



## Early View

Original article

# Effects of exercise and airway clearance (PEP) on mucus clearance in cystic fibrosis: a randomised cross-over trial

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## **Title page**

### **Title**

Effects of exercise and airway clearance (PEP) on mucus clearance in cystic fibrosis: a randomised cross-over trial

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**Take home message:** Exercise and PEP therapy significantly improved mucus clearance in CF, but PEP therapy cleared more mucus than exercise alone.

**Abbreviated title:** Exercise and mucus clearance in CF

**Key words:** Cystic fibrosis; exercise; airway clearance; physiotherapy; positive expiratory pressure (PEP); mucus clearance

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## **Abstract**

Exercise improves mucus clearance in people without lung disease and those with chronic bronchitis. No study has investigated exercise alone for mucus clearance in cystic fibrosis (CF). The aim was to compare treadmill exercise to resting breathing and airway clearance with Positive Expiratory Pressure (PEP) therapy, on mucus clearance in adults with CF.

Fourteen adults with mild to severe CF lung disease (FEV<sub>1</sub> 31-113% predicted) completed a three-day randomised, controlled, cross-over trial. Interventions were 20 minutes of: resting breathing (control); treadmill exercise at 60% of the participant's peak oxygen consumption; and PEP therapy (including huffing and coughing).

Mucus clearance was measured using the radioaerosol technique and gamma camera imaging.

Treadmill exercise improved whole lung mucus clearance compared to resting breathing (mean difference 3%, 95%CI 2 to 4), however exercise alone was less effective than PEP therapy (mean difference -7%, 95%CI -6 to -8). When comparing treadmill exercise to PEP therapy, there were no significant differences in mucus clearance from the intermediate and peripheral lung regions, but significantly less clearance from the central lung region (likely reflecting the huffing and coughing that was only in PEP therapy).

It is recommended to include huffing and coughing to maximise mucus clearance with exercise.

## Text

### Introduction

People with cystic fibrosis (CF) produce large amounts of thick mucus that is not cleared normally from the lungs, resulting in mucus retention and chronic lung damage.[1]

Treatments to improve mucus clearance, including airway clearance therapies, remain a cornerstone of the standards of care and the respiratory management of CF lung disease.[2-5]

These time consuming therapies are required daily and adults with CF report performing an average of 108 minutes of treatment each day, with the majority of time taken up with airway clearance and exercise.[6] Exercise may aid secretion clearance in patients with CF,[7-9]

however there is no conclusive evidence to show if exercise can act as a substitute for established airway clearance treatments. Treatment time and burden for patients with CF would be reduced if exercise could replace airway clearance time whilst still reaping the known benefits of exercise training.[10]

The most widely accepted technique to measure mucus clearance is the assessment of bronchial mucus transport, using a radioaerosol technique and imaging with a gamma camera.[11, 12] Mucus clearance measured in this way is not confounded by sputum that is swallowed or saliva mixed with expectorated sputum. Individual physiological studies, using this measurement procedure, have investigated the effects of exercise and airway clearance techniques on mucus clearance, compared to no intervention. Exercise has been shown to significantly increase mucus clearance in people without lung disease[13] and in adults with chronic bronchitis.[14] Several airway clearance techniques have been shown to significantly increase mucus clearance in adults with CF, including “conventional chest physiotherapy” (postural drainage combined with percussion and vibration),[15, 16] the forced expiratory technique (FET, which is relaxed breathing and huffing),[17, 18] and positive expiratory

pressure (PEP) therapy.[17, 18] No specific airway clearance technique has been shown to be significantly more effective than other techniques.[15-19] Participants, however, report higher preference for techniques that can be done independently, such as with the use of PEP devices, compared to techniques performed on the patient by a health care professional or carer, such as percussion and vibration.[20]

To date, no study has measured the effect of exercise alone on mucus clearance in adults with CF, nor compared exercise alone to an established airway clearance technique. For this study we have chosen to use PEP therapy as the airway clearance technique, as it has demonstrated both good efficacy and patient preference.[20] Therefore, the aim of this study was to determine the effect of treadmill exercise, compared to resting breathing (control) and PEP therapy, on mucus clearance and subjective responses in adults with CF. We hypothesised that treadmill exercise would be more effective than no intervention (control) and that treadmill exercise would be similarly effective to PEP therapy.

## Materials and Methods

### **Study design**

A randomised, cross-over trial was conducted with four visits (Figure 1). Participants were recruited from the Adult CF Clinic at Royal Prince Alfred Hospital, Sydney, Australia and an advertisement in the CF NSW newsletter. On Visit 1, lung function and exercise capacity assessments were made prior to randomisation. Intervention order (one intervention per Visit 2, 3 and 4) was determined by computer-generated randomisation, performed by a person not involved in the interventions and stored in sealed, sequentially numbered opaque envelopes. The three interventions were: constant-load treadmill exercise; PEP plus the forced expiratory technique (FET) (from now on referred to as PEP therapy); and resting breathing (control).

Each intervention was performed for 20 minutes.

In order to standardise procedures, Visits 2, 3, and 4 were scheduled at the same time in the afternoon, separated by at least 48 hours and within a two-week period (during which medication, airway clearance and exercise regimens were unchanged). Participants were also asked to withhold routine mucolytic therapy, and not perform any airway clearance or exercise until after completion of all procedures on a trial day. Participants withheld beta-agonist medication for at least eight hours before any study visit, unless they had a history of exercise-induced bronchoconstriction. Those participants took 200µg of salbutamol via metered dose inhaler and spacer 30 minutes before the peak treadmill test on Visit 1 and the interventions on Visits 2, 3, and 4. Outcomes were measured before, immediately after the interventions and during a 60-minute period following the interventions. All outcome measures were later analysed by an assessor blinded to the intervention.

This trial was registered with the Australian and New Zealand Clinical Trials Registry (#ACTRN12608000287336). Research procedures were approved by the Sydney South West Area Health Service Ethics Committee (RPAH Zone) (Protocol X08-0030) and participants provided written informed consent prior to data collection.

## **Participants**

Patients were eligible for inclusion if they were at least 17 years old, had a confirmed diagnosis of CF (genetic testing and/or previous positive sweat test) and their treating physician deemed them to be clinically stable.[21] Patients were excluded if they had received a lung transplant, were infected with *Burkholderia cepacia* complex, were pregnant or if they had exceeded the dose constraints from radiation exposure additional to normal clinical



management, according to the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) Code of Practice for exposure of humans to ionizing radiation for research purposes (RPS8, 2005). Characteristics of participants (height, weight, spirometry, lung volumes and treadmill peak exercise capacity) were measured on Visit 1 to describe the sample and individually prescribe the treadmill exercise intervention.

### **Interventions**

For the exercise intervention, participants exercised on the treadmill for 20 minutes at a constant work rate equivalent to 60% of the peak oxygen consumption ( $\text{VO}_2$ ) achieved in the incremental peak treadmill test on Visit 1. This intensity and duration were chosen to replicate a typical prescription used for exercise training.[22]

The PEP therapy intervention consisted of breathing through the PEP device for 15 breaths, followed by relaxed and deep breathing, huffing and coughing, according to the FET.[23]

This cycle was repeated six times. The PEP hole diameter was chosen for each participant that achieved 10-20cmH<sub>2</sub>O in mid expiration.[24] Participants were taught to use the mouthpiece PEP device (PARI PEP System II, PARI, Starnberg, Germany) by a senior physiotherapist. If participants used PEP on a regular basis, any corrections to their technique were made if necessary.

