 percent confidence intervals

13.4. Hypertonic Saline and Quality of Life

Hypertonic Saline has been shown to significantly improve QOL using the CFQ-R adolescent and adult version in two other studies (5, 6). In the *Elkins et al* study, there were significant improvements from HS therapy in the Role domain (7.3 points, P = 0.04), Emotion domain (4.8 points, P = 0.03), and the Health domain (5.3 points, P = 0.01) (6). In the *Donaldson et al* study, the Respiratory domain improved with HS therapy as compared to HS plus amiloride mean +/- SE (82.3+/- 3.1 versus 70.0 +/-3.1, p=0.01) (5). These significant improvements in the CFQ-R were not mirrored in our study. One potential explanation for this discrepancy is the milder pulmonary disease in our study participants as reflected by better patient reported QOL (see Table 13). However, another explanation which is somewhat related to the first may be our young population. Of the nineteen study participants, only four completed the adolescent and adult version of the CFQ-R indicating that they are 14 years of age or older. The remaining fifteen patients completed the pediatric versions. Therefore, it is possible that the improvement in lung function from HS inhalation as measured by LCI and

spirometry in our study was perceived differently by the younger patients accounting for the lack of improvement in the CFQ-R. This may also account for the discrepancy between the treatment effect data and the significance of the correlations with the LCI.

In our trial, although not significant, HS therapy worsened CFQ-R scores in the Body and Digestion domains by more than eight points each. The possible harmful effects of HS therapy may have contributed in part to our findings. The salty taste of HS may explain the perception of eating problems and nausea, vomiting and increased cough are all possible explanations for an increase in abdominal pain. In addition, a more negative personal outlook could be the result of any of the above mentioned side effects of HS therapy thus accounting for a worsening Body domain score.

The *Elkins et al* publication also reported on the CFQ-R, parent version (6). The Digestion domain was significantly higher in the control group as compared to the HS group which is the opposite of what we found in our study (6). Despite the significant treatment effect, the Digestion domain did not correlate with the LCI, our primary outcome measure. For the Digestion domain parents are asked to answer "always, often, sometimes or never" to the following three questions: 1) My child has gas 2) My child has diarrhea 3) My child has abdominal pain. As such, the relevance of this domain to HS inhalation therapy is questionable.

Table 13: Baseline CFQ-R and CFQ-R Parent Respiratory Domain Scores for Our Trial and Two Previous Publications

	Baseline 1 Respiratory Domain Score	**Baseline 2 Respiratory Domain Score**
Our Trial		
CFQ-R	78+/-14	77+/-13
CFQ-R Parent	84+/-13	78+/-15
Elkins et al		
CFQ-R	65+/-17	
CFQ-R Parent	73+/-11	
Donaldson et al		

CFQ-R	73.3+/-22	74.7+/-20

Mean ± Standard Deviation

13.5. Study Limitations

13.51 Limitations of the Study Design

There were several limitations of the current study based on the study design. This was a cross-over trial which allows the response of a subject to HS to be contrasted with the same subject's response to IS. Removing patient variation in this way makes crossover trials more efficient than parallel trials. However, the principal drawback being that the effects of one treatment may "carry over" and alter the response to subsequent treatments. Although, we demonstrated in our study that there was no carry-over effect (baseline values before each treatment periods were not different), these tests have limited power and cannot rule out a type II error (wrongly concluding there is no carry over effect) (50).

The HS and IS were identical in appearance of the solution, volume and packaging but the inhalation solutions had different tastes with HS being much saltier. The investigators did attempt to mask the taste of IS and HS by adding quinine sulphate as per the *Elkins et al* publication (6). However, the solutions could be differentiated despite the addition of quinine sulphate so the decision was made to not add quinine sulphate. Therefore, the study participants were not blinded and were able to taste the difference between the two study solutions. Once patients have tasted both solutions they would be able to differentiate between the two solutions and 94% of patients did indeed correctly guess their treatment allocation. Given this lack of blinding one would have expected differences in overall compliance for HS and IS. However,

in our trial, there was no significant difference in compliance for the HS and IS treatment periods or between HS inhalation in treatment period 1 or 2.

