Measuring Voluntary and Reflexive Cough Strength in Healthy Individuals

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ABSTRACT

Introduction: Several studies have evaluated citric acid cough reflex in healthy individuals and neurologically impaired patients. These studies have been instrumental in providing evidence for its use as a validated tool in bedside swallowing evaluations, enabling the identification of patients at risk of silent aspiration. However, inter- and intra-rater reliability for perceptual measurements of strength of coughing is sub-optimal and there are no established objective methods for measuring strength of reflexive coughing. The aim of this study was to objectively evaluate voluntary and reflexive cough strength in healthy individuals.

Methods: Fifty-four healthy individuals, aged 50 years and over, participated in this study. Participants performed 'strong' and 'weak' voluntary coughs and underwent suppressed cough reflex testing using the face-mask method and incremental doses of citric acid at doses of 0.4, 0.8, 1.2 and 1.8 Mol/L. Peak and area under the curve (AUC) measurements were taken for pressure, airflow, and acoustics. Twenty-nine sets of data, where participants produced a C2 response to three doses of citric acid, were included for final analysis.

Results: Repeated-measures ANOVA revealed a significant trial effect for reflexive cough, with the second cough in the C2 sequence being smaller than the first, for all 6 measures (pressure: peak $p < .01$, AUC $p < .01$; flow: peak $p < .01$, AUC $p < .01$; acoustic: peak $p = .04$ and AUC $p < .01$). However, there was no significant effect of citric acid dose on strength of reflexive coughing (pressure: peak $p = .65$, AUC $p = .86$; flow: peak $p = .95$, AUC $p = .10$; acoustic: peak $p = .93$, AUC $p = .93$). Repeated-measures ANOVA also revealed a significant effect of type of voluntary cough, with strong coughs having greater values than weak coughs for all 6 measures ($p < .01$). There was also a significant trial effect of voluntary cough in the C2 sequence, with the second cough being weaker than the first for 4 of the measures ($p < .01$ for peak and AUC pressure; $p < .01$ for peak flow and AUC acoustic). Paired t-tests demonstrated that strong voluntary coughs were stronger than reflexive coughs for all measures ($p < .01$).

Conclusions: This research suggests that all six outcome measures were sensitive to measuring changes in coughing strength. The significant difference between strong voluntary coughs and reflexive coughs supports existing research suggesting that assessment of voluntary coughing strength does not provide accurate information about the strength of the reflexive coughing. There was an effect of cough sequence, cough 1 or 2, for both voluntary and reflexive coughing. This might indicate that the first reflexive cough is the most

important in terms of airway protection and clearance. There was no effect of concentration of citric acid on strength of suppressed coughing: once the reflex was elicited, coughing strength was stable regardless of stimulus dose. This suggests that either cortical augmentation of voluntary coughing produces a response much greater than the reflexive airway protective response, or that cortical control cannot suppress sensitivity of coughing, but can suppress strength of coughing, at increasing concentrations of citric acid. Alternatively, it may imply that a suppressed cough reflex is an all-or-nothing response, but there is potential that a true reflexive cough, stimulated by aspiration, would show a dose-response effect.

ABBREVIATIONS

ANOVA	analysis of variance
AUC	area-under-the-curve
C2	2 coughs without intervening inspiration, threshold response
C5	5 coughs without intervening inspiration, supra-threshold response
CDHB	Canterbury District Health Board
CR	cough reflex
CRT	cough reflex testing or cough reflex test
EMG	electromyography
ERS	European Respiratory Society
FEES	Fibreoptic Endoscopic Evaluation of Swallowing
fMRI	functional magnetic resonance imaging
LER	laryngeal expiratory reflex
MCA	middle cerebral artery
NBM	Nil-by-mouth
NC	natural cough
RAR	rapidly adapting receptors
RC	reflexive cough (both the LER and CR) or reflexive coughing
SAR	slowly adapting receptors
SC	suppressed cough
sEMG	surface electromyography
VC	voluntary cough or voluntary coughing
VFSS	Videofluoroscopic Swallowing Study

1 Introduction

Dysphagia, or swallowing difficulties, can occur as a result of congenital impairment or can be acquired secondary to neurological impairment. Dysphagia can have a significant impact on both quality of life and safety; if food and drink are aspirated into the lungs it can result in aspiration pneumonia which can have serious, and even fatal, consequences. Coughing plays an important role in protecting the lungs from aspiration, and when impaired can result in disastrous consequences for the individual. Cough reflex testing in the neurologically impaired population, particularly the stroke population, has been shown to be highly effective in identifying people with impaired cough sensitivity, who are at risk of silent aspiration (aspiration without coughing) and developing pneumonia (Miles, Moore, et al., 2013). As yet, there is no established method of objectively assessing strength of reflexive coughing which is vital to provide a more specific and objective measure of risk of aspiration pneumonia.

This thesis investigates the objective measurement of strength of suppressed reflexive coughing in healthy individuals and compares this to strength of voluntary coughing. Outcome measures included peak and area under the curve (AUC) for pressure, airflow and acoustics. Reflexive coughing was assessed using the face-mask method with nebulized citric acid at doses of 0.4, 0.8, 1.2 and 1.8 Mol/L. The information gained from this study will enhance the evidence-base concerning the differences between reflexive and voluntary cough and explore, for the first time, strength of suppressed reflexive coughing. It will also contribute to the development of a clinically practical approach for objective measurement of strength of reflexive coughing and the establishment of a reflexive coughing strength threshold.

2 Literature Review

2.1 Definition of Cough

Coughing is the body's mechanism for airway protection, and facilitates the removal of both inhaled particulates and secretions from the lungs (Haji, Kimura, & Ohi, 2013). It is a three-stage motor process which consists of an inspiratory phase, a compressive phase which involves high velocity expiratory airflow against a closed glottis, and an expulsive phase where the glottis opens and there is rapid and forceful expiration with the distinctive cough sound (Morice et al., 2007). A cough rarely takes place in isolation but typically occurs in a sequence of coughing events or components (Paul, Wai, Jewell, Shaffer, & Varadan, 2006). A cough can be voluntary or involuntary, and it is the involuntary reflexive cough that specifically act to protect the airway from any inhaled particulates, for example, food and drink aspirated into the lungs (Smith Hammond et al., 2001).

Dystussia is defined as a disordered cough response (Pitts et al., 2014). Coughing can be impaired in different ways. For example, sensitivity of coughing can be disrupted, resulting in reduced or absent response to saliva, food or drink passing the level of the vocal cords into the airway, termed silent aspiration. Coughing strength can also be impaired resulting in a reduced ability to prevent material from entering the airway, or expectorate any aspirated material from the airway (Bianchi, Baiardi, Khirani, & Cantarella, 2012). Both impaired strength and sensitivity of coughing have been shown to be associated with increased risk of aspiration and pneumonia (Miles, Moore, et al., 2013; Smith Hammond et al., 2009).

2.2 Neurological Control of Cough

Voluntary coughing (VC) is mediated by the cerebral cortex as it involves intentional control (Widdicombe, Addington, Fontana, & Stephens, 2011). Cortical regions activated during VC include the supplementary motor cortex, primary motor cortex, sensorimotor cortex, somatosensory cortex, and the midbrain-pons region (Simonyan, Saad, Loucks, Poletto, & Ludlow, 2007). In contrast, reflexive coughing (RC) is controlled primarily by the brainstem and is triggered when a threshold of stimulation is reached in the sensory nerve fibres in the upper airway, larynx, trachea, bronchi, and the lower airway. There are three types of nerve fibres involved in the cough reflex: the slowly adapting stretch receptors (SARs), the nociceptive receptors (C-fibres), and the rapidly adapting receptors (RARs) (Haji

et al., 2013). The SARs are mechanosensors, the C-fibres are chemosensors, and the RARs are mechanosensors stimulated by both mechanical and chemical stimuli. SARs have been found to inhibit respiration and it has been suggested that they regulate the cough reflex rather than directly elicit it (Mazzone, 2004). C-fibres are more numerous than RARs or SARs, and are found both in the intrapulmonary and extrapulmonary airway epithelium. C-fibres are believed to be involved in the elicitation of the RC, but it has also been postulated that they are involved in regulation and inhibition of coughing (Canning, 2006). RARs respond and adapt quickly to any physiological changes in the intrapulmonary and extrapulmonary airways, for example changes in lung inflation or deflation. RARs have only been found to respond to one tussigenic agent, citric acid (Pecova, Javorkova, Kudlicka, & Tatar, 2007). It is believed that RARs are directly activated by the low pH of citric acid resulting in mechanical distortion of the nerve ending (Kollarik & Undem, 2002; Mazzone, 2005). Indirect activation of RARs also occurs via the bronchoconstriction effects of citric acid (Mazzone, 2005). It is still unclear which of these three receptors, individually or collectively, are responsible for the elicitation of the RC and precisely how they work together to regulate it (Canning, 2006; Haji et al., 2013).

Mazzone, Cole, Ando, Egan, & Farrell (2011) investigated neurological control of VC, non-evoked suppressed coughing (where participants were instructed not to cough and coughing was completely suppressed) and evoked cough reflex (where participants were instructed to cough whilst breathing in capsaicin) in healthy individuals undergoing fMRI. They found that during VC and non-evoked suppressed cough the primary motor and somatosensory cortices and the posterior mid-cingulate cortex are activated. Non-evoked suppressed cough produced activation of the pre-supplementary motor area and the caudate. Conversely, evoked RC resulted in activation of the rostral and caudal medulla. These findings support previous research that VC and non-evoked suppressed coughing are cortically controlled and RC is mediated by the brainstem. It would have been useful if they had also included fMRI of evoked suppressed coughing, when suppression of true RC was no longer possible. This would have provided information regarding differences in the neurological control of evoked suppressed coughing and RC.

It has been suggested that the amygdalo-hypothalamo-reticular pathway and supratentorial connections act to modulate brainstem control of the cough reflex, as disruption of these regions following cerebral infarcts can result in dystussia (Addington, Stephens, Widdicombe, & Rekab, 2005). It has also been hypothesized that voluntary

suppression of coughing is mediated by the pyramidal tracts and that any impairment to these tracts could result in a lower suppressed cough reflex threshold (Smith & Wiles, 1998).

2.3 Differences between Reflexive and Voluntary Coughing

A cough reflex (CR) is similar to a VC, in that it is also composed of an inspiratory phase, a compressive phase and an expulsive phase, however it is triggered by sensory stimulation and is brainstem mediated. A laryngeal expiration reflex (LER), is different to a CR in that it is composed of a two phase response: the compressive phase and the expulsive phase (Tatar, Hanacek, & Widdicombe, 2008). This lack of inspiratory phase in a LER is an airway protective mechanism which prevents inhalation of the aspirate into the airway (Widdicombe et al., 2011). Conversely, the inclusion of the inspiratory phase in a CR enables a greater strength for the expulsive phase of the cough to enable more effective clearance of the airway and lungs (Tatar et al., 2008). Vovk et al. (2007) reported that LERs are typically weaker and of shorter duration than CRs, which they postulated is due to a lack of an inspiratory phase.

Reflexive coughs usually appear in a sequence rather than in isolation. When a cough sequence is reflexively triggered, it is usually composed of a mixture of CRs and LERs (Stephens, Addington, & Widdicombe, 2003; Widdicombe & Fontana, 2006). It is difficult to distinguish a CR from an LER in a cough sequence without physiological measures, such as airflow or pressure. Research has found that with increasing strength of tussigenic agent there is increased number of both CRs and LERs (Vovk et al., 2007). They also found that there were fewer LERs than CRs elicited at every concentration of capsaicin. Indeed, 92% of coughs were identified as CRs and only 8% were LERs (Vovk et al., 2007). Given that tussigenic agents usually stimulate the elicitation of a mix of CRs and LERs, when the term ‘cough reflex’ or ‘reflexive cough’ is used it is typically signifying both CRs and LERs (Tatar et al., 2008). Both the LER and the RC are of interest in the dysphagic population, as a person’s ability to reflexively prevent entry into the airway and clear any aspirated material is important for pulmonary safety. For these reasons, when the term RC is used, it refers to the mixture of CRs and LERs that are elicited by sensory stimulation.

Lasserson et al. (2006) compared VC and RC strength in healthy individuals, using surface electromyography (sEMG) of expiratory and accessory muscles, as well as airflow measures. They found the respiratory muscles are activated simultaneously and quickly in RC, whereas muscles are activated sequentially and more slowly in VC. They also discovered

that cough flow rate is greater for VC than for RC. They reported that these differences enable optimum airway protection and clearance in RC and enhanced control and modulation of VC (Lasserson et al., 2006).

Historically, assessment of the cortically-controlled VC has been used to provide information about a person's ability to clear and protect their airway (Smina et al., 2003; Smith Hammond et al., 2009). Indeed, some research has suggested that there is good correlation between strength of VC and risk of aspiration (Smith Hammond et al., 2001). However, Smith Hammond et al. (2001) also reported that there is a lack of specificity in the findings for an individual's relative risk of aspiration. General consensus is now shifting towards the importance of assessing RC, rather than VC, to make judgements about an individual's ability to protect and clear their airway, as this is believed to be more accurate in identifying risk of pneumonia, particularly with regard to involuntary response to aspiration (Addington, Stephens, Phelipa, Widdicombe, & Ockey, 2008; Magni, Chellini, Lavorini, Fontana, & Widdicombe, 2011; Widdicombe et al., 2011).

Both VC and RC can be impaired in similar ways. For example, damage to the vagus or glossopharyngeal nerves can result in desensitisation of the mucosa of the pharynx, larynx and trachea. Desensitisation can also occur secondary to intubation trauma or following increased pooling of secretions in the larynx (Smith & Wiles, 1998). This altered sensation in the upper airway can result in reduced sensitivity of reflexive coughing and reduced urge-to-cough with consequent impaired VC (Widdicombe & Singh, 2006). Patients who have disordered neurological control of respiration have been found to have more variable cough compression times than healthy controls, which results in more inconsistent subglottic pressure build up prior to the expiratory phase. This erratic compression time has been shown to result in reduced coughing strength for patients compared to healthy individuals (Lavorini et al., 2007).

Neurological control for RC is very different than that for VC, implying that neurological impairment typically affects VC and RC in different ways. For example, a study that investigated VC and RC in patients following stroke found that they were independent of each other and that VC was impaired in 79% of right-handed patients who had suffered acute left-sided middle cerebral artery (MCA) infarcts but CR remained unimpaired and no patients developed aspiration pneumonia (Stephens et al., 2003). A similar study investigating VC and RC in stroke patients found that 10% of patients had a weak or absent CR, whereas 20% of patients had an abnormal VC (Addington, Stephens, & Gilliland, 1999). Addington et al.

(2005) also suggest that cerebral infarcts can result in transient, or permanent, 'brainstem shock' which can result in impaired cough reflex.

Ward et al. (2010) investigated the difference between RC and VC in patients following ischaemic hemispheric stroke and in healthy controls. They found that both VC and RC strength, as measured by peak cough flow rate and cough volume acceleration, were more impaired in patients than in healthy controls. However, when they directly stimulated the abdominal respiratory muscles by applying magnetic stimulation to the 10^{th} thoracic nerve, they found that there was no impairment of coughing strength in patients. This implies that stroke can result in impairment of the cortical and involuntary control of coughing strength, but it does not cause impairment at the level of the abdominal expiratory muscles themselves (Ward et al., 2010). Unfortunately, they did not perform statistical analysis to compare their measures of VC and RC strength. As such, conclusions cannot be made about differences in strength following stroke or in healthy individuals.

Research investigating VC and RC in patients with early stages of Parkinson's disease has revealed that patients have a lower strength of VC and RC than healthy controls, as measured by EMG of abdominal muscles; however, they found that there was no difference in cough sensitivity (Fontana, Pantaleo, Lavorini, Benvenuti, & Gangemi, 1998). They hypothesized that this impairment of the motor control of coughing is a result of impairment of neurological control of respiratory muscles.

2.4 Cough Reflex Testing

Many tussigenic agents have been utilized to stimulate cough reflex, including tartaric acid, capsaicin, ultrasonically-nebulized distilled water, and citric acid. Citric acid is known to be a powerful tussigenic agent that stimulates both chemosensors and mechanosensors by acting on both C-fibres and RARs (Mazzone, 2005; Pecova et al., 2007).