For the control intervention, participants received no intervention, i.e. they sat quietly for 20 minutes.

## **Outcome measures**

### *Mucus clearance*

At each Visit 2, 3, and 4, subjects underwent a mucus clearance scan, which involved the inhalation of <sup>99m</sup>Tc-labelled sulphur colloid. The procedures were: 1) radioaerosol inhalation; 2) dynamic imaging over 10 minutes in order to assess initial deposition and baseline clearance of the radioaerosol; 3) 20-minute intervention; 4) dynamic imaging over 60 minutes in order to assess post-intervention mucus clearance.

The primary outcome was mucus clearance, as measured using the radioaerosol technique and dynamic imaging with a double-headed gamma camera (ECAM, Siemens, Illinois, USA) as reported previously by our group.[25-33] The radioaerosol, <sup>99m</sup>Tc-sulphur colloid (CIS-US Inc., Bedford, Massachusetts, USA) was generated by a jet nebuliser (mass median aerodynamic diameter of the particles of 5.5µm, span 1.9; Medic-Aid, Peckham, UK) at 7L/min. Subjects inhaled the radioaerosol with a controlled breathing pattern following a target on a computer screen aimed at maximising deposition in the conducting airways: 450mL tidal volume, short inspiratory time (0.6-1.4s, individually set according to the participant's airway obstruction, with shorter time for those with less airway obstruction), 0.2s inspiratory hold time and 2s expiratory time. The breathing pattern aimed at a peak inspiratory flow of approximately 45 L/min. Approximately five minutes after the radioaerosol inhalation, simultaneous anterior and posterior dynamic imaging were commenced with a 1-minute frame rate and the patient in a supine position. In addition, on Visit 2 only, the participant's lung fields were delineated from a transmission scan with a cobalt-57 sheet source, before any other procedures were undertaken.

All images were decay-corrected to the imaging start time. The anterior and posterior

emission images were combined into geometric mean images. The right lung was divided into central, intermediate and peripheral regions. The central region was a rectangle comprising the middle half of the vertical and horizontal dimensions of the right lung and the intermediate and peripheral regions were concentric bands surrounding the central region.[34] The defined regions were stored as a template for use on each mucus clearance scan. The initial lung radioaerosol distribution was defined in terms of the penetration index, which is the ratio of mean counts per pixel in the peripheral region to mean counts per pixel in the central region, multiplied by 100 (with lower numbers representing a more central deposition of the radioaerosol). The first image was obtained five minutes after the radioaerosol inhalation. The total counts of the whole right lung and defined regions in the dynamic emission geometric mean images were expressed as a percentage of the counts at the end of the 10-minute baseline scan (i.e. 100% retained immediately before the intervention). Activity that had not been retained had been cleared. The mucus clearance scans were later analysed by an assessor blinded to the interventions.

### *Cough*

All coughs (spontaneous and those directed, according to the FET) were manually counted during each 20-minute intervention and 60-minute follow-up recovery period.

### *Sense of chest congestion*

Participants recorded their subjective sense of chest congestion on 10cm visual analogue scales (0=very congested, 10=very clear) immediately before, during and at the end of 60-minutes following the intervention. The visual analogue scales were later measured by an assessor blinded to the interventions.

## **Data analysis**

For the mucus clearance scans, a random coefficients model was fitted using General Linear mixed models to model the relationship between the percentage of retention and time after the 20-minute intervention. The primary outcome was the mucus cleared immediately after finishing the 20-minute intervention, measured by the intercept of the regression coefficient of retention. The secondary outcomes were the mean retention over the 60 minutes following the 20-minute intervention, the subjective sense of chest congestion and the number of coughs. Repeated measures ANOVA were performed on the secondary outcome measures to compare differences between the interventions. Statistical significance was set at  $p < 0.05$ .

Data from a previous study involving eight subjects with chronic bronchitis resulted in a mean within-subject increase in mucus clearance with exercise of 7.5 (SD 5.8).[14] Sample size calculations showed that 13 participants would be required to provide 90% power to detect the anticipated between group differences in the primary outcome measure as significant ( $\alpha=0.05$ ). We sought to recruit 15 participants to allow for a 15% dropout and increase precision around our estimates.

## Results

### **Flow of participants through the study**

Fifteen adults with mild to severe CF lung disease were recruited and 14 completed the study (one participant withdrew after Visit 2 due to an allergic reaction that may have been a delayed response from the inhaled radioaerosol). Participant baseline characteristics are presented in Table 1 and the supplementary material.[35-37] Routine mucolytic therapy was: hypertonic saline only for two participants; rhDNase only for three participants; both hypertonic saline and rhDNase for four participants; both denufosol and rhDNase for one

participant. No participant used mannitol and five participants did not use any mucolytic or osmotic medication. No participant was prescribed a CFTR corrector or potentiator medication. All but one participant were prescribed bronchodilator/s and/or inhaled corticosteroids. Five participants, with a history of exercise-induced bronchoconstriction, took 200µg of salbutamol 30 minutes before the intervention on Visits 2, 3 and 4. All 15 participants performed exercise regularly when well and 14 performed some form of airway clearance routinely (four only exercised; five performed established airway clearance techniques only and five performed a combination of exercise and airway clearance techniques), including eight who performed PEP therapy on a regular basis.

**Table 1.** Participant characteristics

Characteristic ( <i>n</i> =15)	Mean (SD)	Range
Age, <i>yr</i>	27 (9)	18 – 48
Sex, <i>number male (%)</i>	10 (67)	
BMI, <i>kg/m<sup>2</sup></i>	22.2 (2.7)	18.1 – 27.4
FEV <sub>1</sub> , <i>L</i>	2.45 (0.94)	1.16 – 4.78
FEV <sub>1</sub> , <i>predicted %</i>	65 (23)	31 – 113
FVC, <i>predicted %</i>	88 (18)	64 – 119
FEV <sub>1</sub> /FVC	0.61 (0.16)	0.34 – 0.84
RV/TLC, <i>%</i>	32 (11)	10 – 50
Treadmill peak VO <sub>2</sub> , <i>mL/kg/min</i>	36.1 (10.4)	18.9 – 53.7
Treadmill peak VO <sub>2</sub> , <i>predicted %</i>	94 (25)	49 – 136

Mean (SD) and range of participant characteristics. Forced expiratory volume in 1 second (FEV<sub>1</sub>), forced vital capacity (FVC)[37] and treadmill peak VO<sub>2</sub>[35, 36] expressed as a percentage of predicted values. Residual volume (RV) divided by total lung capacity (TLC) reflects the degree of air trapping.

There were no significant differences in the initial distribution of radioaerosol between the study days, with an average penetration index of 27.8 (SD 13.8) for all scans, meaning that approximately 3.5 times the amount of radioaerosol was deposited in the central region compared to the peripheral region. There were no significant differences in pre-intervention

mucus clearance during the 10-minute baseline scan between any of the interventions (Figure 2) and no carry-over or order effect between the interventions was detected.