Compliance with the study drug was measured by the number of returned vials. Other than the first dose, during the twenty-eight treatment periods the patients were not observed by study personnel during the administration of the medication. Therefore, one cannot be sure that the medication was taken correctly or completely or that some ampoules of the study drug were not left at home. However, most young CF patients have experience with inhalational therapies and are either highly motivated themselves and/or have highly motivated parents.

13.52. Limitations of the A priori Treatment Effect

Although significant the mean treatment effect and variability of HS was less than what was anticipated in the sample size calculation *a priori*. This can be explained by milder pulmonary disease and lower baseline LCI among our study participants compared to the previously published paediatric population that we based the sample size calculation on (32). As pulmonary disease worsens and FEV_1 decreases, variability in the LCI increases which may explain why a smaller treatment effect still resulted in a significant treatment effect in this study (29, 33).

13.53. Limitations of LCI as an Outcome Measure

Data from this study would support the utility of LCI in other interventional studies, but many questions remain unanswered at present. The LCI has yet to be linked to mortality and it is therefore unknown if the LCI's improvement was truly a reflection of HS therapy or rather

an epiphenomenon that was present but not connected with disease improvement and survival (33). While we have shown that LCI significantly correlates with FEV_1 and Quality of Life scores, two surrogates of mortality, further longitudinal studies in patients with more significant lung disease are required to establish its link to survival in CF patients (23, 43). This poses a dilemma as mortality is rare in patients with mild disease (though the lifespan is shortened in the majority of patients) and the usefulness of the LCI may vary between patients with mild versus more advanced disease. In addition, our study was limited to a patient population that was able to perform technically adequate spirometry and thus, it has not yet been established as to whether HS therapy is an effective therapy in the infant and preschool population.

13.54. Limitations of the CFQ-R

One of the challenges of using the CFQ-R as an outcome measure for a pediatric study is the number of different versions of the questionnaire based on patient age; we used all four versions of the CFQ-R in our trial. There is no overall composite score for the CFQ-R and the domains are not identical among the four different versions. As such there has been a recent shift towards focusing on the Respiratory Domain of the CFQ-R (44,45). However, patients scores on this domain alone may not be truly reflective of their QOL. As such we included all domains in common to all versions of the CFQ-R in our analysis but the QOL information in our study is still limited as several domains were omitted.

13.6. Future Directions

At present the Minimum Clinically Important Difference (MCID) for the LCI is unknown. Therefore, the next step would be to conduct a larger study in a similar patient population to look at the effect of long-term HS therapy on clinically meaningful outcomes such as pulmonary exacerbations, pulmonary function, quality of life and safety in addition to the LCI. Our study could provide pilot data and be used for the sample size calculation.

Our trial was limited to CF patients with acceptable and reproducible spirometry and a future trial would extend the study to the infant and preschool populations. With the question of whether HS therapy is an effective early intervention strategy in mind, a cohort study should be initiated in which CF infants identified by newborn screening are randomized to HS or IS inhalation and followed forward with regular assessments of lung function using a combination of spirometry and the LCI. Data from our study demonstrates the proof of concept and could be incorporated into the sample size calculation.

14. Conclusions

We have demonstrated for the first time that Hypertonic Saline inhalation is an effective treatment intervention in pediatric CF patients with normal spirometric lung function. We hope that our findings open the door to studying other interventions in similar patients as aside from a cure, early aggressive therapy may be the key to improving survival for people living with Cystic Fibrosis.

15. References

1. Ratjen F, Doring G. Cystic Fibrosis. Lancet. 2003; 361(9358): 681-689.

2. http://www.cff.org/

3. Robinson MB. Mucociliary Clearance in Cystic Fibrosis. *Pediatr Pulmonol.* 2002; 33:293-306.

4. Wark PAB, McDonald V, Jones AP. Nebulised hypertonic saline for cystic fibrosis.[update of Cochrane Database Syst Rev. 2003;(1):CD001506; PMID: 12535409]. *Cochrane Database Syst Rev.* 2005(3):CD001506.

5. Donaldson SH, Bennett WD, Zeman KL, et al. Mucus clearance and lung function in cystic fibrosis with hypertonic saline. *N Engl J Med*. 2006; 354(3):241-250.

6. Elkins M, Robinson M, Rose B, et al. A controlled trial of Long-term Hypertonic Saline in patients with Cystic Fibrosis. *N Engl J Med.* 2006; 354:229-240.