The citric acid inhalation cough challenge was first described in the mid-1950s and has been shown to be reproducible over time with healthy individuals (Morice et al., 2007). In recent years, there has been extensive research investigating the association between response to citric acid and the risk of silent aspiration. Cough reflex testing (CRT) has been shown to have reasonably high sensitivity and specificity in detecting silent aspiration (Miles, Moore, et al., 2013). Furthermore, it is now being used widely in clinical practice in New Zealand and has been found, when used with a management protocol, to substantially reduce the rate of aspiration pneumonia (Davies, Fink, & Huckabee, 2015).

Most of the research investigating CRT has examined natural cough reflex – in this test individuals are given instructions to 'cough if they feel the need to'. However, Hutchings, Morris, Eccles, & Jawad (1993) have demonstrated that reflexive coughing to capsaicin can be voluntarily suppressed. This indicates that either cortical inhibition of RC is possible, or alternatively, that a true RC has not been initiated at all. The potential exists for patients undergoing natural CRT to cough voluntarily – rather than reflexively – during the assessment, because they are aware they are undergoing a cough test (Monroe, Manco, Bennett, & Huckabee, 2014). In order to offset this 'placebo effect', CRT can incorporate the assessment of suppressed coughing, where individuals are instructed 'if you feel the need to cough, try to suppress it'. The rationale for using suppressed coughing as well as, or in lieu of, natural coughing is that there is increased likelihood that any coughs produced are truly reflexive as the person is no longer able to cortically suppress the response (Monroe et al., 2014). The potential drawback of suppressed coughing is that patients with cognitive or communication impairments may not be able to follow instructions to suppress their cough. Research investigating natural and suppressed cough threshold in healthy people revealed that 22% of participants were able to suppress their cough at all concentrations of citric acid up to 2.6 Mol/L, whereas only 5% of participants did not produce a natural cough at the same concentrations. Furthermore, 90% of participants produced a natural cough response by 0.8 Mol/L and 87% of participants produced a suppressed cough response by 0.8 Mol/L citric acid. However, these figures excluded any participants who did not respond to citric acid at any dose, therefore if these numbers were not excluded the percentages would have been 85% and 65% respectively (Monroe et al., 2014). This indicates that some patients may have a pre-morbid disposition to lower cough sensitivity, and that the rate of this is considerably higher when assessing suppressed coughing.

Clinically, the application of the cough reflex test (CRT) varies slightly in different localities. In a recent study conducted by Miles, Zeng, McLauchlan, & Huckabee (2013), the following protocol was used. Patients received 0.9% saline solution to acclimatize them to the test. They were subsequently presented with nebulized 0.8 Mol/L citric acid via a face-mask for up to 15 s. Patients underwent this test a further two times, with a gap of at least 30 s between trials to prevent tachyphylaxis. Perceptual judgement was made regarding whether the person produced a C2 response, two successive coughs not interrupted by inspiration, and whether their coughs are weak (too weak to clear material from the airway) or strong (strong enough to clear material from the airway). If they produced a strong C2 on at least two of the trials, they were deemed to have passed the first stage of the CRT. Patients who passed this

first stage then underwent a further 'suppressed CRT', where individuals were asked to 'try not to cough' during three trials of 0.8 Mol/L citric acid. Again, perceptual judgement of strength of coughing and C2 response were recorded. Those individuals who failed underwent a further suppressed CRT at 1.2 Mol/L.

Addington, Stephens, Widdicombe, & Rekab (2005) highlighted that strength of coughing is an important factor in identifying risk of aspiration pneumonia, as they found that 10% of stroke patients that were rated as having a weak cough went on to develop aspiration pneumonia compared with 15% of patients that were rated as having an 'absent' cough reflex. However, they did not provide clear definition as to what they meant by weak or how this rating was given. Inter- and intra-rater reliability of perceptual judgement of strength of coughing are low (Miles & Huckabee, 2012). In addition, both experienced and inexperienced speech and language pathologists reported that this subjective perceptual rating is difficult.

2.5 Objective Cough Strength Testing

The effectiveness of coughing in clearing material from the airway is likely to be based on a number of factors, including the efficiency and strength of respiratory muscles, the efficacy of the laryngeal muscles, competent vocal cord functioning, rate of inspiratory flow, lung volume, and compression phase (Fontana & Lavorini, 2006; Magni et al., 2011; Smith & Wiles, 1998). It is vital that outcome measures that are chosen to assess strength of coughing incorporate as many of these aspects as possible.

2.5.1 Cough Peak Flow

One of the most widely used clinical measures of coughing strength has been the measurement of voluntary cough peak flow using spirometers or peak-flow meters. Cough peak flow is a reliable measure of the expiratory muscle strength needed for voluntary coughing (Tzani et al., 2014) and a good predictor of a person's ability to protect their airway (Bianchi et al., 2012). For example, Smina et al. (2003) investigated cough peak flow in intubated intensive care patients by placing a peak flow meter in line with the endotracheal tube and asking the patient to cough. They were able to establish an optimum cough peak flow threshold of $\geq$60 L/min. They determined that patients with cough peak flows below this threshold were significantly more likely to fail extubation and 19.1 times more likely to die during their hospital stay.

The gold standard for measurement of cough peak flow is the pneumotachograph (Silverman et al., 2014). Pneumotachography works by measuring the expiratory flow rate during a voluntary cough. Portable peak-flow meters have been found to be highly correlated with pneumotachography in healthy individuals and in patients with neuromuscular disease (Sancho, Servera, Díaz, & Marín, 2004). However, other research has indicated decreased accuracy of portable peak flow meters with peak flows of less than 400 L/min (Jackson, 1995) and that flow measures are often underestimated with this method (Rebuck, Hanania, D'Urzo, & Chapman, 1996).

The research conducted by Smina et al. (2003) also found that 4 of the 9 patients who died during their hospital stay had cough peak flows below the threshold of 60 L/min and were found to be aspirating. This reveals the strong potential for cough peak-flow measurement to identify patients at risk of pulmonary complications as a result of aspiration. The disadvantage of using peak flow meters to measure cough strength is that the device requires a patient to be able to follow instructions; as a result, this method is often not appropriate for use with patients with cognitive or communication impairment (Smina et al., 2003; Smith Hammond et al., 2001). In addition, patients who have facial or labial weakness, as is often seen following stroke, often struggle to produce airtight lip seal around the peak flow meter mouthpiece (Chatwin et al., 2003). Moreover, peak flow meters used in this way are measuring the strength of a voluntary cough. In the dysphagic population, strength of reflexive coughing is of greater interest, as this influences the ability to protect their airway following an aspiration event.

Boitano (2006) hypothesized that the coordinated action of the glottis and the respiratory muscles in the compressive and expulsive phases of coughing are particularly crucial in the production of an effective cough. However, other research has suggested that the glottis has little effect on coughing pressure and strength. As evidence, patients who have undergone laryngectomy and do not have a glottis have been found to have similar cough peak flow as patients with a glottis (Fontana et al., 1999). However, the methodology for this research involved placing an airtight mask over the laryngectomy stoma. It could be hypothesized that this airtight mask would, in effect, serve as a substitute for the glottis and facilitate a build-up of pressure that would not be present with an open laryngectomy stoma.

2.5.2 Thermal Measures of Cough

Drugman et al. (2013) looked at multiple aspects of coughing using various devices. They found that the thermistor was slow-varying but gave the most information about respiratory volume. However, the thermistor is not a reliable detector of coughing as it is easily affected by forced expiration, throat clearing, and background noise. In addition, thermistors provide an indirect measure of airflow during coughing and are highly influenced by temperature and humidity level. Therefore, it is unlikely to provide a reliable measure of strength of reflexive coughing with nebulized citric acid (Farré, Montserrat, Rotger, Ballester, & Navajas, 1998).

2.5.3 Acoustic Measures of Cough

Cough effort or energy can be measured by acoustically recording coughs and determining the integral of the acoustic power spectrum (Morice et al., 2007). Cough intensity can be measured by dividing the total cough effort by the cough count and is thought to be a good indicator of the effectiveness of coughing (Pavesi, Subburaj, & Porter-Shaw, 2001).

Drugman et al. (2013) investigated the accuracy of various cough detection devices. The focus of their study was the specificity and sensitivity of the different devices in measuring the frequency of coughing in varying environmental conditions. However, they also collected data on a wide range of features, including the energy and loudness of the recorded signals and the amplitude of the waveform. They found that when limiting each device to their two best features, the best sensors were the audio microphone followed by the accelerometer positioned on the throat.

Pavesi et al. (2001) assessed audio-vibration signals of coughing using a calibrated accelerometer attached to the suprasternal notch of the patient. Attaching the accelerometer to this location was found to improve accuracy of measurements, as interference from vibrations caused by speech and swallowing was excluded (Paul et al., 2006). They found this methodology to be reliable and effective in obtaining objective measures of coughing, including information about frequency of coughing, cough sequences, cough effort, and cough intensity. In a previous study, the same team used a miniature microphone attached to the lateral aspect of the left nostril to record cough sound. In this study they found that the coughing intensity reduced with increasing distance between the microphone and the source. They reported that coughing intensity was more affected by horizontal distance from the

source than vertical distance. They highlighted the importance of careful positioning of the microphone to ensure reproducibility and consistency within and between subjects. They also reported that coughing intensity was affected by temperature and humidity, which suggests that audio-microphones attached to the nose may not be the best method for examining cough strength when using nebulized citric acid (Subburaj, Parvez, & Rajagopalan, 1996).

Smith Hammond et al. (2009) examined voluntary coughing in stroke patients using an air-tight face mask attached to a pneumotachograph to measure airflow and microphone attached to the face mask to measure sound pressure levels. They found that sound pressure level was associated with aspiration risk, as measured by Videofluoroscopic Swallowing Study (VFSS) or Fibreoptic Endoscopic Evaluation of Swallowing (FEES). Patients who developed aspiration pneumonia had lower average sound pressure levels. However, they also hypothesized that acoustic measures of coughing strength were influenced by the quantity of chest secretions which can result in over-estimation of coughing strength in more productive patients (Smith Hammond et al., 2001).

2.5.4 Electromyography of Cough

Pitts et al. (2014) investigated abdominal, thoracic, laryngeal, and pharyngeal muscle activity using fine-wire electromyography (EMG) on anaesthetized cats. They found that the greater the aspiration risk, the larger the EMG amplitudes in the inspiratory, expiratory, and thyrohyoid muscles. This suggests a potential for a dose effect of citric acid on strength of coughing.

Fontana, Pantaleo, Lavorini, Boddi, & Panuccio (1997) also used EMG of abdominal muscles activity to measure coughing intensity in response to inhaled ultrasonically nebulized distilled water with outputs varying from 0.08 to 4.45 mL/min. They found a positive linear relationship between dose of stimulus and strength of coughing. However, they highlighted the fact that strength of coughing was dependent on more than just expiratory muscle function and effort (Fontana et al., 1997). Cox et al. (1984) also used EMG of abdominal muscles to investigate coughing intensity in response to citric acid and similarly found a dose-dependent increase in coughing intensity.

Vovk et al. (2007) used EMG to investigate LER and CR in healthy participants using capsaicin stimulus. They also found an increase in strength of coughing with greater doses of capsaicin. They found that EMG activity of abdominal and thoracic muscles was proportional to respiratory muscle contraction and correlates strongly with airflow measures. However,

they also reported that methods for analysing EMG measures are high variable which can make between-study evaluations problematic.

There are other reported limitations of using EMG for assessment of coughing strength. Consistency in the placement of electrodes can be challenging, which results in a high degree of variability in intra- and inter-subject measurements (Cox et al., 1984). There are signal variations with greater levels of abdominal fat content, skin resistance, muscle size, and distance between electrodes. These factors result in a high level of variability in coughing strength measures between and within individuals (Pitts & Bolser, 2011). These reports imply that EMG may be a less reliable method of coughing intensity measurement. In addition, some of these studies were utilising EMG to measure coughing intensity in respiratory impairment or chronic cough (Cox et al., 1984; Fontana et al., 1997), where any deficits in strength are likely to be at the respiratory muscle level. In contrast, patients who have impaired coughing secondary to neurological deficit may expect to have deficits at the laryngeal level or with coordination of respiratory muscles and glottal functioning, these deficits can result in a reduction in coughing strength that may not be captured by EMG of abdominal or thoracic muscles (Fontana & Widdicombe, 2007). Therefore, EMG may not be the best measure of coughing strength in patients with neurogenic dysphagia.

2.5.5 Cough Pressures

Coughing strength can also be assessed by measuring intra-abdominal pressures using urethral and rectal catheters. Addington, Stephens, Phelipa, Widdicombe, & Ockey (2008) used this method to assess VC and RC in response to inhaled tartaric acid. They found that area-under-the-curve (AUC) pressure was greater for RC than for VC; however, they were comparing the entire cough sequence which comprised small numbers of VC (mean of 1.8 coughs) with large numbers of CRs (mean 6.0 coughs). If the AUC of one VC was compared with one RC then it is highly likely that the results would be reversed, with VC strength being greater that RC. Interestingly, they observed significantly lower duration of intra-abdominal pressure with VC compared to RC, where glottic abduction occurred during cough producing a coinciding reduction in pressure. They suggests that sustained intra-abdominal pressure during RC is an important aspect of airway protection, as the glottis remains closed except during the very rapid vocal cord abduction during the expulsive phase of cough. They also concluded that recording the AUC of a cough measure is important to provide information

about an entire cough, as the pressure over the duration of a cough can vary between VC and CR (Addington et al., 2008).

It has been demonstrated that cough gastric pressures are normal in patients following stroke, whereas respiratory measures of coughing strength, including flow rate and expired volume, are reduced (Addington et al., 1999). It was hypothesized that gastric measures of coughing are not indicative of actual coughing strength as they do not encompass measurement of impairment at the upper airway.

It is also possible to assess coughing strength by measuring intra-oesophageal pressures using pressure transducers (Young, Abdul-Sattar, & Caric, 1987) or a balloon catheter (Pitts et al., 2014). However, these methods, along with EMG and intra-abdominal pressure measurements are considered to be clinically impractical.

Nasal pressure cannulas are used to measure air pressure fluctuations that take place during inspiration and expiration. They have been widely used in sleep studies and have been shown to be reliable and sensitive for detection of apnoea and hypopnea (Thurnheer, Xie, & Bloch, 2001). Nasal pressure measures of respiration have been shown to be comparable with facemask pneumotachography, which is the gold standard quantitative measurement for respiration in sleep (Thurnheer et al., 2001). They have also been shown to be more sensitive than thermocouples or thermistors to subtle changes in airflow and airway resistance, indicating the potential of pressure measurements for use in cough strength testing[1]. Szeinberg et al. (1988) investigated cough capacity in patients with muscular dystrophy and reported that this maximal expiratory pressure provides more accurate representation of coughing strength than forced vital capacity or peak expiratory flow rates. Moreover, Fontana et al. (1999) found no significant difference in cough expiratory flow in individuals with laryngectomy and healthy individuals and hypothesized that the closure of the glottis during the compressive phase of a cough is not important to cough peak flow. This implies that measures of cough pressure, which is likely to incorporate information about the compressive phase of a cough, may provide more accurate measures of the efficacy of coughing.

[1] Rapoport, D., Norman, R., & Nielson, M. (2001). *Nasal Pressure Airflow Measurement An Introduction*. *Pro-Tech Services, Inc*.

2.6 Gaps in the Research

Cough reflex testing provides information about the sensitivity of the cough reflex and enables the identification of patients who are at risk of silent aspiration with high sensitivity and specificity (Miles, Moore, et al., 2013). However, subjective judgements of strength of reflexive cough are unreliable which has highlighted a need for a reliable objective assessment (Miles & Huckabee, 2012; Spinou & Birring, 2014). This is vital so that CRT can provide more thorough information about the patient's reflexive coughing and enable more precise identification of patients at risk of developing aspiration pneumonia.

Despite many studies into strength of voluntary coughing using a variety of measures, there has been limited research investigating the strength of reflexive cough. Several studies have examined dose effects on strength of reflexive coughing using sEMG and found a positive relationship (Cox et al., 1984; Fontana et al., 1997). However, as reported previously, this method is clinically impractical and has the potential to provide inaccurate results for the neurogenic dysphagic population.

Investigations into cough peak flow have predominantly targeted voluntary coughing using a closed system, with an airtight mask or a mouthpiece (Smith Hammond et al., 2001, 2009; Ward et al., 2010). Using the assessment of VC to make judgements about a person's ability to reflexively protect their airway is problematic. Assessment of VC only provides information about damage to the cortical control of coughing and respiratory muscle weakness; it gives no information about the brainstem-mediated control of RC, which is of particular interest with regards to aspiration risk (Widdicombe et al., 2011). The other potential problem with using measures of VC to judge aspiration risk, is that assessment requires the person to understand and follow instructions, whereas as RC can be assessed without needing a specific level of cognition or communication skills (Widdicombe et al., 2011). A few studies have investigated RC strength utilizing measures of airflow, via a mouthpiece attached to a pneumotachograph, and reported greater strength with increasing dose of stimulus (Fontana et al., 1997; Vovk et al., 2007). The general consensus is that measures of flow are easy, non-invasive, inexpensive and the most clinically practical methods for measurement of coughing strength (Spinou & Birring, 2014).