### **Treatment descriptors**

Pulse rate, oxygen saturation and treatment descriptors (work rate and perceived intensity during treadmill exercise; [38, 39] PEP hole diameter and average expiratory pressure) for the 20-minute interventions are presented in Table 2. Treadmill exercise intensity was rated somewhat severe for breathlessness and strong/heavy for perceived exertion. All treatments were well-tolerated with no adverse events.

**Table 2.** Treatment descriptors

	PR (bpm)	SpO <sub>2</sub> (%)	Treatment descriptors
Control	83 ± 14	98 ± 2	resting breathing
Treadmill	134 ± 14	96 ± 3	5.7 km/hr ± 0.6 at 5% incline ± 3, dyspnoea 4 ± 2, RPE 5 ± 2
PEP	89 ± 12	97 ± 3	3.8 mm ± 0.5, 20 cmH <sub>2</sub> O ± 4

Data are presented as mean ± standard deviation for group values of the pulse rate (PR) and oxygen saturation (SpO<sub>2</sub>), and treatment descriptors (treadmill speed and incline, modified Borg dyspnoea [39] and modified 0-10-point rate of perceived exertion (RPE); [38] PEP hole diameter and average expiratory pressure). Treadmill work rate was set at the speed and incline equivalent to 60% of the participant's peak VO<sub>2</sub> achieved on Visit 1 of the study. PEP hole diameter was chosen for each participant that achieved 10-20cmH<sub>2</sub>O in mid expiration.

### **Effect of exercise**

Treadmill exercise cleared significantly more mucus during the intervention compared to control for the whole right lung, intermediate and peripheral regions (Table 3 and Figure 2) but there was no significant difference in the mucus cleared from the central region. When including the 60-minute follow-up period, treadmill exercise cleared significantly more mucus compared to control for the whole right lung, intermediate and peripheral regions (Table 3 and Figure 2), but significantly less mucus compared to control for the central region. The

improvement in mucus clearance with exercise was primarily achieved during the 20-minute intervention, as there was no persistent benefit in the 60-minute follow-up period.

There was a non-significant trend for participants to do more spontaneous coughs during treadmill exercise compared to control, but there were no differences in the number of coughs between the interventions in the 60-minute follow-up period (Table 4).

There were no significant differences in the change in sense of chest congestion following treadmill exercise compared to control either immediately after or 60 minutes following the intervention (Table 5).

**Table 3. Mucus clearance**

Outcome	Interventions			Difference between interventions		
	Tread (n = 15)	PEP (n = 14)	Con (n = 14)	Tread minus Con	PEP minus Con	Tread minus PEP
Whole lung						
Immed. post intervention, %	13.4 (7.1 to 19.7)	20.3 (14.0 to 26.7)	10.8 (5.5 to 16.1)	2.6* (1.6 to 3.6)	9.5* (8.5 to 10.5)	-6.9* (-5.9 to -7.9)
60-min post intervention, %	15.4 (9.7 to 21.0)	21.8 (16.2 to 27.5)	14.0 (8.4 to 19.7)	1.3* (0.8 to 1.8)	7.8* (7.3 to 8.3)	-6.5* (-7.0 to -6.0)
Central region						
Immed. post intervention, %	14.2 (6.1 to 22.3)	27.1 (19.0 to 35.2)	14.0 (7.3 to 20.7)	0.2 (-1.2 to 1.6)	13.1* (11.7 to 14.5)	-12.9* (-11.5 to -14.3)
60-min post intervention, %	15.7 (8.4 to 22.9)	28.4 (21.1 to 35.7)	17.1 (9.8 to 24.3)	-1.4* (-2.1 to -0.7)	11.3* (10.6 to 12.0)	-12.7* (-13.4 to -12.0)
Intermediate region						
Immed. post intervention, %	11.4 (6.3 to 16.5)	11.8 (6.7 to 16.9)	6.9 (2.7 to 11.1)	4.5* (3.6 to 5.4)	4.9* (4.0 to 5.8)	-0.4 (-1.3 to 0.5)
60-min post intervention, %	12.9 (8.5 to 17.2)	13.6 (9.2 to 17.9)	9.5 (5.2 to 13.9)	3.3* (2.9 to 3.8)	4.0* (3.6 to 4.5)	-0.7* (-1.2 to -0.3)
Peripheral region						
Immed. post intervention, %	14.2 (6.7 to 21.7)	15.5 (8.0 to 23.0)	7.5 (1.6 to 13.4)	6.7* (5.2 to 8.3)	8.0* (6.4 to 9.6)	-1.3 (-2.9 to 0.3)
60-min post intervention, %	19.4 (12.9 to 25.9)	19.0 (12.5 to 25.5)	13.4 (6.9 to 19.8)	6.1* (5.3 to 6.8)	5.6* (4.8 to 6.4)	0.4 (-0.4 to 1.2)

Mean (95% CI) of mucus clearance immediately after the 20-minute intervention, measured as the intercept of the regression co-efficient of retention, and the mean retention during the 60-minute resting breathing/recovery follow-up period. Tread=treadmill, Con=control.

\* $p < 0.001$ , † $p < 0.01$



**Table 4. Cough**

Outcome	Interventions			Difference between interventions		
	Tread (n = 15)	PEP (n = 14)	Con (n = 14)	Tread minus Con	PEP minus Con	Tread minus PEP
<i>Cough (number)</i>						
During 20-min intervention	16 (23)	73 (59)	4 (6)	13 (-2 to 27)	69* (33 to 105)	-56* (-87 to -26)
During 60-min follow-up	21 (43)	13 (20)	13 (15)	8 (-17 to 32)	0 (-11 to 11)	8 (-10 to 25)

Mean (SD) for group values and mean (95% CI) for between group differences of the number of coughs during the 20-minute intervention and the 60-minute resting breathing/recovery follow-up period. Tread=treadmill, Con=control. N.B. Participants were instructed to cough 18 times and huff 12 times during the PEP therapy intervention.

\* $p < 0.01$

**Table 5.** Sense of chest congestion

Outcome	Interventions									Difference between interventions					
	Pre			Immediately post (Post-0)			60-min post (Post-60)			Post-0 minus Pre			Post-60 minus Post-0		
	Tread (n = 15)	PEP (n = 14)	Con (n = 14)	Tread (n = 15)	PEP (n = 14)	Con (n = 14)	Tread (n = 15)	PEP (n = 14)	Con (n = 14)	Tread minus Con	PEP minus Con	Tread minus PEP	Tread minus Con	PEP minus Con	Tread minus PEP
Chest congestion VAS (cm)	5.2 (1.9)	4.2 (1.9)	5.9 (1.8)	6.1 (1.9)	5.9 (2.2)	6.4 (1.9)	5.4 (2.1)	6.1 (1.7)	6.0 (2.0)	0.5 (-0.3 to 1.3)	1.2 <sup>§</sup> (0.1 to 2.3)	0.7 <sup>§</sup> (0.1 to 1.4)	0.1 (-0.7 to 1.0)	1.8 <sup>*</sup> (0.9 to 2.7)	1.7 <sup>‡</sup> (0.7 to 2.7)

Mean (SD) for group values and mean (95% CI) for between group differences for the sense of chest congestion, measured on a 10cm VAS scale, where higher numbers represent less chest congestion and positive numbers represent a decrease in chest congestion from pre to post intervention. Tread=treadmill, Con=control.

