

Assessment and Modulation of the Sensorimotor Cough Response

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of
Philosophy

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Abstract

Impairments of coughing (i.e. dystussia) and swallowing (i.e. dysphagia) frequently co-occur. This co-morbidity increases the risk of developing aspiration pneumonia. The aim of this research program was to enhance methods of assessment and explore an option for rehabilitation of the sensorimotor cough response. This gave rise to a series of studies that evaluated (1) methods of citric acid cough reflex testing (CRT) (2) modulation of the sensorimotor cough response through sensory stimulation, and (3) the use of acoustic intensity as a measure of cough strength.

There is lack of standardization and inadequate data on test-retest variability of citric acid CRT. These issues necessitated two methodological studies on citric acid CRT. A systematic review that summarized and appraised methods of citric acid CRT across disciplines was completed. Data across studies were translated to standardized units of measurement to streamline comparison across studies. A total of 136 citric acid CRT protocols were retrieved and evaluated by two independent investigators. The study revealed lack of standardization and substandard reporting of instrumentation and citric acid CRT protocols across studies, preventing the full replication of many. It is anticipated that these findings will contribute to the development of standards of methods of citric acid CRT, and highlight implications of methods of citric acid CRT on the outcome of the test.

Test-rest variability of citric acid CRT was evaluated in healthy individuals ($n = 16$) across three alternate days (i.e. Monday, Wednesday, Friday). Methods of citric acid CRT were chosen to optimize test-retest reproducibility. Estimated increases of 0.43 and 0.32 doubling concentrations of citric acid, per day ($p < 0.05$), were identified for natural (NCT) and suppressed (SCT) citric acid cough thresholds, respectively. These data suggest a habituation

effect occurs with repeated exposure to citric acid CRT. Quantification of this habituation effect enabled the effects of a sensory stimulation protocol in the subsequent study to be evaluated against the artefact of repeating the citric acid CRT, which was used as an outcome measure.

A prospective, pseudo-randomized control trial was conducted to evaluate the safety and efficacy of a 4-day sensory stimulation protocol, involving inhalations of distilled water to modulate cough sensitivity in healthy adults ($n = 24$). Evaluation of safety was necessary due to the known risks of bronchoconstriction following distilled water inhalation. Participants were randomly assigned to one of three groups: (1) high intensity stimulation (inhalations of distilled water at high flow rate), (2) low intensity stimulation (inhalations of distilled water at low flow rate), and (3) a control group (inhalations of 0.9% saline). A citric acid CRT was completed at baseline (Day 1), and after the sensory stimulation protocol on alternate days (i.e. Day 3 and Day 5) to determine participants NCT and SCT. The sensory stimulation protocol, and spirometry (to monitor the safety of the distilled water inhalations on the respiratory system) were completed on days two to five. The study revealed that the sensory stimulation protocol did not induce bronchoconstriction in any participant. SCTs changed differently across days in the high and low intensity sensory stimulation groups, compared to the control group ($p < 0.05$). In the control group, citric acid cough thresholds increased across days, resembling the habituation effect observed upon repeated exposure to CRT. In contrast, an absence of habituation to citric acid CRT was observed following both of the sensory stimulation protocols, suggesting a possible sensitization effect of distilled water.

The final two studies represent a clinical adjunct to this research program. There are no clinically applicable, objective measures of cough strength, as it relates to clearance of penetration and/or aspiration. This prompted two studies that used acoustic intensity to (1)

determine a cut-off value of effective/ineffective clearance of penetration and aspiration on videofluoroscopic swallowing studies (VFSS) and (2) compare citric acid induced cough strength between healthy individuals and patients with dysphagia. Acoustic intensity was chosen as a measure of cough strength as it represents a non-invasive, clinically applicable means of measuring coughing. In the first study, patients referred for VFSS were recruited (n = 88). Data were included from patients who coughed to penetration and/or aspiration during their VFSS (n = 13). An important, yet unexpected finding, was that no patient effectively expelled aspirate material from the laryngeal vestibule (n = 10). Coughing expelled penetration (n = 7). However, definitive cut-off values of cough effectiveness could not be made due to the limited number of observations. There are modifications of the study design that must be thoroughly investigated prior to making conclusions regarding the role of coughing in airway clearance of aspirate, and the validity of acoustic intensity as a measure of cough strength.