In summary, there has been minimal research conducted into RC strength testing and there is no currently clinically practical approach to objectively measure RC strength. Furthermore, all RC strength studies have examined natural cough reflex rather than suppressed cough reflex, which is believed to be a more reliable way to elicit a true RC.

Additionally, there have been no reported investigations of coughing strength using direct measures of pressure; however, research supports this as a potentially reliable and accurate outcome measure, and it is vital to explore this.

2.7 Problem Statement

Easy to access and inexpensive methods are available to assess the sensitivity of an individual's coughing using CRT and the strength of a person's VC using portable peak flow meters. However, evidence suggests that VC and RC are quite different and affected differently by neurological impairment. Thus, assessment of VC strength is an unreliable indicator of how effective RC is in protecting the airway and clearing aspirated material from the lungs. At this time, there is no established method of objectively measuring the RC strength of individuals produced during sensory testing. Developing a low-cost, simple method to assess coughing sensitivity and strength simultaneously is likely to provide more comprehensive information about the efficacy of a person's coughing (Widdicombe et al, 2011). This would enable more accurate estimation of an individual's risk of aspiration pneumonia so that this risk can be managed effectively to reduce pneumonia rates, mortality rates, length of hospital stay, and improve quality of life.

2.8 Objective and Hypotheses

The objective of this study was to investigate the use of a pressure transducer, a spirometer, and a microphone to measure the relative strength of the suppressed RC elicited by varying doses of citric acid challenge and compare this to VC strength in healthy individuals.

The following hypotheses were tested:

1. The pressure sensor will provide a sensitive measure of strength of coughing in healthy individuals.
2. The flow rate sensor will provide a sensitive measure of strength of coughing in healthy individuals.
3. The microphone will provide a sensitive measure of strength of coughing in healthy individuals.
4. The pressure sensor will provide the most accurate measure of strength of coughing in healthy individuals.

5. A higher concentration of inhaled citric acid will produce increased RC strength.
6. A strong VC will be stronger than a RC cough stimulated by citric acid.

3 Methodology

3.1 Study Design

A within-subject design was utilized to investigate strength of reflexive and voluntary coughs. Participants underwent a citric acid cough reflex challenge provided via a face mask connected to a nebulizer. Simultaneous measurements of cough pressure, cough flow and cough acoustics were taken in response to citric acid stimulation. VC was also measured using the same equipment in the same conditions.

3.2 Ethical considerations

The University of Canterbury Human Ethics Committee granted ethical approval prior to the commencement of data collection. Subjects gave written consent for the study after having been provided with verbal and written explanations about the procedures (see Appendix 1 and 2) and opportunities to ask questions.

3.3 Participants

Published research investigating coughing strength in healthy participants did not provide appropriate data to support sample size calculation. However, a sample size of 30 or below has previously been adequate to identify significant differences in coughing strength under different conditions; therefore, an initial sample size of 30 was chosen (Chatwin et al., 2003; Cox et al., 1984; Fontana et al., 1997). Exclusion criteria included: individuals below the age of 50, gastro-oesophageal reflux, respiratory conditions, neurological conditions, dysphagia, smokers and people taking steroids, opiates or who had taken codeine-based analgesia in the 24 hours prior to assessment. Preliminary statistics and power analysis were to be completed following acquisition of 30 sets of data. However, due to time constraints, this proposed interim analysis represent the conclusion of this research project.

Participants were recruited via community groups (e.g., Probus groups, Rotary groups and church groups) using the advertisement shown in Appendix 3. Fifty-four healthy individuals, 33 females and 20 males, were initially recruited for the study. However, of these only 29 (20 females and 9 males) were considered suitable for inclusion for data analysis (see Results for information on data exclusion). The participants included had a mean age of 61, with a range of 50 to 84.

3.4 Resources

Citric acid solutions at concentrations of 0.4, 0.8, 1.2 and 1.8 Mol/L, as well as 0.9% saline, were sourced from the University of Canterbury Chemistry Department and appropriately stored. A PulmoMate® Compressor Nebulizer (model 4650I) (DeVilbiss Healthcare LLC, Pennsylvania, US) was used to deliver the citric acid stimulus to participants. A pre-determined free-flow output of 8 L/min and a restricted flow output of 6.6 L/min was utilized to ensure consistency in the dispersal of the citric acid within and between subjects (Morice et al., 2007). This same flow output was also used, to apply nebulized room air only during VCs to ensure identical airflow and pressure conditions.

The AD Instruments physiological pressure transducer (Model MLT844) was connected to a bridge amp (Model ML110) and a respiratory flow head 1000 L (MLT1000L) was connected to a spirometer pod (Model ML311) (Figure 1). These were utilized to collect information on cough pressure and cough flow rate, respectively. A Littmann stethoscope was attached to an Optimus omnidirectional impedance microphone (1KΩ Model 33-3003) to obtain acoustic measures of coughing (Figure 2). All instruments were connected to an AD PowerLab 26T-3819 (model ML856) (Figure 1) and LabChart Version 7.3.7 was utilized to collect and analyse data. A disposable Hudson RCI MICRO MIST® adult, elongated aerosol nebulizer mask and 7-foot Start Lumen® Tubing were used for each participant. This face mask had detachable tubing attached to each port: one connected to the pressure transducer, and one connected to the spirometer flow head (Figure 3). Medizyme proteolytic enzyme cleaner was used to clean all equipment, except the disposable face mask, after each assessment. The full set up of equipment can be seen in Figure 4.

The following equipment preparation was conducted before each procedure:

1. Flow measures:
    a. The spirometer was zeroed and calibrated using the following parameters –
        0.0 mV = 0.0 L/s and 1.0 V = 40.1L/s
    b. Recording range was set at 500 mV
    c. Low pass filter was set at 30 Hz
    d. Anti-alias filter was turned on
2. Pressure measures:
    a. The pressure transducer was calibrated manually using a sphygmomanometer.
    b. Recording range was set at 2 mV
    c. Low pass filter was set at 2 kHz

   d. High Pass DC filter was turned on
   e. Anti-alias filter was turned on
3. Acoustic measures:
   a. No calibration was necessary
   b. Recording range was set at 2 V
   c. Low pass filter was turned off
   d. Anti-alias filter was turned on
4. For all measures the sampling rate was set at 10 kHz.
5. The nebulizer was set as far away from the recording equipment and the participant as possible to prevent artefacts in sound and pressure recordings.

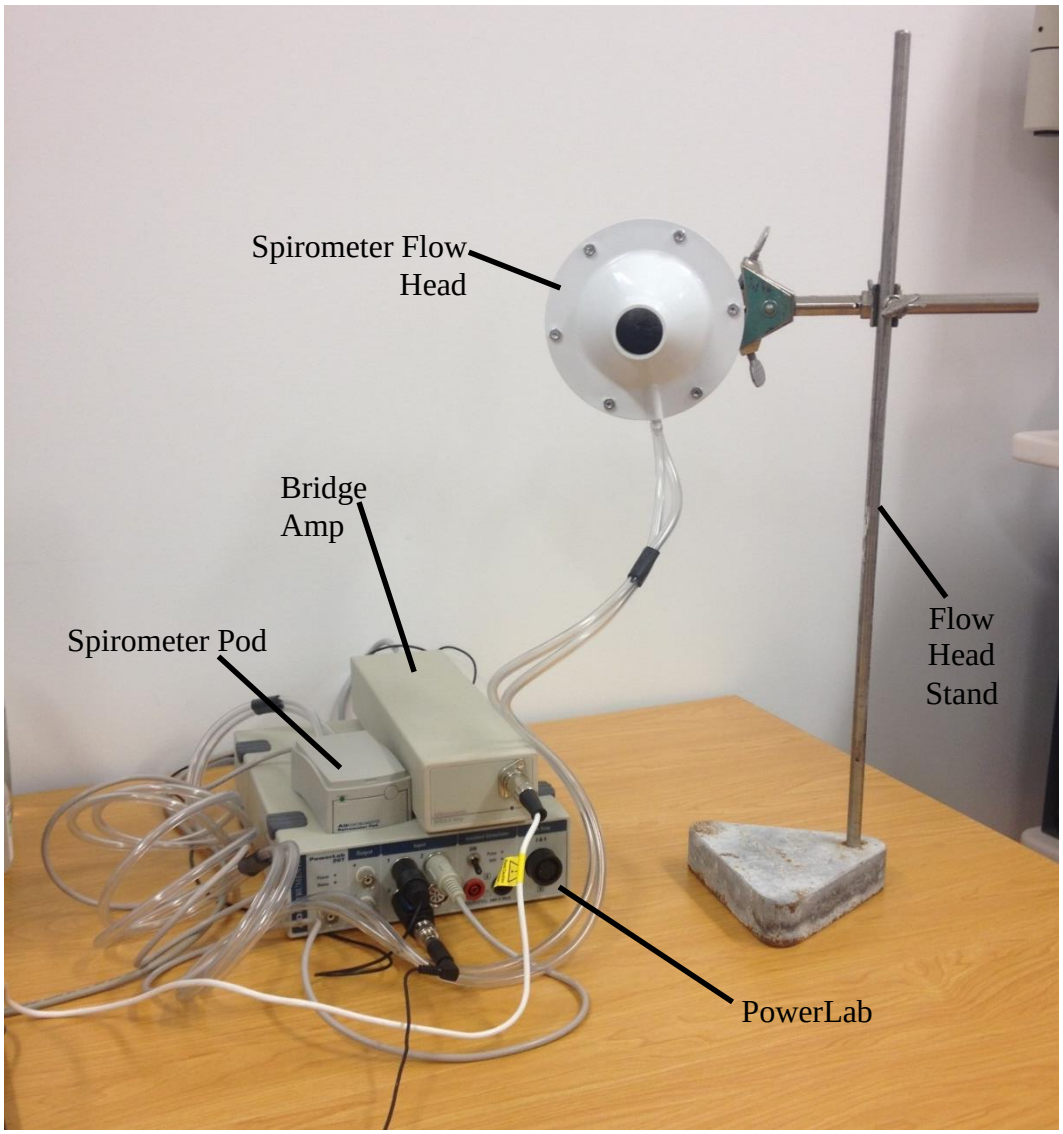


Figure 1. Set-up of spirometer flow head, spirometer pod, PowerLab and bridge amplifier.

Figure 2. Littmann stethoscope attached to microphone on a neck strap.

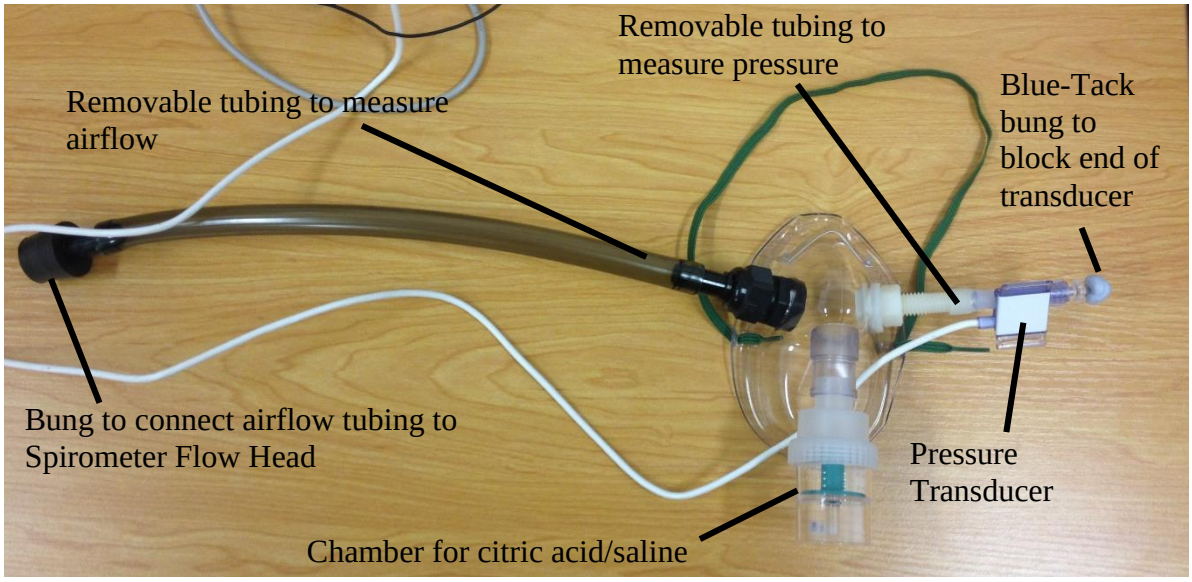


Figure 3. Face mask with tubing attached.

Figure 4. Full set-up of equipment.

3.5 Procedure

A counterbalanced design determined if the participant commenced data collection with voluntary or reflexive coughs. In addition, execution of the type of VC: a strong single cough, two strong coughs, a single weak cough, or two weak coughs, were varied randomly for each participant (see Appendix 4 and 5 for details).

For RC, participants were initially given 0.9% saline solution via a facemask as per the European Respiratory Society (ERS) guidelines on the assessment of cough (Morice et al., 2007). This served to reduce or prevent the 'startle phenomenon', of excessive coughing on the first trial of tussive agent inhalation, which has been reported with capsaicin (Dicpinigaitis, 2003, p. 64). Placebo was also applied after each trial of citric acid to improve blindness of the study by preventing participants from anticipating dosages and consequently giving conditioned responses (Morice et al., 2007).

A counterbalanced approach to dose order of citric acid was not possible due to the tendency for higher concentrations of citric acid to influence subsequently-applied lower concentrations as there is a greater risk of tachyphylaxis with higher doses of citric acid. Therefore, the lower doses of citric acid were administered first, followed by the higher doses, adhering to the ERS guidelines (Morice et al., 2007). Citric acid was administered for no more than 15 s, adhering to current clinical practice, as continual inhalation of citric acid over a period of a minute or more has been shown to result in tachyphylaxis (Morice et al., 2007). Each dose of citric acid was administered once only. When a C2 response was observed on three consecutive and increasing doses of citric acid, no further doses of citric acid were presented.

The following procedures took place with each individual. The face mask was securely placed with the straps tightened; the nose piece was adjusted to reduce movement of the mask during the assessment and minimize air escape. The stethoscope attached to the microphone was placed centrally on the neck over the participant's larynx using a neck strap. A central position was chosen in order to reduce artefact of pulse detection, which frequently occurred when the stethoscope was placed on the lateral aspect of the larynx. For VC, participants were asked to do each of the following whilst the nebulizer was running with an empty chamber:

1. "take a breath in and produce 1 strong cough"
2. "take a breath in and produce 2 strong coughs on one breath"
3. "take a breath in and produce 1 weak cough"

4. "take a breath in and produce 2 weak coughs on one breath"

The different types of cough were modelled for participants. A 60 s rest period was given between each type of cough. If participants felt that they had not carried out the task adequately or if they were observed to perform the coughs incorrectly, for example, if they inhaled between coughs, they were requested to repeat the attempt.

For RC, participants were given the instruction "Breathe in and out through your mouth. If you feel the need to cough, try to suppress it". The placebo was applied through the nebulizer for 15 s. This was also repeated after citric acid doses of 0.4, 0.8, and 1.2 Mol/L. All citric acid trials were administered through the nebulizer for up to 15 s. If a C2 response was not observed at any of the previous doses then citric acid of 1.8 Mol/L was applied through the nebulizer for up to 15 s. With all citric acid trials, as soon as a C2 response was observed the nebulizer was turned off. Again, a rest period of at least 60 s was given after all trials of citric acid or placebo.

The AD PowerLab system was used to record the data obtained from the pressure transducer, the spirometer and the microphone. A video-recording of the procedure was also taken, with the participants' consent, to allow analysis of the coughs post-procedure.

3.6 Data Extraction

3.6.1 Procedure

The pressure and acoustic signals were digitally filtered post-recording to reduce baseline drift. Pressure was filtered using a 2 Hz high-pass digital filter and acoustic using a 20 Hz high-pass digital filter.