7. Ratjen, F. Restoring Airway Surface Liquid in Cystic Fibrosis. *N Engl J Med.* 2006; 354: 291-293.

8. King M, Dasgupta B, Tomkiewicz RP, et al. Rheology of cystic fibrosis sputum after in vitro treatment with hypertonic saline alone and in combination with recombinant human deoxyribonuclease I. *AJRCCM.*. 1997*;* 156(1):173-177.

9. Wills PJ, Hall RL, Chan W, et al. Sodium chloride increases the ciliary transportability of cystic fibrosis and bronchiectasis sputum on the mucus-depleted bovine trachea. *J Clin Invest.* 1997; 99(1): 9-13.

10. Robinson M, Regnis JA, Bailey DL, et al. Effect of hypertonic saline, amiloride, and cough on mucociliary clearance in patients with cystic fibrosis. *AJRCCM*. 1996; 153(5):1503-1509.

11. Robinson M, Hemming AL, Regnis JA, et al. Effect of increasing doses of hypertonic saline on mucociliary clearance in patients with cystic fibrosis. *Thorax.* 1997; 52(10):900-3.

12. Ramsey BW, Pepe MS, Quan JM, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. N Engl J Med. 1999; 340:23-3

13. Eng PA, Morton J, Douglass JA, Riedler J, Wilson J, Robertson CF. Short-term efficacy of ultrasonically nebulized hypertonic saline in cystic fibrosis. *Pediatr Pulmonol*. 1996 ;21(2):77-83.

14. Suri R, Metcalfe C, Lees B, Grieve R, Flather M, Normand C, et al. Comparison of hypertonic saline and alternate-day or daily recombinant human deoxyribonuclease in children with cystic fibrosis: a randomised trial. *Lancet.* 2001 20;358(9290):1316-21.

15. Ballmann M, von der Hardt H. Hypertonic saline and recombinant human DNase: a randomised cross-over pilot study in patients with cystic fibrosis. *J Cyst Fibros*. 2002;1(1):35-7.

16. Suri R, Marshall LJ, Wallis C, Metcalfe C, Shute JK, Bush A. Safety and use of sputum induction in children with cystic fibrosis. *Pediatr Pulmonol.* 2003;35(4):309-13.

17. Subbarao P, Balkovec S, Solomon M, Ratjen F. Pilot study of safety and tolerability of inhaled hypertonic saline in infants with cystic fibrosis. *Pediatr Pulmonol.* 2007;42(5):471-6.

18. Aitken ML, Greene KE, Tonelli MR, Burns JL, Emerson JC, Goss CH, et al. Analysis of sequential aliquots of hypertonic saline solution-induced sputum from clinically stable patients with cystic fibrosis. *Chest.* 2003;123(3):792-9.

19. De Boeck KA, M. Vandeputte, S. Sputum induction in young cystic fibrosis patients. *Eur Respir J*. 2000;16:91-4.

20. Al-Saleh S, editor. North American Cystic Fibrosis Conference; 2007; Anaheim, California.

21. Dellon EP, Donaldson SH, Johnson R et al. Safety and tolerability of inhaled hypertonic saline in young children with cystic fibrosis. *Pediatr Pulmonol*. 2008; 43(11): 1100-6

22. Gelb AF, Williams AJ, Zamel N. Spirometry. FEV1 versus FEF25-75. *Chest* 1983; 84: 473-4

23. Corey M, Farewell V. Determinants of mortality from cystic fibrosis in Canada, 1970–1989, *Am J Epidem* 1996; 143**:** 1007–17.

24. Konstan MW, Hilliard KA, Norvell TM, et al. Bronchoalveolar lavage findings in cystic fibrosis patients with stable, clinically mild lung disease suggest ongoing infection and inflammation. *AJRCCM*. 1994; 150: 448-454.

25. Khan TZ, Wagener JS, Bost T, et al. Early pulmonary inflammation in infants with cystic fibrosis. *AJRCCM.* 1995; 151: 1075-1082.

26. Dakin CJ, Numa AH, Wang HE, et al. Inflammation, infection and pulmonary function in infants and young children with cystic fibrosis. *AJRCCM*. 2002; 165: 904-910.

27. Ranganathan SC, Dezateux C, Bush A, et al. Airway function in infants newly diagnosed with cystic fibrosis. *Lancet.* 2001; 358: 1964-1965.