\*  $p < 0.001$ , ‡  $p < 0.01$ , §  $p < 0.05$

### **Effect of PEP therapy**

PEP therapy cleared significantly more mucus during the intervention compared to control for the whole right lung and all lung regions (Table 3 and Figure 2). When including the 60-minute follow-up period, PEP therapy cleared significantly more mucus compared to control for the whole right lung and all lung regions (Table 3 and Figure 2). The improvement in mucus clearance with PEP therapy was primarily achieved during the 20-minute intervention, as there was no persistent benefit in the 60-minute follow-up period.

There were significantly more coughs with PEP therapy compared to control during the intervention but no significant difference in the 60 minutes following the interventions (Table 4).

There was significantly more improvement in sense of chest congestion following PEP therapy compared to control both immediately after the intervention and 60 minutes following the interventions (Table 5).

### **Effect of exercise compared to PEP therapy**

Treadmill exercise cleared significantly less mucus during the intervention compared to PEP therapy for the whole right lung and central region but there was no significant difference in the mucus cleared from the intermediate or peripheral regions (Table 3 and Figure 2). When including the 60-minute follow-up period, treadmill exercise cleared significantly less mucus compared to PEP therapy for the whole right lung, central and intermediate regions but there was no significant difference in the mucus cleared from the peripheral region.

There were significantly fewer coughs with treadmill exercise compared to PEP therapy

during the intervention (Table 4). There was no significant difference in the number of spontaneous coughs in the 60 minutes following the interventions (Table 4).

There was significantly less improvement in sense of chest congestion following treadmill exercise compared to PEP therapy both immediately after the intervention and 60 minutes following the interventions (Table 5).

### Discussion

This is the first study to have measured mucus clearance, using the gold standard inhaled radioaerosol technique, with exercise alone in adults with CF. The main findings were that although treadmill exercise significantly increased mucus clearance from the whole lung compared to no intervention, treadmill exercise was significantly less effective compared to PEP therapy. There were no significant differences, however, in the amount of mucus cleared from the intermediate and peripheral lung regions when comparing treadmill exercise and PEP therapy.

These results demonstrate that exercise alone does act as an effective independent airway clearance technique for adults with mild to severe CF lung disease in clearing mucus from the intermediate and peripheral lung regions. Importantly, however, less mucus was cleared from the central lung region following treadmill exercise alone compared to PEP therapy, most likely due to the FET component in PEP therapy that involved directed huffing and coughing. Participants did approximately 60 more coughs during the PEP intervention than during treadmill exercise, which in turn had approximately 10 more coughs than during the control intervention. The absence of a matched “cough control” day in our study prevents determining how much the improvement in mucus clearance was due to treadmill exercise

and PEP breathing, compared to the benefits achieved with cough alone. Previous studies have demonstrated that FET alone is an effective treatment [17, 40] and interventions that include FET and/or directed coughing clear mucus predominantly from the central regions.[16, 18, 41] Perhaps if FET or directed coughing had been included with the treadmill intervention there would have been more mucus cleared from the central region.

The baseline mucus clearance (i.e. that achieved during the resting breathing, control, intervention) in this study was very similar to that reported in the most comprehensive study of adults with CF (in our study approximately 15% cleared from the whole lung one hour post inhalation of the radioaerosol compared to 14% clearance in Robertson et al, 2000).[28] This amount of mucus clearance in people with CF is about half that of the group of healthy aged-matched control participants, which was reported as 28% clearance from the whole lung.[28] Despite the vast majority of the participants in our study carrying out well-established airway clearance routines (14/15 were prescribed mucolytic and/or osmotic medication and 14/15 performed some form of airway clearance regularly), it would appear that mucus clearance remains markedly reduced in adults with CF, further highlighting the ongoing need to optimise airway clearance therapies.

The improvements in mucus clearance with exercise and PEP therapy were primarily achieved during the 20-minute intervention. There was no persistent benefit or acceleration of mucus clearance created by the treatments in the 60-minute follow-up period, which is consistent with every previous study that has included a follow-up period after an airway clearance treatment in adults with CF.[15-18] In contrast, the mucolytic and osmotic medications hypertonic saline[27, 29] and mannitol,[30] improve mucus clearance immediately following the inhalation and continue to accelerate mucus clearance in the

follow-up period. One clinical implication is that, as there is no continued benefit to mucus clearance after the initial effects of airway clearance interventions, people with CF will need to regularly perform these treatments to maintain adequate mucus clearance.

The improvement in whole lung mucus clearance with exercise (approximately 3% more than during the control intervention) seen in our study was less than the 8-9% improvement found in earlier studies in people without lung disease[13] and adults with chronic bronchitis.[14] The improvement in the peripheral lung region mucus clearance, however, was similar (about 7% in our study compared to 6%).[14] It is not clear why there was a discrepancy in the effect for the whole lung, as participants in our study exercised at a similar intensity to those in the other two studies.[13, 14] The baseline mucus clearance in our study was greater than that seen in those with chronic bronchitis (in our study approximately 15% cleared from the whole lung one hour post inhalation of the radioaerosol compared to 10% clearance in those with chronic bronchitis).[14] Perhaps as most of the participants in our study already had well-established airway clearance routines, there was less potential for an improvement in mucus clearance with exercise. Another possibility is that in our study radioaerosol deposition was predominantly in the central lung region, which had the least improvement in mucus clearance, and thus may have led to a lowering of the overall whole lung mucus clearance, due to the greater relative contribution of this region. It should also be noted that as the mucus clearance from the peripheral and intermediate lung regions was greater with exercise than control, more mucus would have been entering the central region during the exercise intervention, which would likely lower the net mucus clearance from this region.

The improvement in whole lung mucus clearance with PEP therapy in our study (approximately 10% more than during the control intervention) is similar to the 5-20%

improvement that has been reported previously.[17, 18, 42] Also similar to previous research, the main increase in mucus clearance was noted in the central lung region.[18] Interestingly, trials that have compared PEP therapy to FET or directed coughing alone have shown no between group differences in mucus clearance[17, 43] or dry weight of expectorated sputum[44] immediately following the PEP interventions. Also, another study that investigated PEP therapy compared to PEP alone (i.e. no FET or directed coughing) found that although there were no between group differences in lung function, expectorated sputum weight or exacerbation frequency, participants reported PEP alone to be ineffective in clearing mucus.[45] In combination, these studies would suggest that the FET component of PEP therapy makes an important contribution to the efficacy of PEP therapy.

Exercise cleared significantly less mucus than PEP therapy for the whole lung and central lung region (approximately 7% and 13% respectively) in our study, with no significant differences in the intermediate or peripheral lung regions. The only other study which compared exercise alone to PEP therapy found that exercise alone produced significantly less sputum than PEP therapy.[46] This study, however, has only been published in abstract form and lacks details on CF participant characteristics, exercise intensity, PEP protocol and results data. Two studies have compared exercise plus FET or directed coughing to PEP therapy and found no between group differences in mucus clearance[19] or expectorated sputum weight.[47] It needs to be noted, however, that all of these studies, including our study, involved participants who were not taking CFTR modulators. Considering the likely improvement in mucus clearance with these highly effective therapies, it is possible that the effects of airway clearance techniques may change once patients with CF are routinely prescribed these new medications.