C2 responses were identified using the airflow waveform to ensure that there was no inspiration between the first and second cough. C2 responses were recorded for each of three consecutive doses and labelled as 'dose 1', 'dose 2', and 'dose 3'. This means that for some participants threshold dose, or dose 1, was 0.4 Mol/L, and for others it was 0.8 Mol/L, depending on their individual responses. The following events of interest were then identified and extracted for each of the VCs and for the first and second cough of the C2 response for each citric acid dose:

- The cough peak flow, the cough peak pressure and the peak amplitude of the cough acoustics for each VC and for the C2 response for each citric acid challenge.

- The area under the curve (AUC) of the cough flow rate, the rectified cough pressure, and the rectified acoustic waveform.

In order to extract these data the following procedures were implemented:

- ‘s’ markers were manually placed up to 1 s before the beginning of activity in the rectified acoustic waveform for each cough
- ‘e’ markers were manually placed up to 1 s after the end of activity in the rectified acoustic waveform for each cough.
- ‘b_1’ marker was placed during the first placebo trial and ‘b_2’ marker was placed 3 s later, in order to mark a baseline for the rectified acoustic waveform where there was nebulizer running but there was no coughing or vocalizations.
- A macro was written for PowerLab which calculated the mean and standard deviation of this baseline. The code then calculated a threshold by adding 7 times the standard deviation to the baseline. This threshold was then utilized to place ‘start’ and ‘end’ markers. The macro found the ‘s’ marker, searched up to 1 second after this marker and placed a ‘start’ marker as soon as the rectified acoustic waveform rose above the threshold. Likewise, it found the ‘e’ marker and searched up to 1 s backwards and placed an ‘end’ marker as soon as the rectified acoustic waveform rose above the threshold (see Figure 5 for an example of marker placement).
- For some coughs, no ‘start’ or ‘end’ were detected, as the waveform did not rise above the threshold. In these cases the ‘start’ and ‘end’ markers were manually placed where the airflow or pressure first rose above and returned to baseline (see Figure 6 for an example).
- For other coughs, the rectified acoustic waveform did not closely match the airflow and pressure measures and resulted in substantial clipping of the airflow and/or pressure waveforms. In cases where this clipping included loss of the peak of either flow or pressure, the ‘start’ and/or ‘end’ markers were manually modified. In these instances, ‘start’ and ‘end’ markers were manually placed where the airflow or pressure first rose above and returned to baseline (see Figure 7 for an example).
- Once ‘start’ and ‘end’ markers were placed, a further macro was utilized to place the peak and AUC information for each outcome measure into the software datapad.

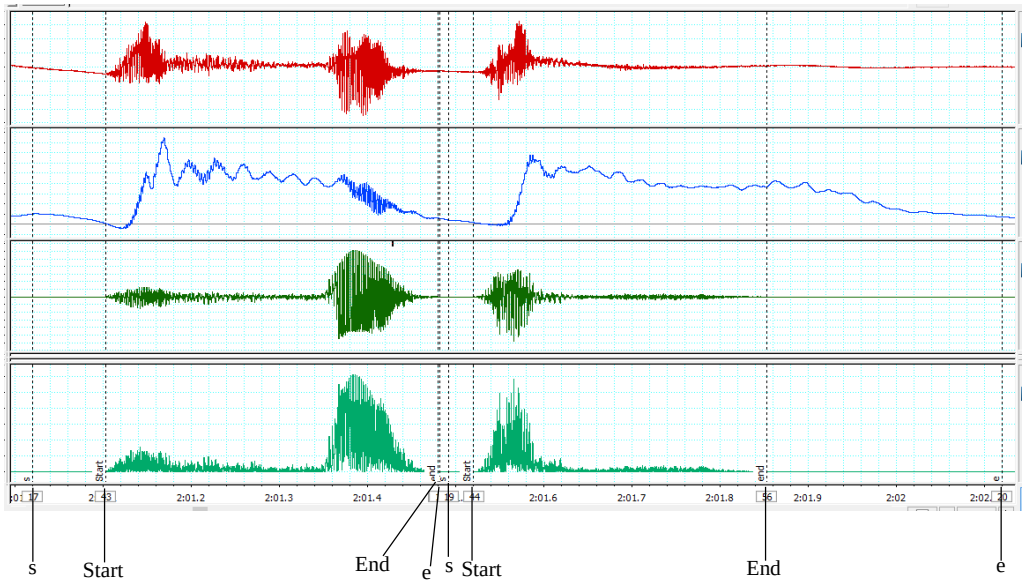


Figure 5. Automatic marker placement for two strong voluntary coughs from participant 14B. 'Start' and 'End' markers have been automatically placed within the manually placed 's' and 'e' markers. The 1st waveform is pressure (red), 2nd waveform is airflow (blue), 3rd waveform is raw acoustic (dark green), and 4th waveform is rectified acoustic (pale green).

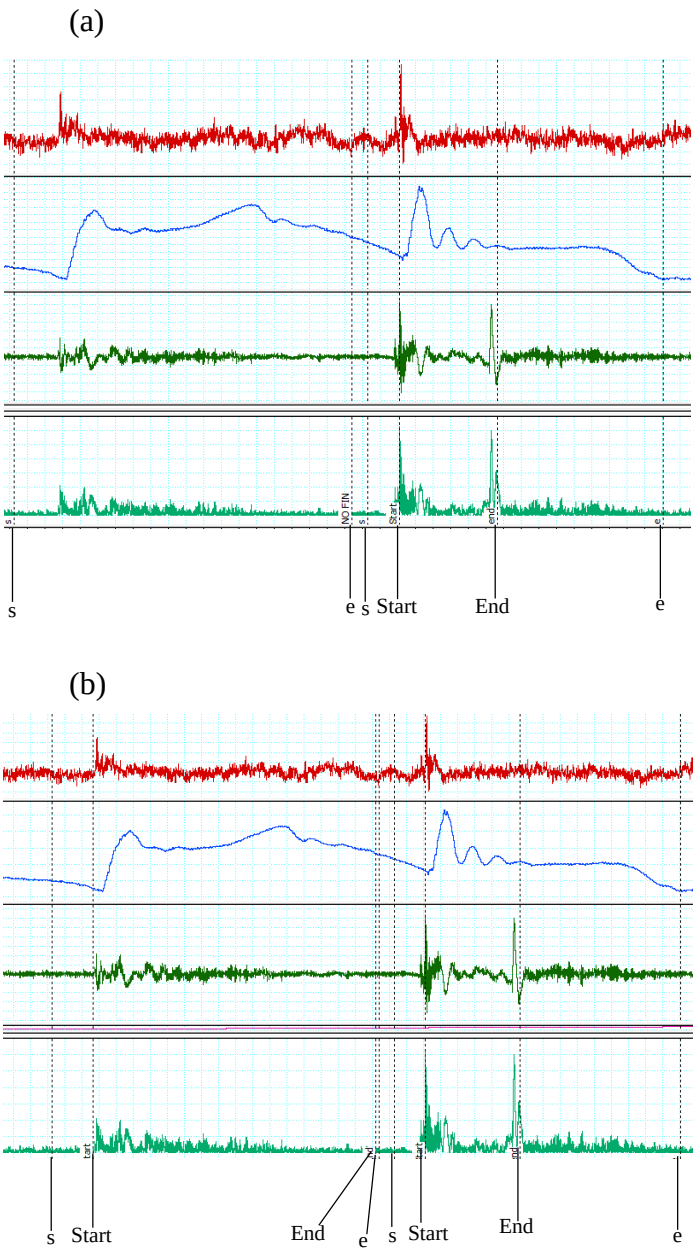
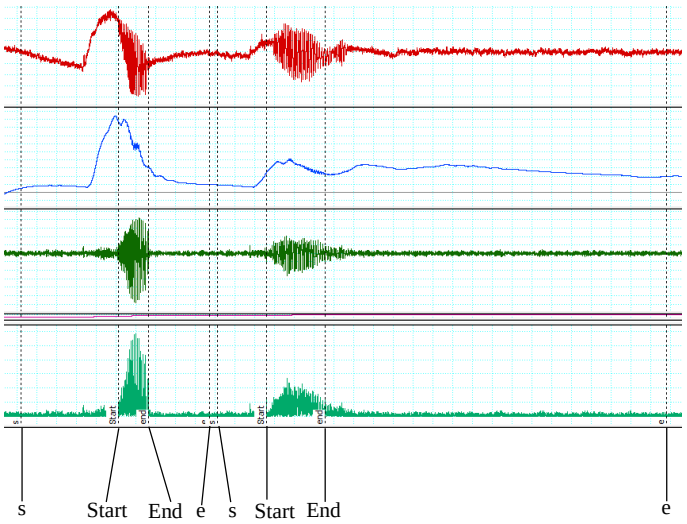


Figure 6. Manual placement of markers for citric acid 1.8M/L for participant 50B: (a) 'Start' and 'End' markers were placed for cough 2 but no 'Start' or 'End' markers were placed for cough 1 as the rectified acoustic waveform did not go above threshold. (b) 'Start' and 'End' markers were manually placed for cough 1 where the airflow and pressure started to rise above baseline. The 1st waveform is pressure (red), 2nd waveform is airflow (blue), 3rd waveform is raw acoustic (dark green), and 4th waveform is rectified acoustic (pale green).

a).



b).

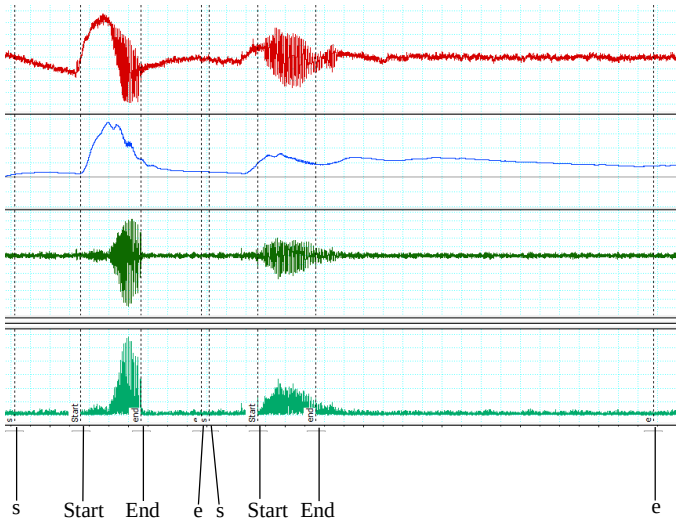


Figure 7. Manual placement of markers for two voluntary weak coughs from participant 16D: (a).'Start' and 'End' markers were placed for both cough 1 and 2 but the start marker clips the peak of the pressure and flow. (b) 'Start' marker was manually placed for cough 1 where the airflow and pressure started to rise above baseline. The 1st waveform is pressure (red), 2nd waveform is airflow (blue), 3rd waveform is raw acoustic (dark green), and 4th waveform is rectified acoustic (pale green).

3.6.2 Rationale

Provisional analysis of the data revealed that the pressure waveform closely mirrored the acoustic waveform, both characterized by high frequency waveform that passes

frequently into the negative. It was hypothesized that this was likely due to small changes in pressure at the level of the vocal cords during a cough as a result of the Bernouilli Effect creating negative pressure in the glottis (Tao, Jiang, & Zhang, 2006; Van Den Berg, Zantema, & Doornenbal, 1957). Therefore, peak and AUC measurements were derived from the rectified pressure waveform. Utilizing the rectified waveform enabled all the energy of a cough captured by the pressure transducer to be analysed. Likewise, the rectified acoustic waveform was used to obtain peak and AUC measurements.

There were many factors to take into consideration when choosing which outcome measure to select for the macro to use to place automatic 'start' and 'end' markers. The airflow waveform tended to begin slightly before pressure and acoustic measures and finish a considerable time after they finished. Therefore, airflow should have been an ideal candidate for selection. However, the highly fluctuating baseline, due to inspiration and expiration, rendered it unsuitable for automatic placement of cough markers. The pressure waveform and the rectified pressure waveform, once a high-pass digital filter was applied, had stable baselines and potential for selection. However, on trialling the pressure waveform and the rectified pressure waveform for automatic cough marker placement, it became apparent that the pressure waveforms tend to be considerably shorter than the acoustic and airflow measures and there was substantial clipping of other waveforms. The rectified acoustic waveform was thus chosen for automatic placement of 'start' and 'end' markers. Using this waveform provided the least amount of clipping of other waveforms and was felt to be the optimum choice.

The decision to use seven times the standard deviation of the baseline in order to calculate the threshold was made after running trials with the macro using different threshold calculations. Using 7xSD gave the most consistent placement of 'start' and 'end' markers with the least amount of clipping of pressure and flow measures.

Where the automatic markers clipped peaks of airflow or pressure, or were not placed at all, markers were manually placed. The 'start' markers were placed whenever pressure or flow first started to rise above the baseline. The rationale for this was that on some occasions the flow commenced before pressure, whereas at other times pressure commenced before flow. Without exception, cough flow duration was longer than cough pressure duration and, therefore, 'end' markers were always manually placed where airflow measure approaches the baseline.

3.7 Data Analysis

The data were then further analysed using the IBM SPSS Statistics software (version 22). Repeated-measures analysis of variance (ANOVA) was used to compare the peak values and the AUC for the different doses of citric acid and for the different types of VC for each of the three measures: pressure, flow, and acoustics. Analysis of sphericity was evaluated using Mauchly's test of sphericity, when sphericity was violated either the Greenhouse-Geisser or Huynh-Feldt estimates of sphericity were used to correct the degrees of freedom.

Paired t-tests were utilized to compare the third dose of citric acid to strong voluntary coughs. The root mean square error (RMSE) was used to estimate, on average, how far the modified data, taken by manually placing markers, deviated from the unmodified data from automatic placement of markers. A p-value of < 0.05 was considered as significant with a 95% confidence interval. A bivariate correlation analysis was conducted to compare the Pearson's correlation coefficient of the six different outcome measures.

4 Results

Twenty-nine sets of data were included in the study for analysis. Of the 24 sets of data excluded from analysis, 2 were due to equipment failure, 6 were due to human error, and 16 were due to absent C2 response at three consecutive and incremental doses of citric acid. Thirty-two percent of the participants, 50% of whom were male, did not show the expected C2 response.

4.1 Reflexive Cough

4.1.1 Pressure

The means and standard errors for peak pressure and AUC pressure are shown in Figures 8 and 9. For both measures there was a significant main effect of cough sequence (1st or 2nd cough in a sequence) (Peak: $F(1) = 11.88$, $p < .01$, $1\text{-}\beta = .91$; AUC: $F(1) = 30.76$, $p < .01$, $1\text{-}\beta = 1.00$). On average, the second cough was weaker than the first (Peak: mean 1st cough = 2.27 mmHg, mean 2nd cough = 1.84 mmHg, $p < .01$; AUC: mean 1st cough = 0.08 mmHg.s, mean 2nd cough = 0.05 mmHg.s, $p < .01$). There was no significant effect of dose for either measure (Peak: $F(2) = 0.44$, $p = .65$, $1\text{-}\beta = .12$; AUC: $F(1.77) = 0.12$, $p = .86$, $1\text{-}\beta = .07$).

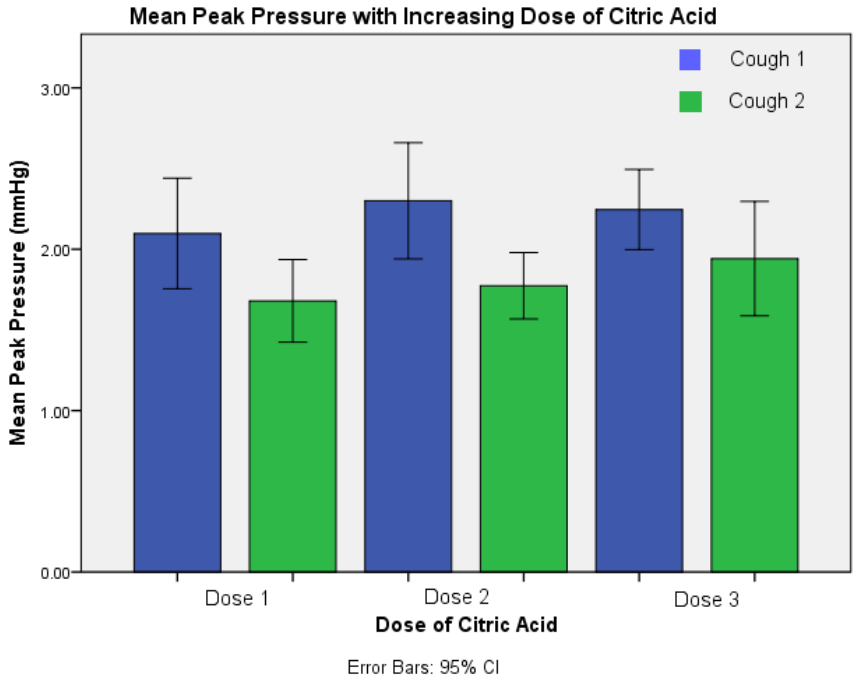


Figure 8. Mean peak pressure for reflexive cough.