28. Accurso FJ. Introduction: the rational for early intervention in cystic fibrosis. *Pediatr Pulmonol.* 1997; 24: 132–133.

29. Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. *Eur Respir J.* 2003; 22(6): 972-979.

30. Aurora P, Wade A, Whitmore P, et al. A model for predicting life expectancy of children with cystic fibrosis*. Eur Respir J.* 2000; 16: 1056–1060.

31. Macklem PT, Mead J. Resistance of central and peripheral airways measured by a retrograde catheter. *J Appl Physiol.* 1967; 22: 395–401.

32. Hogg JC, Macklem PT, Thurlbeck WM. Site and nature of airway obstruction in chronic lung disease. *N Engl J Med.* 1968; 278: 1355–1360.

33. Aurora P, Gustafsson P, Bush A, et al. Multiple breath inert gas washout as a measure of ventilation distribution in children with cystic fibrosis. *Thorax.*2004; 59(12): 1068-73.

34. Aurora P, Bush A, Gustafsson P, et al. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. *AJRCCM.* 2005; 171(3): 249-256.

35. Kraemer R, Blum A, Schibler A, et al. Ventilation inhomogeneities in relation to standard lung function in patients with cystic fibrosis. *AJRCCM.* 2005; 171(4): 371-8.

36. Lum S, Gustafsson PM, Ljungberg H, et al. Early detection of cystic fibrosis lung disease: multiple-breath washout versus raised volume tests. *Thorax.* 2007; 62(4): 341-7

37. Horsley AR, Gustafsson P, Macleod KA, et al. Lung Clearance Index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. *Thorax.* 2008; 63: 135-140

38. Gustafsson PM, De Jong PA Tiddens HA, et al. Multiple-breath inert gas washout and spirometry versus structural lung disease in cystic fibrosis. *Thorax.* 2008; 63**:** 129-34

39. Davies JC, Cunningham S, Alton EW, et al. Lung clearance index in CF: a sensitive marker of lung disease severity. *Thorax* 2008; 63**:** 96-7

40. Robinson PD, Cooper P, Van Asperen P, et al. Using index of ventilation to assess response to treatment for acute pulmonary exacerbation in children with cystic fibrosis. *Pediatr Pulmonol.*, in press

41. World Health Organization. Preamble to the constitution of the World Health Organization. Basic Documents, 26th ed. Geneva, Switzerland; 1976.

42. Gee L, Abbott J, Hart A, et al. Associations between clinical variables and quality of life in adults with cystic fibrosis. *J Cyst Fibros.* 2005;4: 59-66

43. Abbott J, Hart A, Morton AM, et al. Can Health Related Quality of Life Predict Survival in Adults with CF. *AJRCCM.* 2009; 179: 54-58

44. Retsch-Bogart GZ, Quittner A, Gibsonn RL, et al. Efficacy and Safety of Inhaled Aztreonam Lysine for Airway Pseudomonas in Cystic Fibrosis. *Chest* 2009; 135: 1223-1232

45. Quittner A, Modi A, Wainright C, et al. Determination of the Minimal Clinically Important Difference Score for the Cystic Fibrosis Questionnaire-Revised Respiratory Symptom Scale inn Two Populations of Patients with Cystic Fibrosis and Chronic *Pseudomonas aeruginosa* Airway Infection. *Chest* 2009; 135: 1610-1618

46. Miller M, Hankinson J, Brusasco V, et al. Standardisation of spirometry, *Eur Respir J.* 2005; 26: 319-38.

47. Manual Scoring Instruction for the CFQ-R, Alexandra Quittner, obtained through email via Ivette Cruz (icruz@miami.ir.miami.edu).