In the second acoustic intensity study, patients with dysphagia (n = 12) and aged-matched healthy controls (n = 16) were recruited. Audio recordings of citric acid induced coughing were completed for all participants. The study revealed a difference in the acoustic intensity of citric acid induced coughing between the two groups ($p < 0.05$). Healthy individuals were found to have a *louder* cough to CRT than patients with dysphagia. Whether this translates to a functional difference between the two groups, in terms of *strength* of cough, remains to be directly tested. The issues detailed above, encountered when attempting to validate acoustic intensity for airway clearance in patients with dysphagia on VFSS, prevent inference of the current results to functional cough strength.

In summary, this research program enhances understanding of assessment and modulation of the sensorimotor cough response and provides important groundwork for future studies. Future

research should evaluate test-retest variability of citric acid CRT and the safety and efficacy of the distilled water sensory stimulation protocol in patients with dysphagia. Additionally, the role of coughing in expelling aspiration from the airway, and the validity of acoustic intensity in predicting effective/ineffective clearance, should be further investigated.

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Preface

This PhD thesis conforms to the referencing style recommended by the American Psychological Association Publication Manual (6th ed.) and spelling recommended by the Oxford Dictionary (<https://www.oxforddictionaries.com>). The research for this thesis was carried out between February 2016 and March 2019, while the candidate was enrolled in the Department of Communication Disorders at the University of Canterbury. The research presented in this thesis was carried out at the University of Canterbury Rose Centre for Stroke Recovery and Research at St George's Medical Centre, Christchurch Hospital, and Burwood Hospital. The research was supervised by Dr Phoebe Macrae, Prof Maggie-Lee Huckabee, and Dr Michael Epton.

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Wallace, E., Guiu Hernandez, E., Macrae, P. (2018, September). Quantifying test-retest variability urge-to-cough (UTC) in healthy individuals. Poster presentation. European Society for Swallowing Disorders (ESSD), Dublin, Ireland.

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Wallace, E. Macrae, P., Huckabee, M. L. (2017, January). Validation of acoustics as a proxy measure for cough strength in patients with dysphagia. Platform presentation. Stroke Rehab Conference: From No-Tech to Go-Tech, Christchurch, New Zealand.

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List of Abbreviations

ACE	Angiotensin-converting enzyme
AUC	Area under curve
CN	Cranial Nerve
COPD	Chronic obstructive pulmonary disease
CRT	Cough reflex test
CSE	Clinical swallowing evaluation
EMG	Electromyography
ERS	European Respiratory Society
FEES	Fibreoptic endoscopy evaluation of swallowing
fMRI	Functional magnetic resonance imaging
HDEC	Health and disability ethics committee
LER	Laryngeal expiration reflex
NCT	Natural cough threshold
NA	Nucleus ambiguus
NTS	Nucleus tractus solitarius
PD	Parkinson's Disease
PAS	Penetration-aspiration scale
RAR	Rapidly adapting receptor
RLN	Recurrent laryngeal nerve
SCT	Suppressed cough threshold
SAR	Slowly adapting receptor
SLN	Superior laryngeal nerve
UES	Upper esophageal sphincter
VFSS	Videofluoroscopic swallowing study

**SECTION I. INTRODUCTION AND
LITERATURE REVIEW**

CHAPTER 1: Introduction

This research program details five studies that aimed to enhance methods of assessment and rehabilitation of the sensorimotor cough response. Section I provides a literature review, outlining the relationship between coughing and swallowing (Chapter 2). It details how impairment of both mechanisms frequently co-occur following neurological injury, increasing the risk of negative outcomes, such as aspiration and mortality. The neurophysiology of coughing is detailed in Chapter 3. The afferent, central and efferent pathways of the sensorimotor cough response are outlined, providing rationale for the methods of assessment and sensory stimulation used in the subsequent research studies. In addition to this general literature review, each section of this thesis includes a more focused literature review related to the specific research questions address in those sections.