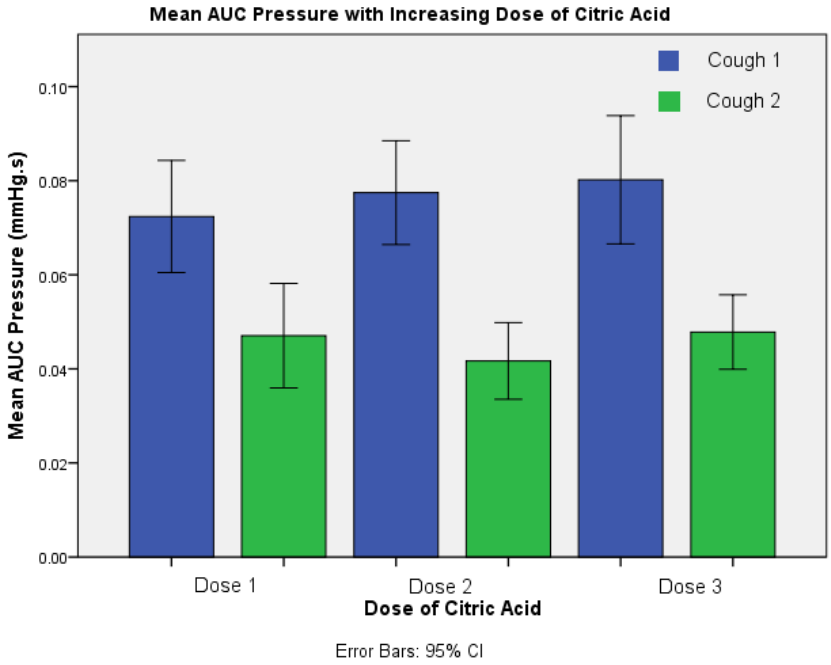


Figure 9. Mean AUC pressure for reflexive cough.

4.1.2 Flow

Figures 10 and 11 outline the means and standard errors for peak and AUC flow. There was a significant effect of cough sequence (1st or 2nd cough in a sequence) for both measures (Peak: $F(1) = 33.25$, $p < .01$, $1\text{-}\beta = 1.00$; AUC: $F(1) = 10.65$, $p < .01$, $1\text{-}\beta = .88$). On average, the first cough was stronger than the second (Peak: mean 1st cough = 0.95 L/s, mean 2nd cough = 0.67 L/s, $p < .01$; AUC: mean 1st cough = 0.15 L, mean 2nd cough = 0.10 L, $p < .01$ L). There was no significant effect of dose for either measure (Peak: $F(2) = 0.05$, $p = .95$, $1\text{-}\beta = .06$; AUC: $F(2) = 2.37$, $p = .10$, $1\text{-}\beta = .46$).

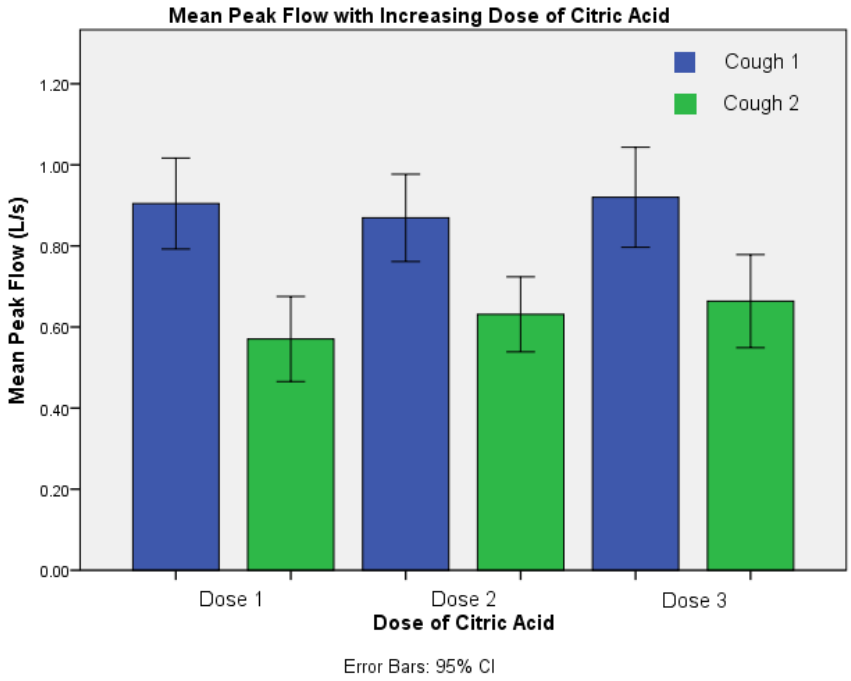


Figure 10. Mean peak flow for reflexive cough.

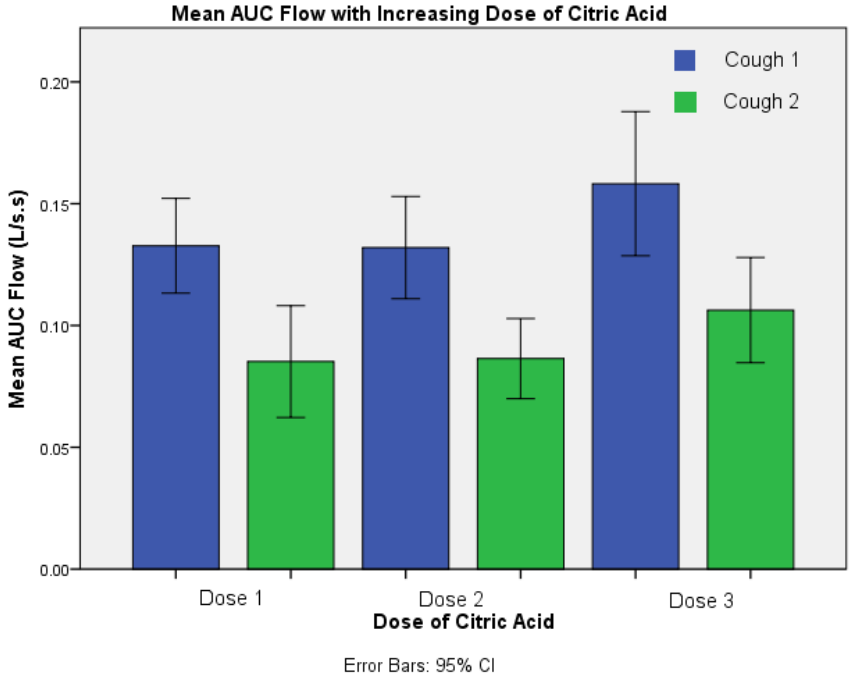


Figure 11. Mean AUC flow for reflexive cough.

4.1.3 Acoustic

Figures 12 and 13 demonstrate the means and standard errors for peak and AUC acoustic. There was a significant main effect of cough sequence (1st or 2nd cough in a sequence) for both measures (Peak: $F(1) = 4.51$, $p = .04$, $1\text{-}\beta = .54$; AUC: $F(1) = 10.94$, $p < .01$, $1\text{-}\beta = .89$). On average, the first cough was stronger than the second (Peak: mean 1st cough = 0.41 V, mean 2nd cough = 0.36 V, $p = .04$; AUC: mean 1st cough = 0.02 V.s, mean 2nd cough = 0.01 V.s, $p < .01$). There was no significant effect of dose for either measure (Peak: $F(1.73) = 0.56$, $p = .93$, $1\text{-}\beta = .06$; AUC: $F(1.75) = 0.56$, $p = .93$, $1\text{-}\beta = .06$).

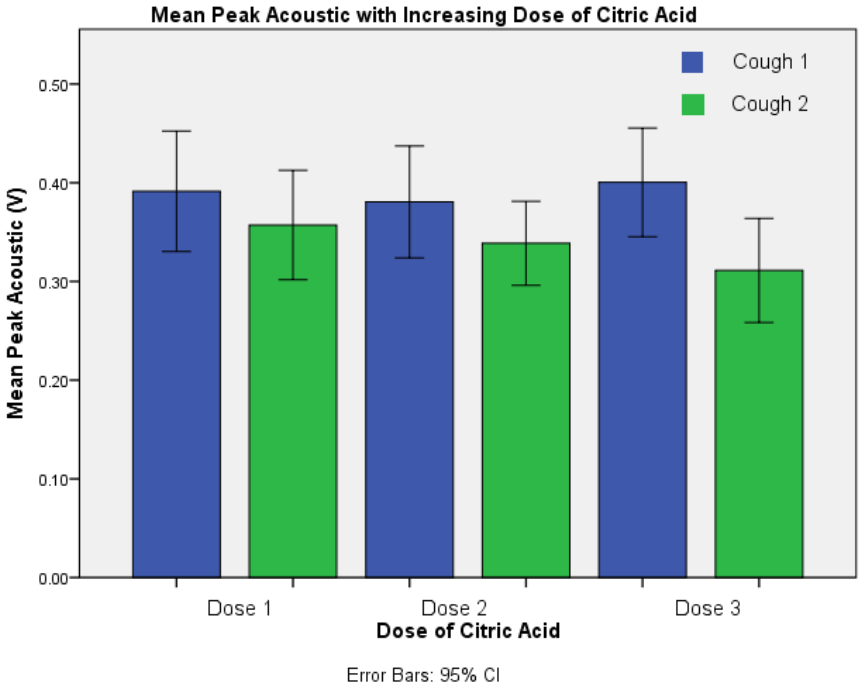


Figure 12. Mean peak acoustic for reflexive cough.

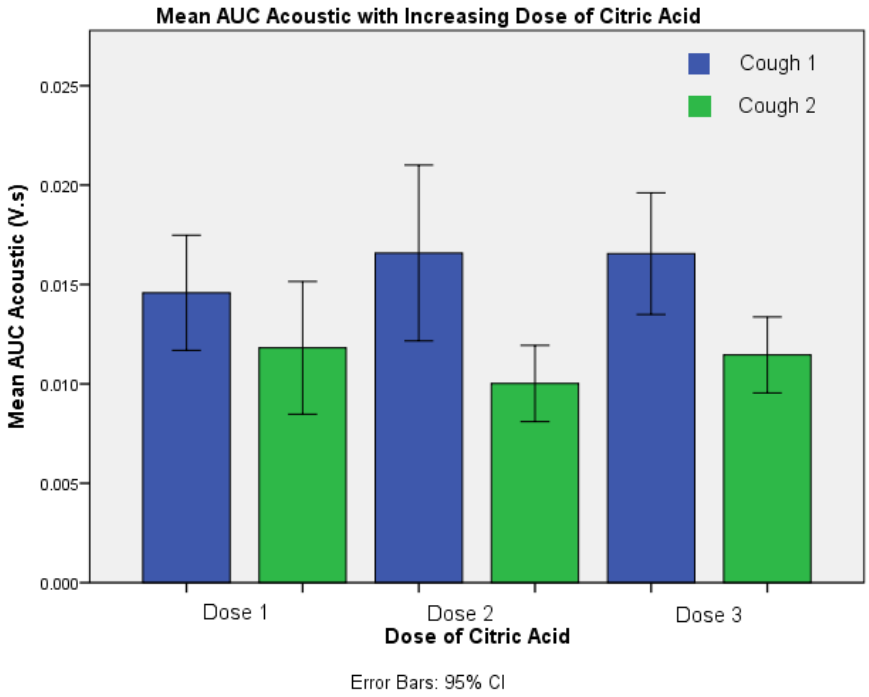


Figure 13. Mean AUC acoustic for reflexive cough.

4.2 Voluntary Coughs

4.2.1 Pressure

The peak and AUC pressure means and standard errors are displayed in Figures 14 and 15. Both measures demonstrated a significant main effect of type of VC, strong or weak (Peak: $F(1) = 57.73$, $p < .01$, $1\text{-}\beta = 1.00$; AUC: $F(1) = 54.09$, $p < .01$, $1\text{-}\beta = 1.00$). This indicates that strong coughs are objectively stronger than weak coughs (Peak: mean weak cough = 1.85 mmHg, mean strong cough = 6.87 mmHg, $p < .01$; AUC: mean weak cough = 0.11 mmHg.s, mean strong cough = 0.28 mmHg.s, $p < .01$). There was also a significant main effect of cough sequence (1^{st} or 2^{nd} cough in a sequence) for both measures (Peak: $F(1) = 16.48$, $p < .01$, $1\text{-}\beta = .98$; AUC: $F(1) = 59.47$, $p < .01$, $1\text{-}\beta = 1.00$). This shows that the first cough is stronger than the second cough (Peak: mean 1^{st} cough = 4.85 mmHg, mean 2^{nd} cough = 3.87 mmHg, $p < .01$; AUC: mean 1^{st} cough = 0.24 mmHg.s, mean 2^{nd} cough = 0.14 mmHg.s, $p < .01$). There was a significant interaction between type of cough and cough sequence for both measures (Peak: $F(1) = 27.04$, $p < .01$, $1\text{-}\beta = 1.00$; AUC: $F(1) = 27.04$, $p < .01$, $1\text{-}\beta = 1.00$). This demonstrates that there is a greater difference between cough 1 and cough 2 for strong coughs than for weak coughs (Peak: mean difference strong = -1.75

mmHg; mean difference weak = –0.21 mmHg; AUC: mean difference strong = –0.15 mmHg.s; mean difference weak = –0.03 mmHg.s). Peak pressure also exhibited a significant interaction between cough sequence and gender for peak pressure ($F(1) = 11.30$, $p < .01$, $1\text{-}\beta = .90$). This revealed that the decrease in strength from cough 1 to cough 2 was greater for males than for females (males mean difference = –1.52 mmHg; females mean difference = –0.44 mmHg).

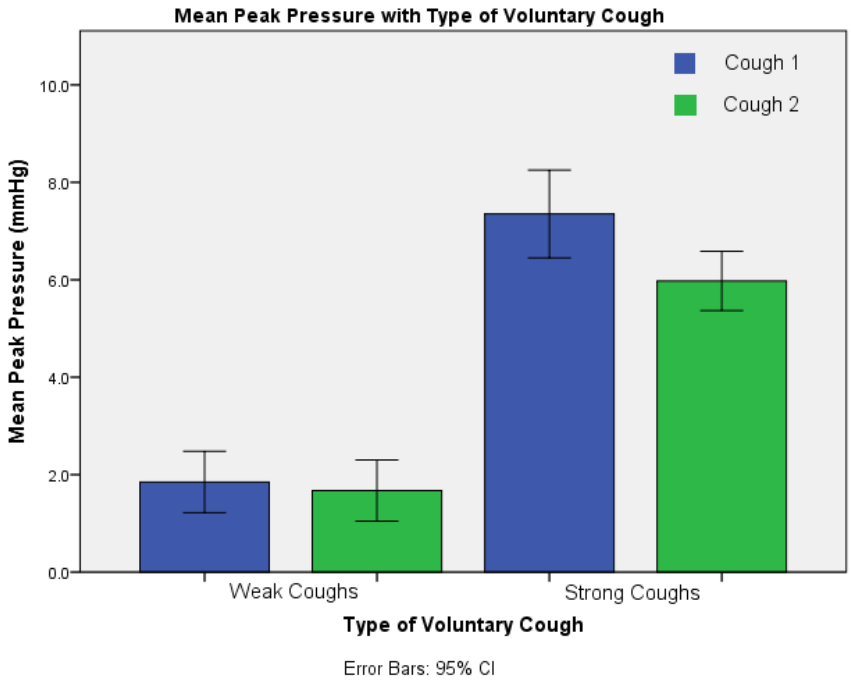


Figure 14. Mean peak pressure for voluntary cough.