This study has demonstrated that a single bout of treadmill exercise improved whole lung mucus clearance compared to no intervention, however exercise alone was less effective than the well-established airway clearance technique of PEP therapy, which includes huffing and coughing. In terms of the effects of the two interventions on the different lung regions, there were no differences in the amount of mucus cleared from the peripheral and intermediate regions, yet significantly less mucus cleared from the central region with exercise alone. This difference was most likely due to the huffing and coughing in the FET component of PEP therapy. In clinical practice, therefore, it would be recommended to include FET with exercise if the aim were to improve central and whole lung mucus clearance. Longer term studies investigating exercise (with huffing and coughing) as a stand-alone airway clearance technique are required to determine if it is as effective as established airway clearance techniques on clinically-important outcomes, such as exacerbation frequency, quality of life and exercise capacity, which are related to morbidity and mortality in CF.



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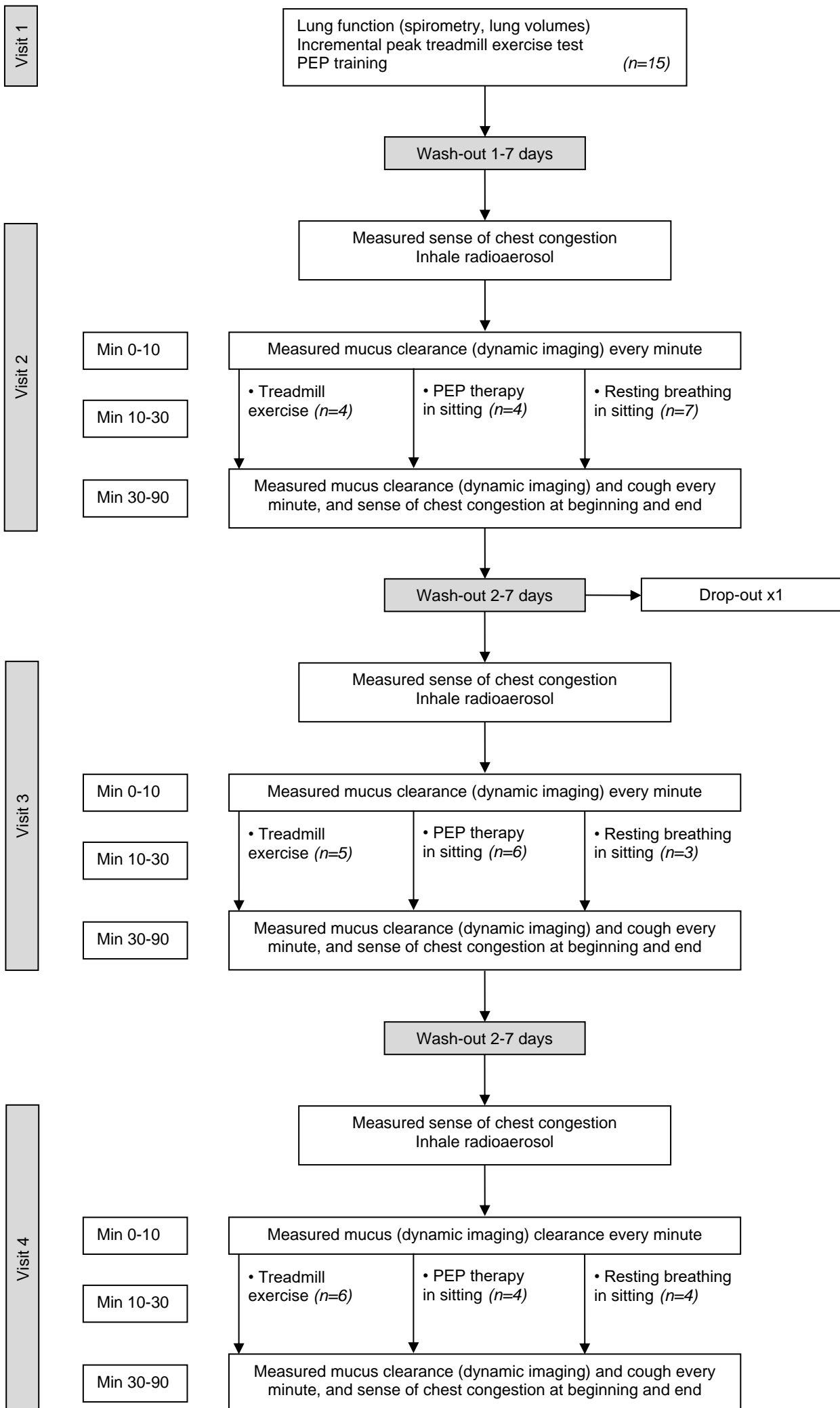
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## **Figure legends**

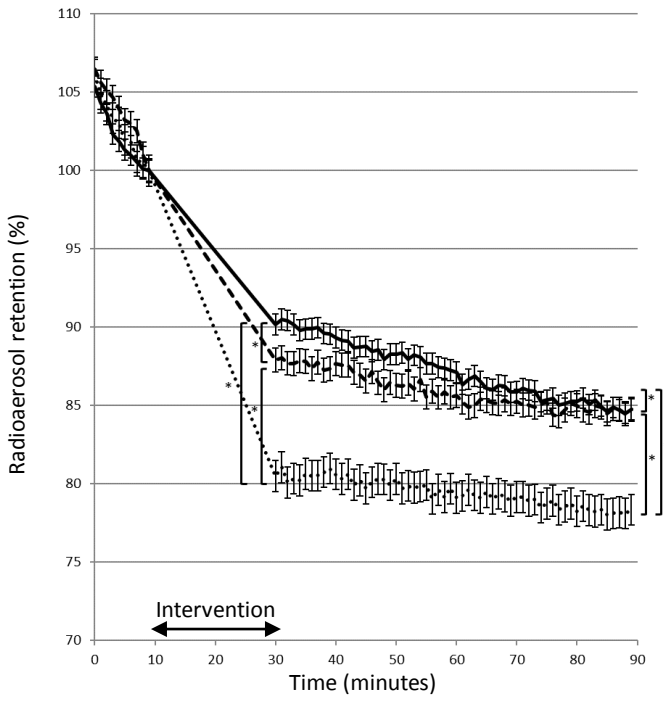
**Figure 1:** Design and flow of participants through the trial.

**Figure 2:** Mucus clearance scans for the control (solid line), treadmill exercise (dashed line), and PEP therapy (dotted line) interventions (mean  $\pm$  SE), expressed as percentage of radioaerosol retention immediately before the intervention. a) Whole right lung; b) Central lung region; c) Intermediate lung region; d) Peripheral lung region.