Section II is dedicated to methodological studies on citric acid CRT. Chapter 4 provides a literature review on citric acid CRT methodology, with emphasis on the European Respiratory Society (ERS) guidelines for citric acid CRT. This chapter offers insight into the lack of standardization and limited data on test-retest reliability of the citric acid CRT. These limitations imposed restrictions on the use of the citric acid CRT as a viable outcome measure for the subsequent study (Chapter 7), and provided the rationale for the two methodological studies. In order to gain insight into methods of citric acid CRT used in published literature, a systematic review of instrumentation and citric acid CRT protocols used across disciplines was conducted (Chapter 5). This study is presented first, as it outlines a number of methodological considerations that are important for understanding the methods and results of the subsequent studies. Chapter 6, presents a study quantifying test-retest variability of the citric acid CRT. The methods of citric acid CRT utilized in this study were chosen to optimize test-retest

reproducibility, based on ERS guidelines for citric acid CRT. The overarching aim of this study was to quantify the variability of repeated citric acid CRT to enable the effects of a sensory stimulation protocol in Chapter 7 to be evaluated against any variance or order effects associated with repeating the test.

Section III (Chapter 7) describes a study that evaluated the safety and efficacy of a 4-day sensory stimulation protocol to enhance cough sensitivity. It begins with a literature review of research related to sensory rehabilitation, and outlines the lack of rehabilitation options for patients with laryngeal sensory deficits and silent aspiration. A framework of sensory stimulation in the limb literature is described, and provides the foundation on which the current sensory stimulation protocol is based. Inhalations of distilled water were used in an attempt to modify cough sensitivity. However, published literature reports risks of bronchoconstriction with inhalation of distilled water in patients with asthma. Thus, the primary aim of this study was to evaluate the safety of the sensory stimulation protocol in healthy individuals. The efficacy of the stimulation protocol at enhancing cough sensitivity (as measured by the citric acid CRT) was evaluated as a secondary objective.

Section IV provides a clinical adjunct to this research program, seeking to provide clinicians with an objective measure of cough strength. Chapter 8 provides a literature review related to limitations of current methods of cough strength evaluation, and the advantages of using acoustic intensity as a clinically applicable means of measuring cough strength. Chapter 9 represents the first study in the literature to objectively measure the strength of a sensorimotor cough response to penetration and aspiration. The study aimed to measure the cough strength (in decibels) that is required to expel penetration and aspiration from the laryngeal vestibule during video-fluoroscopic swallowing studies (VFSS) in patients with dysphagia. Chapter 10

compares the acoustic intensity of citric acid induced coughing in healthy individuals to patients with neurogenic dysphagia, in an attempt to overcome the challenges associated with subjective evaluation of citric acid induced coughing.

In Section V, conclusions are made regarding implications of this research program and directions for future research. In particular, directives for further enhancing methods of citric acid CRT, to hone the test to evaluating specific neural pathways of coughing, are highlighted. The findings from Chapter 7 provide the foundation for future studies to evaluate the safety and efficacy of the sensory stimulation protocol in patient populations with laryngeal sensory deficits and silent aspiration. Lastly, the impact of methodological limitations on the findings of Chapter 9 and 10 suggest that further research is necessary to determine the role of coughing in expelling aspiration from the airway, and to validate acoustic intensity as a measure of cough strength.