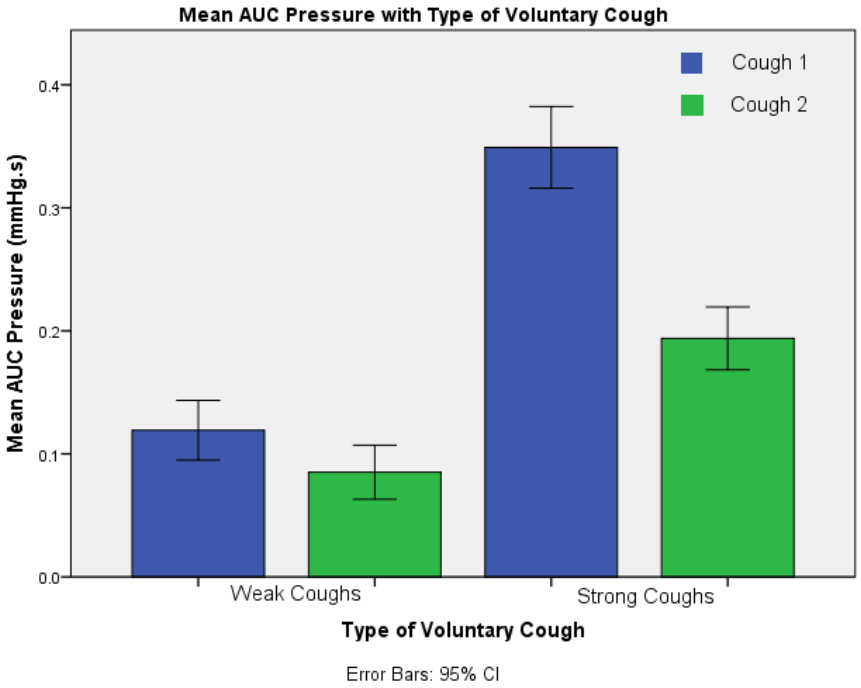


Figure 15. Mean AUC pressure for voluntary cough.

4.2.2 Flow

Figures 16 and 17 reveal the means and standard errors for peak and AUC flow. There was a significant main effect of type of VC, strong or weak, for both measures (Peak: $F(1) = 20.54$, $p < .01$, $1\text{-}\beta = .99$; AUC: $F(1) = 44.40$, $p < .01$, $1\text{-}\beta = 1.00$). This indicates that the strong coughs were quantitatively stronger than the weak coughs (Peak: mean weak cough = 1.24 L/s, mean strong cough = 1.72 L/s $p < .01$; AUC: mean weak cough = 0.19 L, mean strong cough = 0.30 L $p < .01$). There was also a significant main effect of cough sequence (1^{st} or 2^{nd} cough in a sequence) for peak flow ($F(1) = 66.77$, $p < .01$, $1\text{-}\beta = 1.00$) revealing that the first cough was stronger than the second cough (mean 1^{st} cough = 1.65 L/s, mean 2^{nd} cough = 1.31 L/s, $p < .01$). There was also a significant interaction of cough sequence and gender for both measures (Peak: $F(1) = 4.80$, $p = .04$, $1\text{-}\beta = .56$; AUC: $F(1) = 10.47$, $p < .01$, $1\text{-}\beta = .88$). For peak flow, the difference in cough strength between cough 1 and cough 2 was greater for females than for males (males mean difference = –0.26 L/s; females main difference = 0.44 L/s). However, for AUC flow, for females there was a decrease in cough strength from cough 1 to cough 2, whereas for males there was an increase (male mean difference = 0.05 L; female mean difference = –0.06 L). There was also a significant interaction effect of type of cough and cough sequence for peak flow ($F(1) = 6.28$, $p = .02$, 1-

β = .68). This demonstrated that there was a smaller difference in strength of weak coughs, between cough 1 and cough 2, than for strong coughs (mean difference weak = –0.23 L/s; mean difference strong = –0.47 L/s). There was also a significant interaction effect of type of VC and gender for AUC flow (F(1) = 10.64, p < .01, 1-β = .88). This revealed that the difference in weak and strong coughs was greater for females than for males (female mean difference = 0.18 L; male mean difference = 0.06 L).

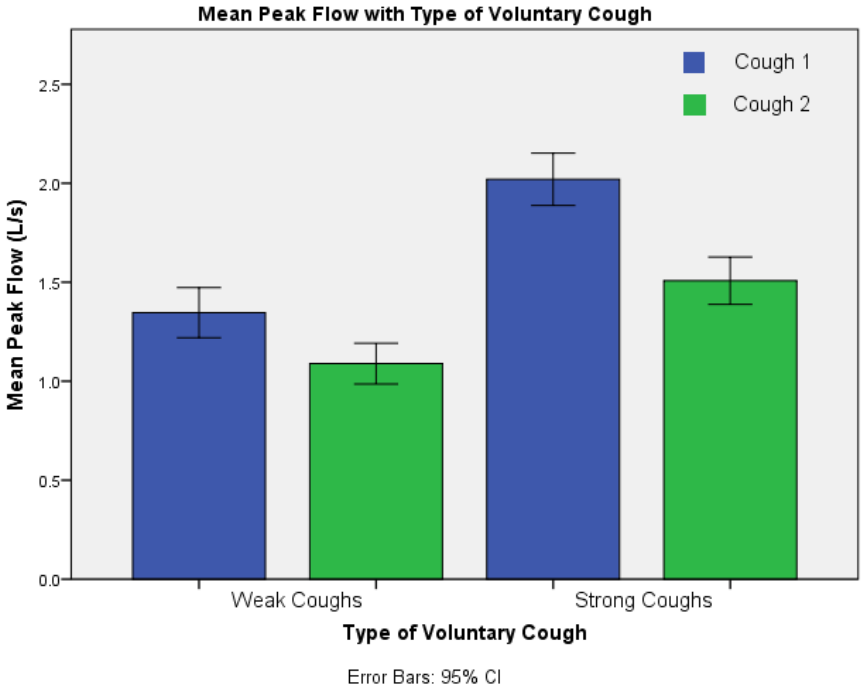


Figure 16. Mean peak flow for voluntary cough.

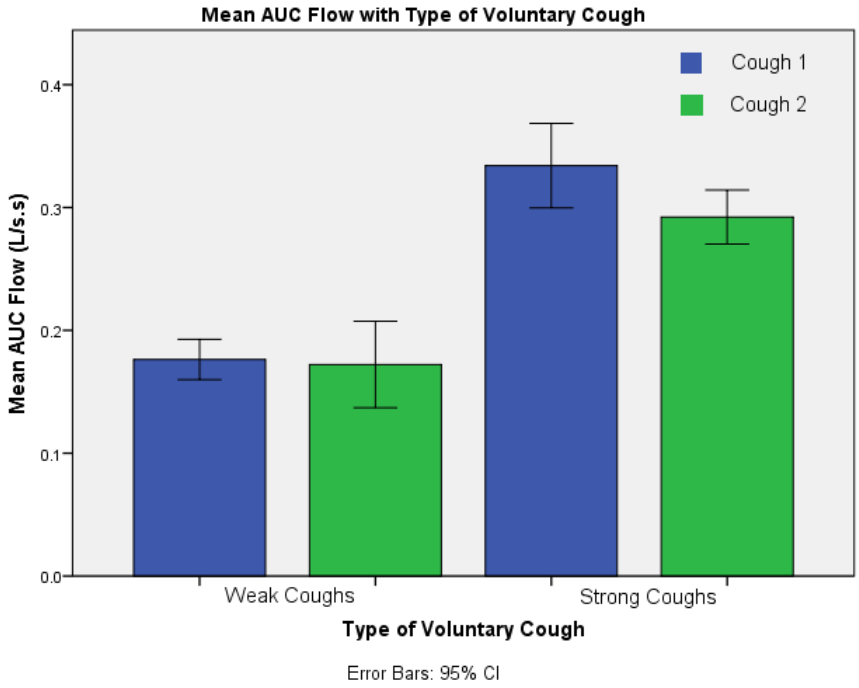


Figure 17. Mean AUC flow for voluntary cough.

4.2.3 Acoustic

The means and standard errors for peak and AUC acoustic are displayed in Figures 18 and 19. There was a significant main effect of type of VC, strong or weak, for both measures (Peak: $F(1) = 41.59$, $p < .01$, $1\text{-}\beta = 1.00$; AUC: $F(1) = 58.98$, $p < .01$, $1\text{-}\beta = 1.00$). This demonstrates that strong coughs were objectively stronger than weak coughs (Peak: mean weak cough = 0.40 V, mean strong cough = 0.65 V $p < .01$; AUC: mean weak cough = 0.02 V.s, mean strong cough = 0.05 V.s $p < .01$). There was also a significant main effect of cough sequence for AUC acoustic ($F(1) = 8.77$, $p < .01$, $1\text{-}\beta = .82$), showing that the first cough was stronger than the second cough (mean 1st cough = 0.04 V.s, mean 2nd cough = 0.03 V.s, $p < .01$). There was a significant interaction of type of voluntary cough and cough sequence for AUC acoustic ($F(1) = 16.76$, $p < .01$, $1\text{-}\beta = .98$). This result revealed a greater difference in strength between cough 1 and cough 2 for strong than for weak coughs (mean difference strong = –0.02 V.s; mean difference weak = –0.005 V.s).

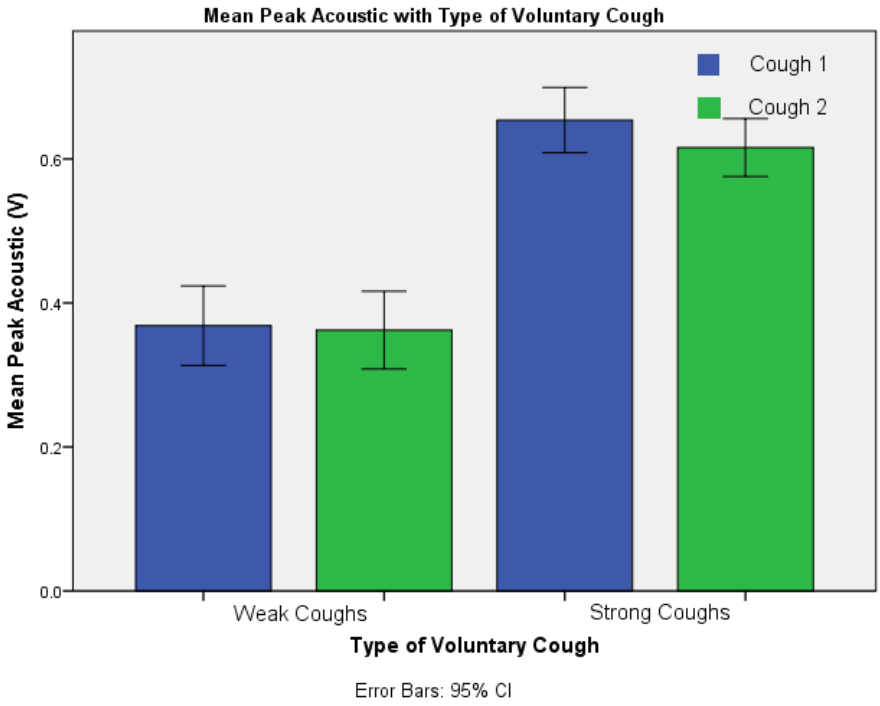


Figure 18. Mean peak acoustic for voluntary cough.

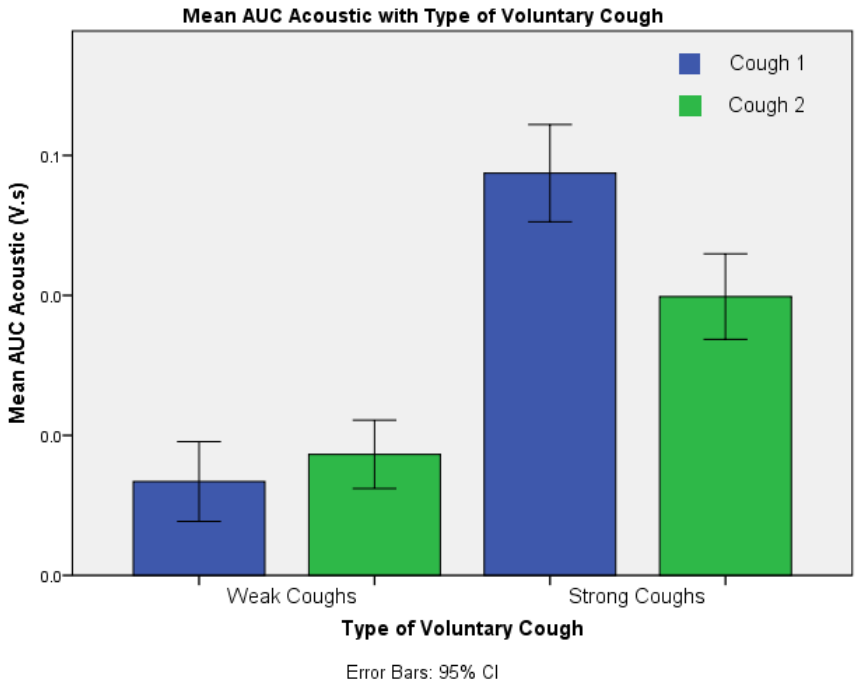


Figure 19. Mean AUC acoustic for voluntary cough.

The means and standard deviations for all reflexive and voluntary coughs are also shown in Appendix 7 and the repeated measures ANOVA results are also shown in Appendix 8.

4.3 Reflexive Cough versus Voluntary Cough

As discussed earlier, the label ‘dose 1’ was the first concentration of citric acid that triggered a C2 response in an individual, ‘dose 2’ was the second concentration of citric acid administered and ‘dose 3’ the third. Although there was no significant difference in the strength of response to the three doses, dose 3 was arbitrarily chosen for comparison with strong voluntary coughs. Paired t-tests comparing cough 1 and cough 2 at dose 3 with cough 1 and cough 2 of the strong voluntary coughs revealed that, on average, participants’ strong VCs were significantly stronger than their RCs at the highest dose of citric acid (see Table 1 for details of findings).

Table 1. Differences between reflexive cough 1 and 2 at citric acid dose 3 with voluntary cough 1 and 2 for each outcome measure

Measure	Cough Number	Mean Difference	Standard Deviation	Standard Error	t (28)	Sig.	Cohen’s d
Peak Pressure (mmHg)	1st cough	-5.106	3.846	0.714	-7.1	.000	1.33
	2nd cough	-4.034	3.097	0.575	-7.0	.000	1.30
AUC Pressure (mmHg.s)	1st cough	-0.269	0.135	0.025	-10.7	.000	1.99
	2nd cough	-0.146	0.103	0.019	-7.6	.000	1.42
Peak Flow (L/s)	1st cough	-1.100	0.780	0.145	-7.6	.000	1.41
	2nd cough	-0.844	0.638	0.118	-7.1	.000	1.32
AUC Flow (L)	1st cough	-0.176	0.149	0.028	-6.3	.000	1.18
	2nd cough	-0.186	0.142	0.026	-7.1	.000	1.31
Peak Acoustic (V)	1st cough	-0.254	0.300	0.056	-4.5	.000	.85
	2nd cough	-0.305	0.247	0.046	-6.6	.000	1.23
AUC Acoustic (V.s)	1st cough	-0.041	0.034	0.006	-6.5	.000	1.21
	2nd cough	-0.028	0.028	0.005	-5.5	.000	1.00

4.4 Comparisons of Measures

Bivariate correlations of the measures for reflexive and voluntary coughs are shown in Table 2. For both RC and VC conditions, the highest correlations are between peak and AUC of the same measures. With regards to different measures, the highest correlation for VC and RC is between AUC pressure and peak flow, and AUC pressure and AUC flow. Acoustic measures correlate poorly with all other measures for RC and VC.

Table 2. Correlations between six measures for reflexive and voluntary cough.

	Reflexive Cough		Voluntary Cough	
	Pearson's Correlation (r)	Sig.	Pearson's Correlation (r)	Sig
Peak pressure vs AUC pressure	.628	p < .01	.836	p < .01
Peak pressure vs Peak flow	.571	p < .01	.474	p < .01
Peak pressure vs AUC flow	.377	p < .01	.440	p < .01
Peak pressure vs Peak acoustic	.334	p < .01	.436	p < .01
Peak pressure vs AUC acoustic	.313	p < .01	.422	p < .01
AUC pressure vs Peak flow	.757	p < .01	.613	p < .01
AUC pressure vs AUC flow	.756	p < .01	.595	p < .01
AUC pressure vs peak acoustic	.265	p < .01	.420	p < .01
AUC pressure vs AUC acoustic	.351	p < .01	.559	p < .01
Peak flow vs AUC flow	.802	p < .01	.770	p < .01
Peak flow vs peak acoustic	.001	p = .99	.290	p < .01
Peak flow vs AUC acoustic	.026	p = .73	.288	p < .01
AUC flow vs peak acoustic	.099	p = .20	.447	p < .01
AUC flow vs AUC acoustic	.112	p = .14	.406	p < .01
Peak acoustic vs AUC acoustic	.812	p < .01	.694	p < .01

4.5 Marker Placement Error

Three coughs out of a total of 348 coughs from the 29 data sets had their start and/or end markers placed manually as automatic placement could not be applied when the rectified

acoustic waveform did not exceed the threshold. An additional eight coughs had their start and/or end markers manually adjusted as the automatic placement caused peak clipping of pressure and/or flow waveforms. These eight coughs were analysed using root mean square error (RMSE) analysis to investigate the differences between the results gained by the automatically placed markers and the manually placed markers (see Table 3 for results of pressure measures and Table 4 for results of flow measures). These results indicate that the difference in peak pressure and AUC pressure values between the modified and unmodified values is very small, 0.06 RMSE and 0.05 RMSE, respectively (with a value of 0 representing perfect agreement in values). However, for the values for peak flow and AUC flow were much higher, with RMSE of 0.73 and 0.17 respectively. This indicates that incorrect placement of the markers has most effect on the flow measures.

