**ONLINE DATA SUPPLEMENT**

**METHODS**

**Subjects**

Healthy adult subjects and clinically stable patients, with cystic fibrosis were studied. Ethical approval was given by the Research Ethics Committee King’s College Hospital NHS Trust and the informed, written consent was obtained from all subjects. None of the CF patients were taking oral corticosteroids at the time of the study.

**Pulmonary function**

Spirometry [FEV1, and slow vital capacity (VC)] was measured in all subjects [S1] (Vitalograph Ltd. Buckingham, UK). Additionally in the CF patients peak expiratory flow (PEF), total lung capacity (TLC) and residual volume (RV) measured by body plethysmography [S2] (Masterscreen Body Box, Jaeger, Frieburg, Germany) and earlobe blood gas samples were obtained with the patient resting and breathing room air and analysed using a calibrated earlobe gas analyser (Chrion Diagnostics, Medfield, USA).

**Electromyographic measurements**

sEMGpara and EMGdi were recorded at rest and during exercise in all subjects. sEMGpara was recorded from bipolar surface electrodes (Kendall, Tyco healthcare, Germany) placed 3cm bilaterally from the midpoint of the sternum in the second intercostal spaces (positive electrode on the right side of the chest). The reference electrode was placed on the lateral aspect of the clavicle [S3]. EMGdi was recorded from the crural diaphragm using a multipair oesophageal electrode catheter [S4-7]. The electrode catheter consisted of nine consecutive recording coils. Each coil was 10mm in length with 0.5mm space between adjacent recording electrodes. The external diameter of the catheter was 2mm [8].

All EMG signals were amplified and band pass filtered between 10 Hz and 3kHz (Biomedical Amplifier Pclab-3808, Guangzhou Yinghui Medical) and subsequently acquired by computer running Chart software (version 5.4, ADInsturments Pty, Castle Hill, Australia) with analogue to digital sampling at 2kHz (Power Lab 16s, ADInsturments Pty, Castle Hill, Australia). Post acquisition band pass filtering between 20Hz and 1 kHz was applied to all recordings using the acquisition software. Peak root mean square per breath was calculated and averaged over one minute. EMG recordings at rest and during exercise were normalised to the EMG signal obtained during a maximal volitional manoeuvre. Four different maximal volitional manoeuvres were performed; inspiratory capacity (IC), maximal static inspiratory pressure (PImax), maximal sniff pressure and maximal voluntary ventilation for fifteen seconds (MVV). Each manoeuvre was repeated 5 times and irrespective of manoeuvre, the numerically largest EMG signal was used for normalisation[S6].

**Respiratory flow and pressure**

Respiratory flow at the mouth was measured using a Fleisch type pneumotachograph (PK Morgan Ltd, Kent, London) attached to either a mouthpiece (PK Morgan Ltd, Kent, London) during measurements performed at rest or to a full-face mask (Hans Rudolph, Kansas City, UAS) during exercise. A nose clip was used in conjunction with the mouthpiece. The pneumotachograph was attached to a differential pressure transducer (MP45, Validyne Corp, Northridge CA USA). Airway pressure (Paw) was measured using a second differential pressure transducer (MP45, Validyne Corp, Northridge CA USA) and the signals from both differential pressure transducers were amplified (CD280 Carrier amplifier, Validyne Corp, Northridge CA USA).

Diaphragm force was assessed as the transdiaphragmatic pressure (Pdi) measured using a dual pressure transducer tipped catheter (Gaeltec, Dunvegan, Isle of Sky, Scotland) with the proximal pressure transducer positioned in the mid oesophagus (oesophageal pressure, Poes) and the distal transducer in the stomach (gastric pressures (Pgas)). Correct positioning of the gastric transducer was confirmed by positive pressure generation during inspiration while the position of the oesophageal transducer was checked by comparing Pes to Paw during an occluded inspiratory effort [S9]. Close agreement of Pes and Paw indicated that the balloon was correctly located in the lower third of the oesophagus and intrathoracic pressure could be reliably estimated.