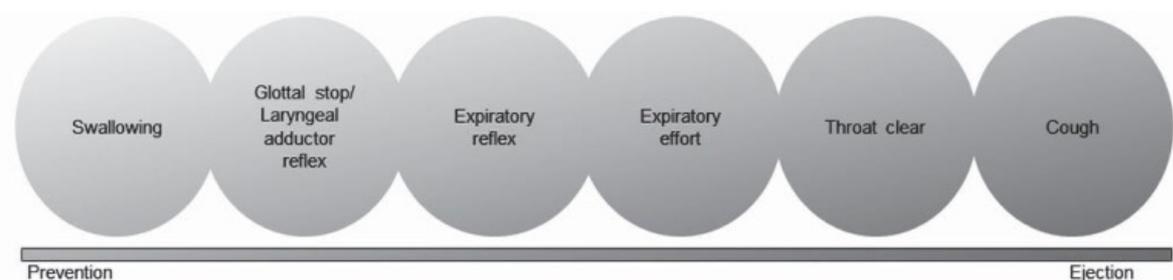
CHAPTER 2: The Relationship Between Swallowing and Coughing

Swallowing is the process by which a bolus is safely transported from the oral cavity into the stomach (Jean, 2001). In doing so, it plays two important roles. Firstly, as a critical component of the ingestive process, swallowing can be considered part of the alimentary (or digestive) system (Gestreau, Milano, Bianchi, & Grelot, 1996; Jean, 2001). Swallowing also protects the upper respiratory tract from aspiration of food, fluids and secretions (Jean, 2001), and within this framework, swallowing can be considered part of the respiratory system (Pitts, 2014; Troche, Brandimore, Godoy, & Hegland, 2014). This latter view of swallowing has particular relevance to this thesis that focuses on the assessment and rehabilitation of coughing in response to failed airway protective mechanisms during swallowing.

The process of swallowing is complicated by the shared anatomical pathway for air and food through the pharynx, which creates significant risk for airway compromise (Martin-Harris, 2006; Pitts, 2014). Due to the importance of pulmonary protection for survival, the body is equipped with a range of airway protective and airway clearance mechanisms (Bolser, Pitts, Davenport, & Morris, 2015). Airway protective mechanisms *prevent* food or liquids entering the laryngeal vestibule (i.e. penetration) and proceeding into the trachea (i.e. aspiration) during swallowing (Bolser et al., 2015). Airway clearance mechanisms, such as coughing, occur *in response* to food or liquids in the airway, i.e. in the event that airway protective mechanisms fail during swallowing (Bolser et al., 2015). Recently, a theoretical framework for understanding the relationship between different levels of airway protection in humans was proposed (Troche, Brandimore, Godoy, et al., 2014). Airway protective and airway clearance mechanisms occur along a continuum, with coughing and swallowing at opposing ends (Figure

1) (Troche, Brandimore, Godoy, et al., 2014). In order to understand what drives the transition from protective to clearance mechanisms, the complexity of swallowing - and significant potential for airway invasion - must be appreciated.

Figure 1: Continuum of airway protective and airway clearance mechanisms. From “A framework for understanding shared substrates of airway protection” by Troche, M. S., Brandimore, A. E., Godoy, J., & Hegland, K. W. (2014), *Journal of Applied Oral Science*, 22(4), p. 252. CC BY-NC.



2.1 The Complexity of Swallowing

The anatomical configuration of the aerodigestive tract demands a rapid and precise cascade of airway protective mechanisms to prevent misdirection of food, fluid or saliva into the airway during swallowing. Airway protection begins in the oral phase of swallowing, but differs for liquid and solid boluses. For liquids, swallowing related apnoea, vocal cord approximation and arytenoid adduction are observed upon introduction of a liquid bolus into the mouth (Dua, Ren, Bardan, Xie, & Shaker, 1997; Hiss, Strauss, Treole, Stuart, & Boutilier, 2004; Martin-Harris et al., 2005; Ohmae, Logemann, Kaiser, Hanson, & Kahrilas, 1995; Shaker, Dodds, Dantas, Hogan, & Arndorfer, 1990). Lower viscosity and larger bolus volumes, which present increased risk of airway invasion, evoke earlier swallowing related apnea and laryngeal vestibule closure in healthy individuals (Hiss et al., 2004; Humbert et al., 2018). This highlights the importance of oral afferents in timely airway protection. For solids, portions of masticated