Table 3. RMSE for peak pressure and AUC pressure for each of the eight coughs

Adjusted Cough	Peak Pressure mmHg (automatic markers)	Peak Pressure mmHg (manual markers)	Peak Pressure Difference mmHg	AUC Pressure mmHg.s (automatic markers)	AUC Pressure mmHg.s (manual markers)	AUC Pressure Difference mmHg.s
1	0.977	0.977	0.000	0.027	0.077	0.049
2	2.086	2.086	0.000	0.030	0.047	0.018
3	0.929	1.104	0.175	0.014	0.061	0.047
4	0.153	0.178	0.025	0.017	0.028	0.011
5	2.289	2.289	0.000	0.007	0.077	0.069
6	3.772	3.772	0.000	0.027	0.111	0.084
7	3.512	3.512	0.000	0.042	0.098	0.056
8	1.252	1.252	0.000	0.022	0.059	0.037
Root Mean Square Error			0.062			0.052

Table 4. RMSE for peak flow and AUC flow for each of the eight coughs

Adjusted Cough	Peak Flow L/s (automatic markers)	Peak Flow L/s (manual markers)	Peak Flow Difference L/s	AUC Flow L (automatic markers)	AUC Flow L (manual markers)	AUC Flow Difference L
1	0.902	0.942	0.040	0.047	0.092	0.045
2	0.671	0.706	0.035	0.012	0.133	0.121
3	0.459	0.459	0.000	0.015	0.093	0.078
4	0.189	0.224	0.036	0.052	0.093	0.041
5	0.385	1.427	1.042	0.001	0.263	0.262
6	0.502	1.870	1.368	0.002	0.209	0.207
7	1.050	2.093	1.043	0.006	0.278	0.272
8	0.820	1.279	0.459	0.013	0.184	0.171
Root Mean Square Error			0.730			0.173

5 Discussion

It is vital to develop objective measures of reflexive cough strength to identify patients at risk of aspiration pneumonia. This study investigated measures of pressure, airflow, and acoustics for clinical application for assessment of coughing strength. All outcome measures were sensitive to detecting differences in strength of cough; but neither peak amplitude or AUC were superior in quantifying strength. Based on effect sizes and correlation, peak flow and AUC pressure appear to provide the best sensitivity of measurement. Acoustic measures had significantly lower effect sizes and lower correlation with pressure and flow. There was a significant difference between strength of VC and RC in healthy individuals, which highlights that assessment of VC does not provide accurate information about the function of RC. Therefore, assessment of an individual's ability to protect their airway from aspiration must be made directly from assessment of RC. Furthermore, there was no dose-response for suppressed RC strength. However, there was a cough sequence effect for both VC and RC, with second coughs being weaker than the first.

This research is unique in investigating cough strength in suppressed RC. The absence of dose-response conflicts with previous research examining cough strength in natural RC. This generates many questions regarding the differences between natural and suppressed RC and which will provide the most accurate information about cough effectiveness.

5.1 Reflexive Cough

It was hypothesized that as the dose of citric acid increased there would be a corresponding increase in strength of cough response. This hypothesis was based on previous research that found positive dose-response relationships when investigating natural cough testing with increasing doses of tussigenic agent (Cox et al., 1984; Fontana et al., 1997; Vovk et al., 2007). However, our results strongly indicated that there was no significant difference in the magnitude of cough response to different doses of citric acid. This outcome might be explained by the protocol of assessing suppressed cough rather than natural cough. It is postulated that suppressed cough is a closer approximation to true reflexive cough with no cortical involvement, as the individual can no longer voluntarily control their response to citric acid (Monroe et al., 2014). However, these findings suggest that although the sensitivity of suppressed coughing is truly reflexive, the individual may retain cortical control of the strength of true reflexive coughing and that in trying not to cough, strength is stifled.

Observations during data collection suggest that this may be true; most participants' RCs were perceptually considered as weak to the investigator. Additionally, once the nebulizer was switched off and the participant was aware that the test was finished, and it was no longer necessary to suppress coughing, the perceptual strength of coughing subjectively increased. Anecdotally, it appeared that once the nebulizer was switched off, both the peak and duration of coughs increased. Conversely, it could be argued that these subsequent coughs, after stimulus is removed, are not true RCs and cortical control of coughing has been re-established.

An alternative hypothesis for the lack of effect of dose on strength of coughing in suppressed CRT, is that in a true RC the strength elicited is an all-or-nothing response that is not dependent upon stimulus dose. The dose-effect observed in natural CRT could be explained by cortical augmentation of the all-or-nothing reflexive response. This would mean that any threat to the airway, whether a small amount of water or a large solid bolus, would result in the same strength of coughing in order to protect and clear the airway. Another explanation could be that, rather than any true RC being an all-or-nothing response, it is the true RC stimulated by citric acid that produces this standard response. Lasserson et al. (2006) emphasized that a tussigenic agent does not identically replicate an aspiration event. Therefore, cough responses in this study may not present the equivalent of a true cough response to aspiration. Pitts et al. (2014) demonstrated that coughing strength in cats is greater when the risk of aspiration is larger. This implies that although no dose effect of citric to coughing strength was identified, there is potential for the existence of a dose effect with actual aspiration.

No hypothesis was made regarding any cough sequence effect (1^{st} or 2^{nd} cough in a sequence) on strength of individual coughs. However, there was a main effect of cough sequence with all outcome measures, with the second cough in the sequence being weaker than the first. This finding is consistent with other research that has shown that strength of coughing decreases over the course of a cough sequence (Fontana & Widdicombe, 2007; Lasserson et al., 2006; Vovk et al., 2007). This is likely due to decreasing lung volume as air is expelled during each cough, leading to less volume to contribute to subsequent coughs. This decreased lung volume also results in decreased expiratory muscle length which leads to reduced muscle force and consequently decreased airflow velocity (Vovk et al., 2007). Although it might be assumed that these weaker coughs are less effective in clearing the airway, it has been hypothesized that there is greater compression in the distal and smaller airways which results in greater linear velocity enabling clearance of substances from the

smaller air passages (Young et al., 1987). However, this clearance of the smaller airways is less important with regards to aspiration, where the key factor is prevention of entry and immediate expectoration from the upper, larger airways. Therefore, it is hypothesized that it is the first cough in a sequence that is key in airway protection and clearance of aspirate.

5.2 Voluntary Cough

The finding that volitionally produced strong coughs were indeed objectively stronger than volitionally produced weak coughs demonstrates that these outcome measures are sensitive to provide a relative measure of strength of coughing, as hypothesized. As with reflexive coughs, second coughs were found to be weaker than first coughs for measures of peak pressure, AUC pressure, peak flow, and AUC acoustic. This similar finding is to be expected, as the amount of residual air left in the lungs decreases with subsequent coughs (Vovk et al., 2007).

Measures of peak pressure, peak flow, and AUC flow revealed variable relationships between cough sequence and gender. In particular, AUC flow unexpectedly revealed that in males the second cough is stronger than the first. The differences observed between genders with each measure highlight the variability of the outcome measures. It is suspected that there is greater error in AUC flow measures, compared to other outcomes, due to clipping of the waveform secondary to start and end marker placement (see section 5.5 for further discussion of this). This clipping of waveforms may have resulted in this irregular finding, and it could be concluded that the flow waveform start and end markers should be placed individually to minimize error. Additionally, it emphasizes the necessity for further research investigating effectiveness of coughing to aspiration so that the best outcome measure can be selected.

Some of our measures, peak pressure, AUC pressure, peak flow, and AUC acoustic, demonstrated a larger decrease in strength between cough 1 and 2 for strong coughs than for weak coughs. This is likely due to a greater volume of air required to produce strong coughs than for weak coughs, resulting in less residual lung volume for the second cough.

The relationship between type of cough and gender with AUC flow reveals a greater difference in strength between weak and strong coughs for females than in males. This relationship is only observed in one of the six outcome measures suggesting that this may be an anomaly. Again, this may be due to suspected inaccuracies in AUC flow measures, and this requires further investigation.

5.3 Reflexive Cough versus Voluntary Cough

The finding that strong VCs are stronger than RCs is similar to results revealed by Lasserson et al. (2006), who showed that sEMG measures of respiratory muscle activity were greater for VC than for RC stimulated by tartaric acid. They hypothesized that this may be due to smaller lung volumes for RC compared to VC, where there is usually inspiration before the cough. It has already been suggested that reflexive airway protection status should not be determined from the assessment of VC (Addington et al., 2008; Stephens et al., 2003). The rationale for this is that RC is more complex and physiologically different than VC. Furthermore, VC is predominantly cortically controlled whereas RC is controlled by the rostral and caudal medulla (Mazzone et al., 2011). This implies that neurological impairment could result in very different outcomes for VC versus RC as shown by Fontana et al. (1998) who found that RC was more impaired than VC in patients with Parkinson's disease. Our research further supports this, as VC strength is likely to be much stronger than a RC. Therefore, determining that VC is strong does not signify that RC will be strong enough to be effective.

5.4 Outcome Measures

A key intent of this study was to identify a salient measure of cough strength that could be easily implemented in clinical practice. All measures were sensitive to cough strength, but measures of AUC pressure and peak flow exhibited the largest effect sizes and strongest correlations indicating they have the best potential for clinical application. The results suggest that acoustic measures, using the methodology of a microphone attached to a stethoscope, may be less accurate and sensitive than pressure and flow measures. This is unfortunate, as acoustic measures are likely to be less expensive and more clinically practical compared to pressure and flow. Nonetheless, it is clear that further work is needed in order to verify the sensitivity and specificity of these measures to identify coughing that is ineffective in clearing aspirate.

5.5 Error in Marker Placements

By modifying the marker placement, where pressure or flow peaks were clipped, it was possible to ensure that peak amplitudes for all measures were 100% accurate. Had these

markers not been moved, substantially different results for those coughs would have been observed, particularly for peak flow, which was most affected by clipping.

It was important to look at AUC as well as peak measures as it provides a measure of strength of the entire duration of the cough rather than just one point within the cough. Lasserson et al. (2006) described AUC as the 'total activity' of a cough. This measure is important as some coughs are of greater duration than others and it may be that this increased duration of a cough is part of its strength and effectiveness in clearing (Addington et al., 2008). These results do not identify any advantage of either AUC or peak in terms of measurement of strength. However, this could be further investigated by studying the effectiveness of cough response to aspiration using the same outcome measures. The RMSE analysis shows that for both pressure and flow, the biggest error for the eight modified coughs was for peak flow. It is likely that there was some error in AUC measures of flow for all coughs analysed, this is due to flow measures tending to finish later than either pressure or acoustic measures, resulting in clipping of the end of waveforms. However, the automatic maker systems should have ensured relatively consistent error between coughs and between participants.

5.6 Exclusions

Recent research by Monroe et al. (2014) evaluated natural cough threshold and suppressed cough threshold in healthy individuals. They found that 65% of participants triggered a suppressed C2 response by a citric acid dose of 0.8 Mol/L. In order for participants in this study to be included they had to produce a suppressed C2 at a consecutive three doses, thus requiring a response at or before doses of 0.8 Mol/L, 1.2 Mol/L, and 1.8 Mol/L. Therefore, on the basis of this normative data, it is expected that approximately 35% of participants would not produce a C2 response at 0.8 Mol/L, and thus would be excluded from analysis. These research findings align with those of Monroe et al. (2014).

When excluding participants who did not produce a C2 response, this decision was made on the basis of the airflow data. Where inhalation between cough 1 and 2 was observed to occur on the airflow waveform, the cough was marked as a fail. It is interesting to note that clinical decisions are made on a perceptual basis as to whether or not inhalation occurs between cough 1 and 2. It is likely some participants who were excluded on the basis of airflow data would have been included from perceptual judgement of C2 response.

5.7 Limitations

The protocol used a non-airtight facemask, the benefits of this were that these masks are cheap, accessible, and already being used in clinical settings. However, the fact that the mask was not airtight, resulting in air escape around the edges of the mask, means that flow and pressure measurements are not absolute measures of coughing strength, but relative measures. However, what is clinically required is a consistent, relative measure of RC strength that will allow judgement of effectiveness of coughing using consumable materials that are readily available and inexpensive. The face-mask set-up utilized in this study demonstrates the potential to provide this.

Pressure and flow measures were taken simultaneously, which will have resulted in a decreased magnitude of pressure values secondary to air escape via the flowhead. However, this set-up was important, as it allowed us to directly compare all outcome measures for the same coughs. As discussed above, it provided a relative measure of pressure adequate for a comparative assessment of coughing strength.

Consistent placement of the stethoscope for acoustic measures was difficult due to small anatomical differences between participants; for some people, despite manipulation of placement, the microphone detected carotid pulse which may have affected the outcomes. Furthermore, the use of a stethoscope connected to a microphone, rather than just using a microphone, means that the majority of the sound detected will have been internal sounds, whereas a microphone predominantly detects external sounds. The measurement of internal sounds is likely to be more affected by inter-participant variability due to anatomical differences. It would be useful in future research to trial the use of a microphone taped to the suprasternal notch or held at a set distance from the facemask in order to increase inter-participant reliability.

As discussed previously, there may be some clipping of waveforms, particularly for pressure and flow, as a consequence of the methodology utilized for marker placement. Unfortunately, any method for placement of markers which chooses a start and end of a cough for all waveforms would inevitably result in clipping of some of the waveforms, as the coughs do not begin and end in precisely the same place for each outcome measure. However, the benefits of choosing a start and end placement for all measures is that they can be directly compared. The benefit of an automatic placement system also ensures that there will be consistency in the clipping of AUC measures and that the study is repeatable.

5.8 Clinical Implications

This study confirms research advocating the importance of assessing strength of RC, rather than VC, in the dysphagic population. The development of a method to objectively measure strength of RC is vital to enable clinical judgement regarding the effectiveness of RC in protecting and clearing the airway. Measurements of pressure, flow, and acoustics all show promise for such objective measurements.

There is no dose-response to cough strength for suppressed cough, suggesting that perceptual or objective measures of this could be obtained from any dose of citric acid to indicate adequacy of airway protection. However, if coughing strength is cortically controlled, strength of suppressed cough may not accurately represent the reflexive cough response to aspiration. Based on these findings, it is recommended that perceptual judgements of coughing strength should be made with great caution. These findings suggest that most healthy individuals would perceptually present with a weak suppressed RC and, thus, fail CRT. Therefore, further investigation into strength of both natural and suppressed RC is required. Additionally, when making perceptual judgements of coughing strength, given that the second cough is usually weaker than the first, it would also be wise for clinicians to make their judgement of coughing strength on the first rather than any subsequent coughs.

5.9 Future Directions

It would be useful to repeat this study with the addition of measurement of natural cough reflex using the same equipment set-up. This would provide more information about whether or not a dose effect is seen in natural CRT. The lack of dose effect observed in suppressed CRT could be due to cortical inhibition of strength of coughing, or due to RC being an all-or-nothing response, or it could be a result of citric acid stimulus not providing a true simulation of aspiration. In order to further elucidate this, the following studies are proposed:

1. Investigation of natural and suppressed RC strength to aspiration would provide information regarding cortical inhibition of strength of coughing in suppressed CRT. This could be achieved utilizing this study's face-mask set-up whilst injecting different volumes of water onto the vocal cords to simulate aspiration in healthy individuals. This would also clarify whether an RC triggered by aspirate is an all-or nothing response, or

whether it increases with greater threat to the airway. It should also enable the establishment of a cough reflex strength threshold which could be used to identify patients who have ineffective coughing. Finally, it would help to clarify which measure of strength most accurately identifies ineffective coughing.