The flow and pressure signals were recorded and displayed in real time on a computer running a Labview software application (National Instruments, Austin TX, USA) with 100 Hz analog to digital sampling (DAQ 16XE-50, National Instruments, Austin TX USA). Tidal volume was obtained by digital integration of the flow signal and Pdi by digital subtraction of oesophageal pressure from gastric pressure by the recording software.

From the recording of respiratory flow and pressure tidal volume (Vt), inspiratory time (Ti), total time for the breath (Ttot), respiratory rate, the mean transdiaphragmatic, and oesophageal pressure per breath (meanPdi) and the transdiaphragmatic (PTPdi) and oesophageal (PTPes) pressure time products, were measured [S10, 11]. The diaphragm tension time index (TTI) is a measure of the load imposed upon and the capacity of the diaphragm TTI was calculated as the meanPdi/Pdimax x Ti/Ttot, where Pdimax was the maximum inspiratory transdiaphragmatic pressure[S12, 13].

**Study protocol**

Subjects attended the muscle laboratory on one occasion. Lung function testing was performed first and then the pressure transducer and oesophageal EMG catheters were inserted nasally and positioned. The nose was anaesthetised with 2-3 sprays of topical anaesthetic Xylocaine spray containing 10mg of lidocaine (Astrazeneca UK Ltd.) Both catheters were lubricated (KY jelly, Johnston and Johnston, France) and introduced via the nostril into the nasopharynx and swallowed down the oesophagus into the stomach with some small sips of water.

Measurements of sEMGpara and EMGdi at rest and during four standardised maximal spontaneous volitional manoeuvres were performed prior to the incremental exercise. The manoeuvres performed were; inspiratory capacity (IC), maximal static inspiratory pressure (PImax), maximal sniff pressure and maximal voluntary ventilation for fifteen seconds (MVV)] [S6]. Ten of the healthy subjects repeated the maximisation manoeuvres on two separate occasions at least 24 hours apart, to assess the reproducibility of the raw resting, maximal and normalised (EMG%max) parasternal EMG recordings. Finally all subjects performed an incremental exercise test to exhaustion.

**Exercise**

All subjects performed an incremental cycle exercise test to exhaustion. The incremental exercise test was performed using an electrically braked cycle ergometer (Ergoselect 100, ErgolineGmbh, Bitz, Germany) and metabolic data were acquired using a Meta Max ergospirometery system (version MMX3B2.1, Cortex Biophysik, Germany). The maximal incremental cycle test consisted of 3 minutes rest, followed by 3 minutes unloaded cycling, the work rate was then increased every 3 minutes by 25 watts in the CF patients and 50 watts in the healthy subjects, with subjects maintaining 50 – 60 revolutions per minute.

The exercise test was terminated if subjects experienced intolerable symptoms of breathlessness, leg fatigue or could no longer maintain the required cycling pace. Towards the end of each minute subjects performed an inspiratory capacity (IC) manoeuvre from functional residual capacity (FRC) and were asked to rate their perceived breathlessness [S14-16] using the modified Borg Scale.

**Data analysis**

All data except Borg score and work rate were normally distributed and expressed as mean and standard deviation (SD). Work rate and Borg scores were expressed as median and inter quartile range (IQR). Differences between healthy subjects and CF patients were assessed using unpaired t-tests for all variables except work rate and Borg scores which were compared using the Mann-Whitney test. The association between sEMGpara%max, EMGdi%max and lung function was examined using Pearson correlation coefficients. The association between Borg breathlessness scores and measured variables during exercise were examined using Spearman correlation coefficients. Reproducibility of sEMGpara was assessed by paired t-test, coefficient of variation and Bland-Altman analysis (S[17](#_ENREF_19)). Bland – Altman analysis was also used to investigate the relationship between the degree of diaphragm and parasternal EMG recruitment during exercise. Statistical analysis of the data was performed using Graph Pad Prism® v5.02 for windows (Graph Pad Prism® Software San Diego California USA). A p value less than 0.05 was considered statistically significant, except for correlation analysis to investigate the relationship between Borg breathlessness scores and measured variables for which a p value less than 0.01 was used to allow for the number of comparisons performed.

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