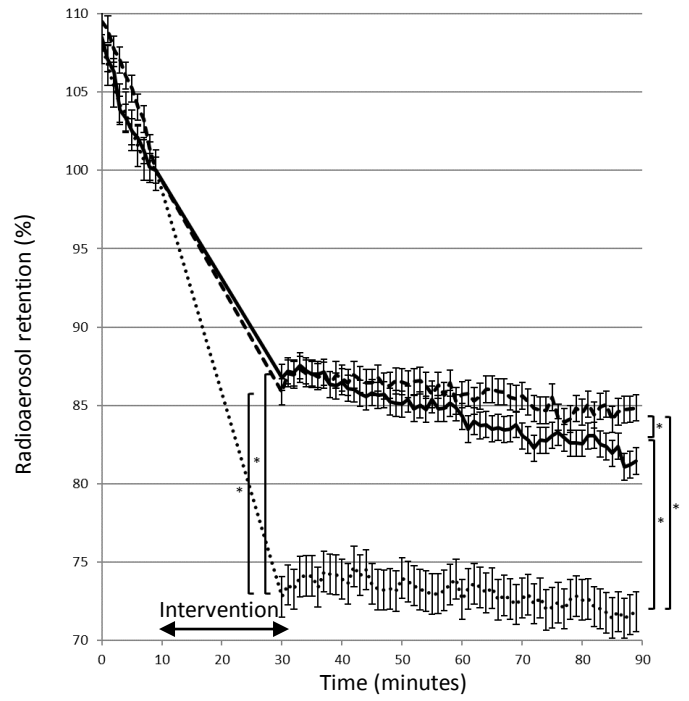
\*  $p < 0.01$



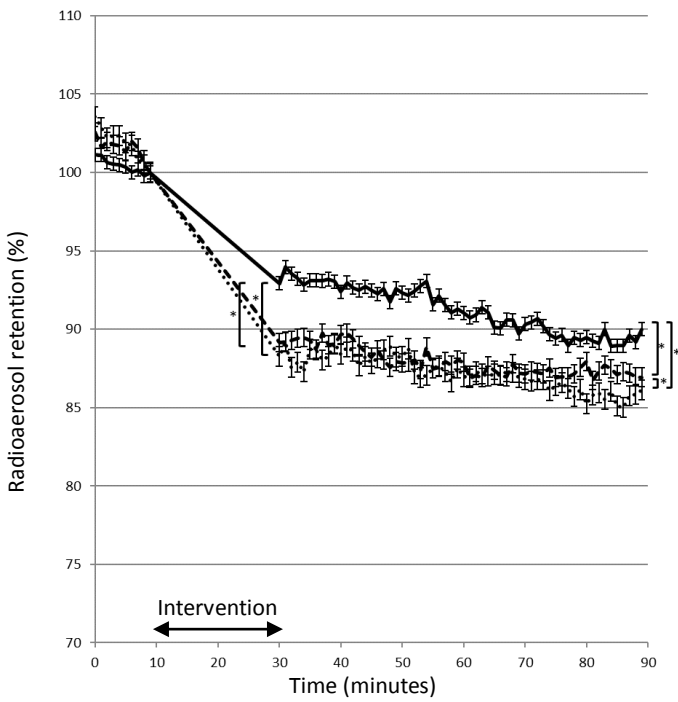
a) Whole right lung



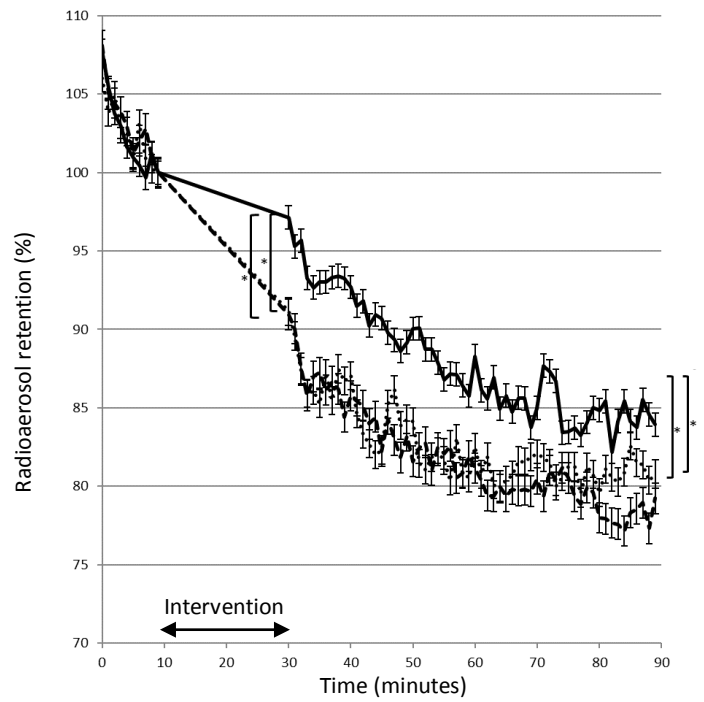
b) Central region



c) Intermediate region



d) Peripheral region



— Control  
- - - Treadmill  
..... PEP therapy  
\*  $p < 0.01$

Item	MCC 01			MCC 02			MCC 03			MCC 04			MCC 05			MCC 06			MCC 07			MCC 08			MCC 09			MCC 10			MCC 11			MCC 12			MCC 13			MCC 14			MCC 15				
	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET	Control	Environ	PEP/FET		
20	95.42	98.84	94.84	85.22	84.78	85.61	92.39	94.94	93.28	91.79	85.23	87.58	81.78	85.32	90.03	97.05	96.41	98.75	87.93	90.27	71.35	82.48	85.62	85.90	92.74	98.68	75.70	95.21	86.36	89.38	82.70	82.57	71.05	91.46	89.80	82.51	95.97	97.51	97.71	88.44	85.20	85.06	drop out	70.39	drop out		
21	100.66	99.05	95.16	87.04	85.64	86.66	93.61	94.57	94.78	91.22	84.87	87.76	81.41	84.29	90.03	97.50	96.72	97.50	87.71	89.69	70.95	81.87	86.82	86.80	93.86	88.50	77.44	95.26	86.17	89.83	82.31	81.78	71.43	91.18	90.53	84.78	96.44	97.84	87.64	88.60	87.71	87.04	85.04	82.69	drop out	71.07	drop out
22	100.23	98.27	96.23	88.28	84.95	87.46	92.93	94.17	92.89	91.55	84.77	87.58	81.01	84.15	89.94	95.02	97.39	94.89	87.33	90.26	70.64	82.01	86.35	85.91	93.98	88.95	77.01	94.72	85.25	89.03	82.96	82.65	70.12	91.49	89.83	82.86	97.70	95.45	87.15	88.58	85.04	82.69	drop out	71.07	drop out		
23	99.30	98.81	95.17	87.39	86.69	87.21	90.47	90.70	93.49	91.02	85.67	86.75	81.36	83.01	85.31	97.48	98.05	95.26	86.08	88.23	71.07	81.41	85.03	86.04	95.05	86.68	77.08	95.65	86.95	88.28	81.53	78.75	89.44	92.09	86.65	83.02	94.98	92.27	94.82	87.89	85.83	84.69	drop out	70.11	drop out		
24	99.02	100.20	95.97	87.48	84.32	88.88	90.80	95.25	91.92	90.89	84.84	86.53	81.46	81.36	85.03	94.79	96.41	96.05	87.66	90.89	70.75	82.12	85.03	86.07	94.53	86.68	77.17	94.26	86.55	88.39	82.77	80.65	89.18	91.57	89.22	82.83	95.27	95.99	96.45	87.60	88.18	84.92	drop out	69.32	drop out		
25	99.95	98.34	96.25	87.41	84.36	87.07	90.83	95.18	91.79	91.85	85.15	85.88	81.66	83.53	93.62	96.58	97.19	96.74	86.75	89.94	71.26	81.08	86.58	86.15	94.57	88.07	78.53	94.84	86.30	89.43	83.74	82.31	90.21	91.13	81.31	94.56	95.28	94.46	87.76	87.64	88.39	drop out	69.84	drop out			
26	99.38	99.76	96.24	89.00	86.09	86.83	89.35	93.06	92.17	90.09	85.09	84.18	79.52	83.38	90.18	97.77	96.69	94.47	86.67	89.69	71.52	81.34	86.18	85.29	94.61	86.68	78.34	94.10	86.19	88.79	80.60	89.10	93.67	80.60	85.10	97.41	95.49	95.08	84.21	86.29	90.14	drop out	70.83	drop out			
27	99.11	100.14	96.09	88.80	86.08	86.78	90.71	93.29	92.31	90.78	84.18	84.32	80.62	83.48	90.50	98.29	97.52	95.02	86.55	91.49	73.16	80.78	86.27	85.02	94.61	86.65	79.56	95.22	86.06	86.89	80.52	81.20	70.67	92.37	80.37	82.40	94.95	97.61	86.95	85.23	86.23	84.04	drop out	69.93	drop out		
28	99.28	99.88	96.67	84.94	84.13	85.15	86.26	93.68	94.38	91.66	85.62	84.08	80.52	83.40	85.64	97.21	96.25	94.89	86.43	86.59	71.86	80.65	86.09	85.12	92.85	86.25	78.87	94.43	85.65	88.18	79.26	80.02	70.98	91.89	90.33	82.34	96.41	94.15	86.76	85.61	85.76	85.68	drop out	69.23	drop out		
29	99.20	98.47	97.31	87.77	85.04	86.18	91.32	94.48	93.79	90.33	85.08	83.41	79.79	82.38	90.20	96.85	97.35	95.44	86.85	90.54	72.31	80.62	85.62	85.61	95.22	86.65	79.42	94.11	86.66	87.28	78.23	81.12	74.22	88.19	94.10	96.98	94.23	85.88	87.09	90.76	drop out	70.28	drop out				
30	99.50	99.29	95.67	87.49	83.98	86.35	90.29	94.21	93.33	90.88	83.53	82.89	79.89	83.20	90.42	96.85	98.94	93.68	85.96	91.73	72.19	80.56	86.46	84.71	94.46	86.60	79.10	94.29	86.62	87.78	77.92	81.17	71.35	90.85	91.40	84.48	94.78	94.46	86.82	89.89	85.05	89.61	drop out	68.46	drop out		