2. Validation of this cough reflex strength test could also be carried out by comparing the findings of the test with VFSS findings to analyse which measures correspond best with ineffective and effective coughing. This would also enable the establishment of a reflexive cough strength threshold.

3. Comparison of perceptual judgements of strength and presence or absence of a C2 response against these objective measures of suppressed cough strength would be useful to further explore observations made that suppressed cough is perceptually weak. It is hypothesized that most of these reflexive coughs will be rated as being weak.

4. It would be useful for further research, like that of Mazzone, McGovern, Koo, & Farrell (2009), to investigate the neurological control of suppressed and natural cough to evaluate whether there is a difference and whether there is evidence of cortical involvement in suppressed coughing which might indicate cortical control of strength of RC.

These studies would provide the information required to determine if it is possible to accurately measure effectiveness of RC strength and whether or not this should be done from natural or suppressed cough. They will also enable further evaluation of the sensitivity of the different outcome measures and verification of whether peak amplitude or AUC provides a better measure of effective cough strength and whether any measures are more accurate or consistent than the others.

6 Conclusion

This research is the first step in developing a test that will enable objective measurement of the strength of RC and establish a threshold of effective RC. It has demonstrated that pressure, flow, and acoustic measures are all sensitive to measurement of strength of coughing. However, acoustic measures tend to show lower effect sizes and correlate less with other measures, and peak flow and AUC pressure show the most promise for assessment of cough strength, with the highest effect sizes and correlation. Subsequent coughs in a cough sequence are likely to be lower in strength than the first. Furthermore, there is no effect of increasing citric acid dose on suppressed reflexive cough strength, which highlights the need for further study into both suppressed and natural CRT using this methodology.

Further research is needed to refine the test and establish a threshold for effective cough strength, which will then enable more precise identification and management of patients who are at risk of aspiration pneumonia.

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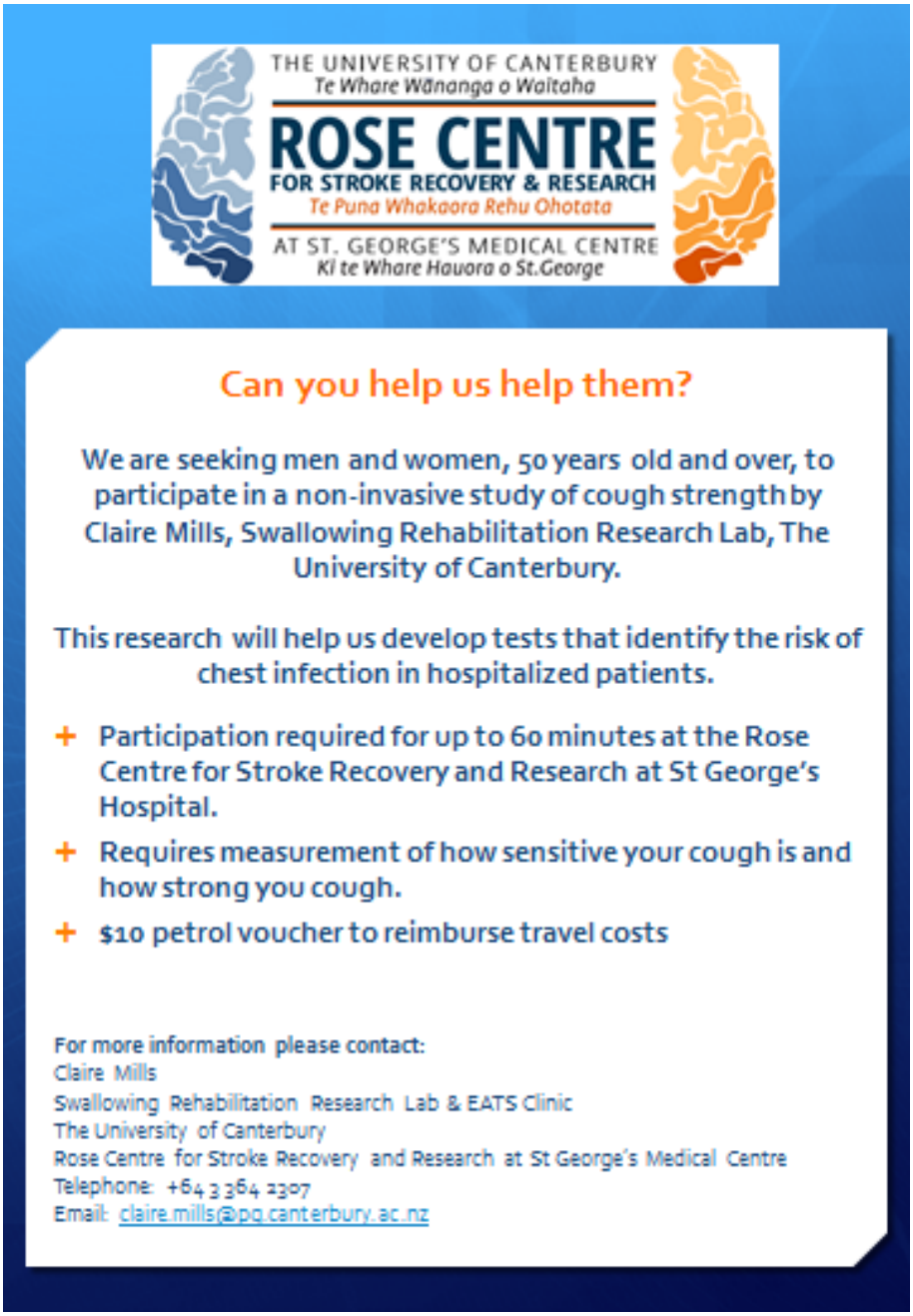
APPENDICES

Appendix 1 –

Advertisement



Appendix 2 –

Information Sheet

Swallowing Rehabilitation Research Lab & EATS Clinic
The University of Canterbury
Rose Centre for Stroke Recovery and Research at St George's Medical Centre
Leinster Chambers, Level One
Private Bag 4737
249 Papanui Road
Christchurch 8140
New Zealand

Telephone: +64 3 364 2307
Email: claire.mills@pg.canterbury.ac.nz

01.05.2015

Measuring Cough Strength in Healthy Individuals using Cough Reflex Testing

I am conducting research as part of my Master's Thesis at the University of Canterbury. The purpose of my research is to develop a method for us to quickly and easily measure the strength of a person's cough. The aimis for this information to eventually be used with patients in hospitals, where it is hoped that measure of their cough strength will help us to identify people who are at risk of chest infections.

You will participate in a cough test, which involves breathing in a variety of mists through a face mask. These mists may, or may not, make you cough. The facemask has sensors that will measure and record the strength of your cough. With your consent, a video-recording will be made of the test. This recording will be used for determining the results of the study and/or teaching. If you choose to consent to video-recording for results only, then the video recording will be deleted on conclusion of the study.

During the cough test you may experience some irritation in your throat. There are no other risks associated with this procedure and these mists have been widely used to stimulate cough production with no serious side-effects. The procedure itself should take 60 minutes in total, with no further follow-up required.

Participation is voluntary and you have the right to withdraw at any stage without providing reason. If you withdraw, none of your information will be retained. The results of the project may be published, but no information that will personally identify you will be used. A Master's thesis is a public document and will be available through the UC Library. You may receive a copy of the project results by contacting me at the conclusion of the project.

To ensure confidentiality, all information relating to you will be given a code number and your consent form will be kept in a locked filing cabinet, accessible only to staff of the Swallowing Rehabilitation Research Laboratory. Video-recordings will be password protected. All identifiable information about you will be destroyed after 10 years.

I will be carrying out the project, as a requirement for my Master's Thesis, under the supervision of Associate Professor Maggie-Lee Huckabee and Professor Richard Jones, who can be contacted at maggie-lee.huckabee@canterbury.ac.nz and richard.jones@canterbury.ac.nz. They will be pleased to discuss any concerns you may have about participation in the project.

This project has been reviewed and approved by the University of Canterbury Human Ethics Committee, and participants should address any complaints to The Chair, Human Ethics Committee, University of Canterbury, Private Bag 4800, Christchurch (human-ethics@canterbury.ac.nz).

If you agree to participate in the study, you are asked to complete the consent form and return it to me.

Thank you for your consideration.
Kind Regards

Claire Mills

Appendix 3 –

Consent Form

Swallowing Rehabilitation Research Lab & EATS Clinic
The University of Canterbury
Rose Centre for Stroke Recovery and Research at St George's Medical Centre
Leinster Chambers, Level One
Private Bag 4737
249 Papanui Road
Christchurch 8140
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Telephone: +64 3 364 2307
Email: claire.mills@pg.canterbury.ac.nz

01.05.2015

Measuring Cough Strength in Healthy Individuals using Cough Reflex Testing

Please Initial Box

1. I confirm that I do not have any of the following: reflux, respiratory condition, neurological condition, swallowing difficulties ☐
2. I confirm that I have not smoked in the past year ☐
3. I confirm that I am not currently taking steroids or opiates and have not taken codeine-based analgesia in the last 24 hours ☐
4. I confirm that I have read and understood the description of the above-named project and have had the opportunity to ask questions. On this basis I agree to participate as a subject in the project, and I consent to publication of the results of the project with the understanding that my anonymity will be preserved. ☐
5. I consent to video-recording of my cough test and for this to be used for:
    a. Research purposes e.g. results of the cough test ☐
    b. Teaching purposes e.g. undergraduates, conference presentations ☐
6. I understand that participation is voluntary and I may withdraw at any time, including withdrawal of any information I have provided should this remain practically achievable. ☐
7. I understand that the project has been reviewed and approved by the University of Canterbury Human Ethics Committee ☐
8. I would like to receive a copy of the summary of results on conclusion of the project ☐

By signing below, I agree to participate in this research project.

*Name:*_____

*Signature:*_____

*Date:*_____

Please return the form to me when attending the clinic for participation in the study.

Appendix 4 –

Format for Counter-balancing of Presentation of Voluntary and Reflexive Coughs

Each participant was given a code with a number relating to the order they were seen, starting with 1, and a letter relating to the presentation of stimulus.

The letters represented the following presentation of stimulus:
A = VCB1, VCB2, VCW1, VCW2, CA
B = VCB2, VCB1, VCW2, VCW1, CA
C = VCW1, VCW2, VCB1, VCB2, CA
D = VCW2, VCW1, VCB2, VCB1, CA
E = CA, VCB1, VCB2, VCW1, VCW2
F = CA, VCB2, VCB1, VCW2, VCW1
G = CA, VCW1, VCW2, VCB1, VCB2
H = CA, VCW2, VCW1, VCB2, VCB1
I = VCB1, VCB2, CA, VCW1, VCW2
J = VCB2, VCB1, CA, VCW2, VCW1
K = VCW1, VCW2, CA, VCB1, VCB2
L =VCW2, VCW1, CA, VCB2, VCB1

Abbreviations:
VCB1 = 1 strong voluntary cough
VCB2 = 2 strong voluntary coughs
VCW1 = 1 weak voluntary cough
VCW2 = 2 weak voluntary coughs
CA = Reflexive coughs (always presented in increasing order of dose)

Appendix 5 –

Example Data Form

Data Collection form for A - VCB1, VCB2, VCW1, VCW2, CA

Task	Detail	Tick when complete
Discuss information sheet		
Discuss and sign the consent form		
Take DOB of participant		
Get signature for petrol voucher		
Attach stethoscope to neck		
Secure face mask		
Set-up video-recording		
Get a strong cough to check audio volume		
Remind: - try not to touch the mask once it's in place - the mist will only be applied for up to 15 seconds - once I've stopped the mist you can take the mask off but try to wait until then if possible - if you want to stop at any point just say - if you want a glass of water please ask		
Start AD lab and video- recording		
Turn Nebulizer on VolCough – 1 strong	Tag VCB1	
60 seconds rest		
Turn Nebulizer on VolCough – 2 strong on one breath	Tag VCB2	
60 seconds rest		
Turn Nebulizer on VolCough – 1 weak	Tag VCW1	
60 seconds rest		
Turn Nebulizer on VolCough – 2 weak on one breath	Tag VCW2	
60 seconds rest		
"Don't hold your breath. Breathe in and out through mouth and if feel need to cough try to suppress it"		
Let them smell CA 1.2 from open container		
60 seconds rest		
Placebo (15 seconds)	Tag P	
60 seconds rest		
CA0.4 (15 seconds)	Tag CA0.4	
60 seconds rest		
Placebo (15 seconds)	Tag P	
60 seconds rest		
CA0.8 (15 seconds)	Tag CA0.8	
60 seconds rest		
Placebo (15 seconds)	Tag P	
60 seconds rest		

CA1.2 (15 seconds)	Tag CA1.2	
60 seconds rest		
Placebo (15 seconds)	Tag P	
60 seconds rest		
CA1.8 (15 seconds)	Tag CA1.8	
Stop AD lab and audio and video recording		
Save recordings		

Appendix 6 –

Table of Means and Standard Deviations for Reflexive and Voluntary Coughs

Stimulus	Cough Number	Peak Pressure mmHg		AUC Pressure mmHg.s		Peak Flow L/s		AUC Flow L		Peak Acoustic V		AUC Acoustic V.s	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dose 1 of Citric acid	**1st**	2.097	1.174	0.072	0.046	0.905	0.555	0.133	0.090	0.391	0.248	0.015	0.012
	2nd	1.680	0.932	0.047	0.028	0.570	0.371	0.085	0.062	0.357	0.264	0.012	0.011
Dose 2 of Citric acid	**1st**	2.300	1.522	0.077	0.048	0.869	0.546	0.132	0.077	0.381	0.269	0.017	0.019
	2nd	1.774	1.079	0.042	0.021	0.631	0.445	0.086	0.059	0.339	0.248	0.010	0.009
Dose 3 of Citric acid	**1st**	2.246	0.988	0.080	0.051	0.920	0.575	0.158	0.124	0.400	0.292	0.017	0.017
	2nd	1.942	1.296	0.048	0.028	0.664	0.519	0.106	0.089	0.311	0.273	0.011	0.012
2 weak voluntary coughs	**1st**	1.849	1.178	0.119	0.068	1.347	0.661	0.176	0.091	0.369	0.279	0.013	0.015
	2nd	1.672	1.112	0.085	0.056	1.089	0.603	0.172	0.118	0.362	0.275	0.017	0.018
2 strong voluntary coughs	**1st**	7.352	3.798	0.349	0.132	2.020	0.734	0.334	0.133	0.654	0.129	0.057	0.035
	2nd	5.976	2.885	0.194	0.097	1.508	0.568	0.292	0.108	0.616	0.172	0.040	0.027

Appendix 7 –

Table of Repeated-Measures ANOVA Results for Voluntary and Reflexive Coughs

Effect		Cough Sequence		Dose	Type of cough	Cough sequence * gender	Type of cough * cough sequence	Type of cough * gender
		RC	VC	RC	VC	VC	VC	VC
Peak Pressure	F	11.877	16.482	0.435	57.726	11.303	27.038	0.239
	p-value	.002	<.001	.650	<.001	.002	<.001	.629
	Effect size	0.078	<.001	-	0.012	-	<.001	-
	Power	.913	.975	.117	1.000	.900	.999	.076
AUC Pressure	F	30.759	59.465	0.121	54.089	0.011	27.038	0.005
	p-value	<.001	<.001	.862	<.001	.916	<.001	.943
	Effect size	1.361	3.401	-	23.110	-	1.040	-
	Power	1.000	1.000	.067	1.000	.051	.999	.051
Peak Flow	F	33.251	66.774	0.050	20.544	4.801	6.275	2.714
	p-value	<.001	<.001	.952	<.001	.037	.019	.111
	Effect size	0.706	0.166	-	1.321	-	-0.011	-
	Power	1.000	1.000	.057	.992	.561	.676	.355
AUC Flow	F	10.649	0.049	2.369	44.402	10.465	1.693	10.643
	p-value	.003	.827	.103	<.001	.003	.204	.003
	Effect size	0.376	-	-	33.472	-	-	-
	Power	.882	.055	.459	1.000	.877	.241	.882
Peak Acoustic	F	4.511	1.043	0.560	41.585	0.116	0.189	1.008
	p-value	.043	.316	.925	<.001	.736	.667	.324
	Effect size	0.018	-	-	8.385	-	-	-
	Power	.535	.167	.058	1.000	.062	.070	.162
AUC Acoustic	F	10.937	8.771	0.560	58.983	.642	16.755	2.457
	p-value	.003	.006	.926	<.001	.430	<.001	.129
	Effect size	0.482	-0.244	-	2.719	-	0.637	-
	Power	.890	.815	0.058	1.000	.121	.976	.327