food are transported from the oral cavity to the pharynx. They gradually accumulate in the oropharynx, valleculae, and even as low as the pyriform sinuses, during continued mastication, until the entire bolus is ready for swallowing - a process known as pharyngeal bolus aggregation (Humbert et al., 2018; Matsuo & Palmer, 2009; Palmer, Hiiemae, Matsuo, & Haishima, 2007). Unlike during ingestion of liquids, the larynx remains open and breathing continues during pharyngeal bolus aggregation of solid textures (Matsuo, Hiiemae, Gonzalez-Fernandez, & Palmer, 2008). As the pharynx is used for respiration *and* food containment simultaneously, there is significant risk for airway compromise. This risk is mitigated by highly effective and efficient sensorimotor control in a healthy system. For example, brief, partial closure of the vocal folds - known as the pharyngo-glottal closure reflex - is associated with entry of a bolus into the pharynx (Dua et al., 1997). This serves as an anticipatory protective reflex to prevent invasion of food or saliva into the airway prior to swallowing, and is dependent on intact pharyngo-laryngeal sensorimotor control. In addition, it is suggested that a healthy system produces an optimized bolus cohesiveness, via mastication and lubrication with saliva, that enables food particles to combine and collect in the valleculae and pyriform sinuses prior to swallowing (Prinz & Lucas, 1997). Cohesion of the bolus ensures small particles are not aspirated into the respiratory tract (Prinz & Lucas, 1997), and depends on adequate sensory input from the oral cavity to inform neural centres in the brain about the state of the bolus (Steele & Miller, 2010).

In the pharyngeal phase of swallowing, a highly repeatable sequence of airway protective mechanisms occur to seal the entrance of the airway as the bolus passes through the pharynx (Jean, 2001). Hyolaryngeal excursion causes the larynx and hyoid bone to move in an superior-anterior direction during swallowing. This is achieved via contraction of the suprahyoid muscles, longitudinal pharyngeal muscles and thyrohyoid muscle (Pearson, Hindson,

Langmore, & Zumwalt, 2013; Pearson, Langmore, Yu, & Zumwalt, 2012). This anterior–superior movement of the larynx is vital for laryngeal vestibule closure, which is achieved via pharyngeal shortening, arytenoid adduction and approximation, and epiglottic inversion (Logemann, Kahrilas, & Cheng, 1992; Matsuo et al., 2008; Vose & Humbert, 2018). Adduction of the true and false vocal folds are independent of laryngeal vestibule closure and serve as a secondary line of defence to prevent entry of material into the lower airway (Ekberg, 1982). The order and timing of these airway protective events can vary with bolus volume, size and viscosity (Ekberg, 1982; Inamoto et al., 2011; Kawasaki, Fukuda, Shiotani, & Kanzaki, 2001; Ohmae et al., 1995; Shaker et al., 1990). A bottom to top closure is most often described, which serves to squeeze misdirected materials from the laryngeal vestibule in the event of inadvertent airway invasion (Ekberg, 1982; Logemann et al., 1992; Ohmae et al., 1995; Shaker et al., 1990; Vose & Humbert, 2018). Numerous structures (tongue, pharynx, larynx, hyoid bone) and physiologic events (arytenoid adduction and approximation, tongue base retraction, epiglottic inversion, hyolaryngeal excursion, pharyngeal constriction) are involved in laryngeal vestibule closure during swallowing (Vose & Humbert, 2018). Given the importance of airway protection for survival, this complexity offers multiple layers of protection (Vose & Humbert, 2018). However, it may also increase the risk of airway invasion if a structure or component is perturbed.