48. Quittner AL, Buu A, Messer MA, et al. Development and validation of the Cystic Fibrosis Questionnaire in the United States: a health-related quality of life measure for cystic fibrosis. *Chest*. 2005; 128(4): 2347-54

49. Rosenfeld M, Emerson J, Williams-Warren J, et al. Defining a pulmonary exacerbation in cystic fibrosis. *J Pediatr*. 2005; 138(1): 105-8

50. Senn SJ. *Cross-over trials in clinical research.* Chichester: John Wiley , 1993.

51. http://hedwig.mgh.harvard.edu/sample_size/size.html

52. http://cClinicaltrials.gov

16. Thesis Committee Membership

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Appendix 1:

Abbreviation List

Legend (Variable Label)	Definition
ANOVA	Analysis of Variance
ASL	Airway Surface Liquid
CF	Cystic Fibrosis
CFQ-R	Cystic Fibrosis Quality of Life Questionnaire -Revised
CFQ-R, P	Cystic Fibrosis Quality of Life Questionnaire -Revised, Parent
CFTR	Cystic Fibrosis Transmembrane Conductance Regular
$FEV_{0.4}$% predicted	Forced Expiratory Volume in 0.4 seconds percent predicted
FEV_{25-75}% predicted	Forced Expiratory Flow at 25 to 75% of the Vital Capacity % predicted
FEV_1% predicted	Forced Expiratory Volume in 1 second percent predicted
FVC% predicted	Forced Vital Capacity
HS	Hypertonic Saline
IS	Isotonic Saline
LCI	Lung Clearance Index
MBW	Multiple Breath Washout
MCC	Mucociliary Clearance
MEF_{25}	Maximal Expiratory Flow at 25% of the forced Vital Capacity
QOL	Quality of Life

Appendix 2

Comparison of Baseline Values For Each Treatment Period for All Secondary Outcome Measures

	P value
Spirometry	
FEV_1% predicted	0.88
FVC % Predicted	0.13
FEF_{25-75}% predicted	0.57
CFQ-R Domains	
Respiratory	0.80
Physical	0.55
Emotion	0.89
Eat	0.22
Treatment Burden	0.88
Social	0.069
Body	0.889
Digestion	0.008
CFQ-R Parent Domains	
Respiratory	0.081
Physical	0.92
Emotion	0.028
Eat	0.21
Treatment Burden	0.85
Body	0.48
Digestion	0.88
Health Perceptions	0.63
Vitality	0.043
Weight	0.18
School	0.18

Appendix 3

Treatment By Randomization Order Interactions for All Secondary Outcome Measures

	P value
Spirometry	
FEV_1% predicted	0.99
FVC % Predicted	0.60
FEF_{25-75}% predicted	0.58
CFQ-R Domains	
Respiratory	0.41
Physical	0.58
Emotion	0.54
Eat	0.44
Treatment Burden	0.77
Social	0.32
Body	0.87
Digestion	0.85
CFQ-R Parent Domains	
Respiratory	0.41
Physical	0.67
Emotion	0.12
Eat	0.022
Treatment Burden	0.95
Body	0.026
Digestion	0.57
Health Perceptions	0.54
Vitality	0.028
Weight	0.53
School	0.34

Appendix 4

Significance of randomization order for all secondary outcome measures

	P value
Spirometry	
FEV_1% predicted	0.47
FVC % Predicted	0.85
FEF_{25-75}% predicted	0.18
CFQ-R Domains	
Respiratory	0.52
Physical	0.43
Emotion	0.58
Eat	0.82
Treatment Burden	0.16
Social	0.37
Body	0.13
Digestion	0.93
CFQ-R Parent Domains	
Respiratory	0.45
Physical	0.45
Emotion	0.18
Eat	0.40
Treatment Burden	0.77
Body	0.74
Digestion	0.61
Health Perceptions	0.90
Vitality	0.51
Weight	0.28
School	0.45

Appendix 5: Timeline for Thesis

- June-July 2007: Draft thesis proposal
- September 2007: Step 1 thesis defence: Form and meet with thesis committee
- December 2007: Health Canada Approval for study protocol and Hospital for Sick Children Research Ethics Board Approval
- January 2008: Step 2 thesis defence
- February 2008: Study protocol revised based on Step 2 defence and submitted to the University of Toronto, Research Ethics Board
- July 2008: Study protocol submitted to the Department of Health Policy, Management and Evaluation (as per Step 2 thesis defence)
- March 2008 to December 2008: Patient recruitment and data collection
- January 2009-March 2009: Data clean-up, LCI analysis and statistical analysis
- April 2009-June 2009: Thesis write-up
- June 2009 –July 2009: Thesis write-up submitted to thesis committee members for review and thesis write-up revised based on thesis committee members' comments
- August 9, 2009: Final copy of thesis to be submitted to thesis committee members, internal and external reviewers and program chair
- September 9, 2009: Thesis defense date tentatively booked