31	99.12	99.02	96.62	84.91	83.77	85.22	92.66	91.49	93.18	90.48	79.28	82.97	80.47	81.76	84.03	96.37	93.70	91.35	79.16	86.15	84.66	84.24	87.01	84.78	84.78	85.22	86.63	94.71	81.89	91.84	91.44	83.88	94.72	91.74	91.44	94.44	95.21	86.86	89.74	88.12	86.54	drop out	69.64	drop out			
32	98.83	100.07	96.51	86.88	84.47	86.82	90.89	94.23	93.83	90.71	83.03	83.03	79.45	82.43	89.80	96.70	97.74	94.51	86.28	89.94	72.96	80.09	85.41	84.13	91.78	98.14	79.19	94.22	84.64	87.32	76.78	82.52	70.31	92.71	88.19	82.47	95.15	97.62	87.16	86.33	88.00	85.33	drop out	69.07	drop out		
33	98.86	99.24	96.85	86.77	84.18	86.56	90.71	92.67	93.05	90.42	83.03	81.57	80.05	82.40	89.66	97.37	97.64	94.09	86.03	89.28	71.84	79.98	86.40	83.68	89.62	86.91	79.20	95.00	86.28	87.42	76.17	80.68	71.14	91.23	89.46	82.37	93.50	95.64	86.20	85.90	85.85	drop out	69.07	drop out			
34	98.46	99.95	96.64	86.74	84.36	87.09	90.31	95.80	93.22	90.19	84.56	82.85	79.21	82.32	89.28	97.65	97.47	93.01	85.49	93.12	72.95	79.40	85.93	84.64	93.48	86.68	80.82	93.48	86.06	87.29	77.41	75.70	71.15	93.29	89.48	82.98	95.97	94.59	85.43	84.64	85.78	drop out	68.22	drop out			
35	98.88	100.24	96.09	86.80	83.01	85.72	90.86	94.33	93.26	91.70	82.80	82.18	79.83	82.72	90.14	96.10	97.88	92.57	86.65	90.71	72.09	79.33	85.03	83.48	89.13	94.39	78.39	93.86	85.42	87.32	77.56	79.96	70.26	90.76	87.17	81.78	94.42	93.68	95.01	85.99	83.36	85.05	drop out	68.16	drop out		
36	98.94	98.91	96.11	86.28	82.90	87.12	90.42	94.58	92.66	91.06	83.08	82.24	79.36	82.58	89.62	96.23	92.12	93.69	85.11	90.26	72.68	79.61	85.27	84.91	90.48	87.07	79.48	93.21	80.61	89.80	90.13	88.80	82.81	94.42	93.62	83.44	85.67	85.21	89.10	87.69	drop out	67.98	drop out				
37	97.19	98.84	96.34	87.29	82.97	87.96	90.91	91.36	93.77	90.64	82.77	82.20	78.90	82.38	93.44	97.66	97.10	93.40	85.31	89.91	72.96	80.27	84.64	84.56	93.50	86.79	79.08	93.65	85.63	86.63	77.06	79.18	89.90	90.87	89.92	82.76	94.24	93.81	87.76	85.22	89.09	93.84	drop out	67.38	drop out		
38	98.66	99.62	96.47	84.14	83.33	87.91	89.98	90.47	93.87	90.19	82.54	81.13	77.95	82.67	84.03	97.01	96.73	93.37	84.99	89.48	72.10	80.23	81.21	84.42	88.78	87.04	81.26	93.11	86.05	87.66	76.28	78.82	70.45	90.24	87.85	82.03	92.18	92.16	92.18	84.28	85.99	90.56	drop out	67.78	drop out		
39	97.98	99.67	96.64	87.08	82.93	85.65	89.40	90.07	93.18	90.46	82.57	81.64	77.69	82.50	85.01	94.89	98.04	92.67	84.21	89.91	72.85	80.34	80.21	84.37	90.75	87.02	81.01	94.09	85.28	87.38	77.47	80.15	70.43	90.42	86.37	79.94	93.37	93.94	93.30	89.59	85.97	89.99	drop out	67.13	drop out		
40	98.95	99.59	98.03	85.03	83.80	86.14	91.31	90.79	92.10	90.92	83.68	83.68	78.91	81.44	85.36	95.92	98.01	95.16	94.80	89.61	72.66	80.33	83.94	85.28	93.23	87.13	78.48	93.90	85.45	87.86	79.82	79.77	71.24	90.69	89.10	92.71	94.19	93.73	94.88	94.88	89.22	85.45	86.89	drop out	66.89	drop out	
41	98.54	99.37	98.04	86.19	82.65	86.41	90.25	90.30	92.06	90.62	83.45	83.45	78.18	82.23	84.77	97.90	98.10	93.97	84.28	90.07	72.40	80.72	80.87	84.51	92.06	86.98	80.06	93.31	85.85	89.07	76.31	79.52	69.58	88.48	89.44	87.24	82.77	94.36	94.20	86.07	85.60	83.82	drop out	66.67	drop out		
42	99.39	99.09	97.40	86.85	82.41	85.76	90.70	89.89	92.17	90.32	83.65	83.76	78.04	81.18	89.86	96.39	98.75	93.13	84.13	89.28	71.40	80.63	79.90	82.65	91.68	87.98	79.14	91.86	85.97	88.23	75.19	80.75	70.32	89.43	89.42	83.23	94.34	94.40	95.64	83.76	87.69	88.67	drop out	66.26	drop out		
43	97.65	99.77	95.73	85.87	82.74	86.69	91.41	89.16	91.62	90.76	82.74	81.02	77.64	82.38	85.93	95.54	98.52																														