Two other respiratory related mechanisms - swallowing related apnoea and breathing-swallowing coordination - offer additional means of airway protection during the pharyngeal phase of swallowing (Martin-Harris, 2006). Swallowing related apnoea refers to cessation of breathing during swallowing, that occurs independent of laryngeal vestibule closure (Hiss & Postma, 2003; Martin-Harris et al., 2005). It highlights the intricate relationship between the respiratory and swallowing systems. The onset and duration of swallowing related apnoea

varies among healthy adults, with reported apnoea interval durations from 0.5 s – 3.5 s across a range of bolus textures and volumes (Martin-Harris, 2006; Martin-Harris et al., 2005). Larger bolus volumes are associated with longer apnoeic intervals (Martin, Logemann, Shaker, & Dodds, 1994). These data suggest that sensory properties of the bolus may influence the apnoeic interval during swallowing, highlighting the importance of afferent input for adequate airway protection. Precise respiratory-swallowing coordination is vital for airway protection (Brodsky et al., 2010). Expiration before and after swallowing is the most common breathing pattern in healthy adults (Martin et al., 1994; Martin-Harris et al., 2005). It serves to expel any misdirected materials from the airway after swallowing, offering an additional airway protective mechanism. Deviation from this respiratory-swallowing pattern is associated with increased risk of airway invasion (Brodsky et al., 2010) This is particularly apparent for initiation or completion of swallowing around an inspiration, which serves to bring air, and potentially misdirected food or fluid particles, into the lungs (Brodsky et al., 2010; Martin-Harris, 2006).

Failure of any of these airway protective mechanisms during swallowing may result in penetration and/or aspiration. In this event, airway clearance mechanisms, such as coughing, act as a safety net by expelling mis-directed material from the upper respiratory tract (Bolser et al., 2015; Hegland, Troche, Brandimore, Davenport, & Okun, 2014; Lee & Birring, 2012; Watts, Tabor, & Plowman, 2016). It's important to remember that this type of coughing is not to be confused with acute or chronic cough associated with respiratory pathology, and refers specifically to coughing as a sensorimotor response to penetration or aspiration.

2.2 Airway Clearance Coughing

Coughing can be broadly defined as “a forced expulsive manoeuvre against a closed glottis, that is associated with a characteristic sound” (Morice et al., 2007, p. 1256). However, not all coughing is the same. An understanding of the differences between cough types (e.g. volitional coughing, reflexive coughing and the laryngeal expiration reflex) is imperative for interpreting the results of studies, and to draw accurate conclusions about airway protective mechanisms. Firstly, voluntary coughing has three phases: inspiratory, compressive and expulsive. It is preceded by an intention or command to cough, rather than a physiologic drive associated with airway irritation (Widdicombe, Addington, Fontana, & Stephens, 2011). In this respect, it is a motor, rather than a sensorimotor response. Others suggest that voluntary coughing can be produced in response to a sub-threshold urge-to-cough, when the capacity for suppression remains (Eccles, 2009). This may have relevance to the type of cough produced in response to conscious perception of trace penetration or accumulation of saliva in the airway. However, most research studies on voluntary coughing in the dysphagia literature align to Widdicombe’s definition, where participants are asked to cough on command, rather than in response to sub-threshold airway irritation. Thus, caution must be made in interpreting these studies to draw inferences about the sensorimotor cough response to penetration or aspiration.

The laryngeal expiration reflex (LER) and reflexive coughing are considered sensorimotor cough responses (Widdicombe et al., 2011). The LER is characterised by rapid closing of the glottis and an expulsive phase in response to chemical or mechanical laryngeal irritation (Fontana & Widdicombe, 2007; Korpas & Jakus, 2000; Widdicombe et al., 2011). It has been experimentally elicited in humans by touching the vocal folds with a nylon wire during laryngoscopy (Korpas, Misik, & Kalocsayova, 1975), and by inhalation of tussive aerosols (Vovk et al., 2007). The absence of an initial inspiration is crucial to the definition of the LER.