Subject ID	FEV <sub>1</sub> (L)	FVC (L)	FEV <sub>1</sub> /FVC (%)	MMF <sub>25</sub> (L)	MMF <sub>50</sub> (L)	TLC (L)	TLC (L) (pred)	VC (L)	IC (L)	FRC (L)	FRC (L) (pred)	RV (L)	RV (L) (pred)	VO <sub>2</sub> (L/min)	VO <sub>2</sub> (L/min)	Typical day SAS Exposure <sup>a</sup> (hr)	Routine Musculars	Prescribed Bronchodilators and Preventers	Bronchodilators and Preventers in Morning of Day 4	β <sub>2</sub> -agonist 30 min Pre-treatment Day 4	Routine Airway Clearance	Regular Exercise	Prevention Order		Penetration Index		Timepoint		PEP				
																							Day 2	Day 4	Control	PEP+PEP	Timepoint	Timepoint	Control	PEP			
MCC_01	3.21	4.25	103	69	1.36	5.23	1.52	1.14	3.39	2.91	1.28	35.4	87	6.3	8.0	4	salbutamol	salbutamol/formoterol	salbutamol/formoterol	salbutamol (2 x 100µg)	PEP, exercise	control	headb	PEP+PEP	26.3	47.5	35.3	5.5	8	4.0	2.0	2.0	
MCC_02	2.94	4.20	111	61	1.31	5.97	1.10	4.09	3.60	2.94	9.6	11.0	1.07	30.5	7.9	2.4	6.1	salbutamol	salbutamol	salbutamol (2 x 100µg)	PEP, exercise	control	headb	PEP+PEP	26.7	24.1	29.1	3.6	8	3.0	1.0	1.0	
MCC_03	1.51	4.41	75	34	0.95	7.29	9.6	3.72	9.5	2.03	5.27	1.30	2.39	19.4	15.1	4.9	7.3	2.0	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, exercise	control	headb	PEP+PEP	34.7	33.8	25.2	6.0	10	4.0	1.0	1.0
MCC_04	1.16	2.93	71	40	0.77	5.26	1.10	2.70	7.1	3.58	1.20	0.80	1.07	27.1	7.0	5.5	2.0	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, exercise/vibration, exercise	control	headb	PEP+PEP	18.0	13.4	15.8	5.0	2	3.0	1.0	1.0	
MCC_05	3.16	5.02	95	63	1.41	7.71	1.10	5.41	3.63	4.04	1.10	2.30	1.37	53.0	1.0	8.7	8.4	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, exercise	control	headb	PEP+PEP	19.4	17.1	12.8	6.8	8	4.0	1.0	1.0	
MCC_06	4.78	6.70	110	84	1.61	7.22	1.48	6.80	4.61	3.21	1.22	1.34	1.20	63.2	12.2	6.4	8.4	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, exercise	control	headb	PEP+PEP	18.0	62.7	76.2	7.0	8	5.0	2.0	2.0	
MCC_07	2.00	2.80	75	69	1.16	4.51	8.0	2.96	7.4	2.12	2.20	7.7	1.50	101	36.2	9.1	6.4	2.0	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, Fluticasone, exercise	control	headb	PEP+PEP	22.5	22.4	26.0	5.1	8	4.0	1.0	1.0
MCC_08	1.90	3.10	74	50	0.92	6.22	1.48	4.60	8.0	4.01	3.01	1.02	1.60	111	20.2	8.1	7.4	2.0	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, Fluticasone, ACBT	control	headb	PEP+PEP	24.1	18.0	16.2	6.8	2	3.0	1.0	1.0
MCC_09	3.47	4.30	84	81	1.37	6.20	1.02	4.55	9.6	3.14	2.13	3.30	1.30	42.4	11.3	4.8	7.4	salbutamol, formoterol, salbutamol	salbutamol, formoterol, salbutamol	salbutamol, formoterol, salbutamol	PEP, exercise	control	headb	PEP+PEP	33.4	30.0	24.2	6.1	8	4.0	2.0	2.0	
MCC_10	2.20	3.10	82	48	0.80	6.80	1.02	4.0	3.00	4.20	3.20	1.20	1.00	111	20.2	8.1	7.4	2.0	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, exercise	control	headb	PEP+PEP	24.1	18.0	16.2	6.8	2	3.0	1.0	1.0
MCC_11	2.02	4.10	82	46	0.80	6.80	1.02	4.0	3.00	4.20	3.20	1.20	1.00	111	20.2	8.1	7.4	2.0	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, exercise	control	headb	PEP+PEP	33.4	30.0	24.2	6.1	8	4.0	2.0	2.0
MCC_12	2.04	3.20	85	80	1.01	4.14	8.0	3.74	1.91	6.4	0.42	3.1	46.0	122	5.4	8.4	8.4	2.0	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, exercise	control	headb	PEP+PEP	17.7	21.0	23.8	5.7	8	3.0	2.0	2.0
MCC_13	2.64	3.20	82	82	1.01	4.59	8.0	2.44	8.0	1.98	5.1	1.03	7.1	32.2	8.4	4.8	6.0	salbutamol, fluticasone	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	PEP, percussive vibration	control	headb	PEP+PEP	33.4	34.2	36.8	5.8	4	3.0	1.0	1.0	
MCC_14	1.34	3.30	64	40	0.89	6.88	9.6	3.48	8.0	1.90	1.22	1.42	1.47	33.1	9.3	7.3	2.4	2.0	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, percussive vibration	control	headb	PEP+PEP	22.5	19.3	21.4	5.8	4	3.0	2.0	2.0
MCC_15	2.00	4.10	119	49	0.90	5.51	1.02	4.09	3.30	1.10	1.18	1.8	8.4	29.1	7.0	2.4	5.0	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol, salbutamol	fluticasone propionate/salbutamol	PEP, percussive vibration	control	headb	PEP+PEP	22.5	19.3	21.4	5.8	4	3.0	2.0	2.0	