It prevents inhalation of foreign bodies further into the airway, and expels material from the supra-glottic space (Korpas & Jakus, 2000; Widdicombe et al., 2011). In this sense, it acts as a specific “anti-aspiration” mechanism (Widdicombe et al., 2011, p. 312). The absence of an initial inspiration also distinguishes the LER from reflexive coughing (Widdicombe et al., 2011). Reflexive coughing is characterised by the same physiologic pattern as voluntary coughing but is preceded by a physiologic drive, or urge to cough (Widdicombe et al., 2011). The advantage of the initial inspiration is that it provides a greater volume and flow of air to expel materials from the lower airways (Widdicombe et al., 2011). However, an inspiration draws materials into respiratory tract and in this sense, it represents a “pro-aspiration reflex” (Widdicombe et al., 2011, p. 314).

While the LER and reflexive coughing are fundamentally distinct (Fontana & Widdicombe, 2007), in reality, both occur within a sensorimotor cough response to chemical or mechanical laryngeal irritation - the LER preceding reflexive coughing (Fontana, 2008; Korpas & Jakus, 2000; Nishino, Tagaito, & Isono, 1996; Widdicombe et al., 2011; Widdicombe & Fontana, 2006). This physiologic pattern is advantageous for the initial expulsion of supra-glottic material, followed by high velocity airflow to expel sub-glottic material (Widdicombe et al., 2011). Physiologic measures of airflow or pressure are required to distinguish between LER and reflexive coughing. Given both mechanisms co-occur in response to airway invasion (Nishino et al., 1996), the term “reflexive coughing” typically signifies both LERs and reflexive cough patterns. It bears mentioning that the term “reflexive coughing” may be inaccurate to describe the sensorimotor cough response to tussigenic aerosols (such as capsaicin and citric acid), as numerous studies demonstrate the role of supra-medullary pathways and the cerebral cortex (Eccles, 2009; Hegland, Bolser, & Davenport, 2012; Hutchings, Eccles, Smith, & Jawad, 1993b; Hutchings, Morris, Eccles, & Jawad, 1993a;

Mazzone, Cole, Ando, Egan, & Farrell, 2011; Mazzone, McLennan, McGovern, Egan, & Farrell, 2007). This is described in detail in Chapter 3. The term ‘sensorimotor cough response’ is used herein to denote coughing preceded by chemical or mechanical laryngeal irritation that may comprise LER and reflexive cough patterns described above.

There is relatively little research on coughing initiated in response to invasion of food or fluid into the airway before, during or after swallowing. This type of coughing is referred to as “cough on swallowing” by Widdicombe and colleagues (2011, p. 315), and is considered a sensorimotor cough response. Coughing to aspiration has been elicited in animal models by dropping water into the larynx, which resulted in a single expiratory effort (recorded with a pneumotachograph), analogous to a LER (Sullivan, Murphy, Kozar, & Phillipson, 1978). In human participants, Nishino et al. (1996) observed an immediate, vigorous response of expiratory efforts, including LERs and reflexive cough patterns, after injecting a small amount (0.5 ml) of distilled water on to the vocal folds of conscious human participants during endoscopy. This is the only study in the literature to document the physiologic pattern of coughing in response to airway invasion in human participants. However, these data are published as part of a review article. The number and/or demographics of the participants included in the study are not reported, and only one example of the physiologic pattern of coughing in response to airway invasion is reported. Thus, these findings should be interpreted with caution. Coughing in response to airway invasion can be observed during video-fluoroscopic swallowing studies (VFSS) in the event of inadvertent penetration or aspiration in patients with dysphagia (Garon, Sierzant, & Ormiston, 2009; Watts et al., 2016). However, no physiological measures of airflow, respiratory muscle activity or gastric/esophageal pressures have been used during VFSS to measure the sensorimotor cough response to aspiration.

2.3 Shared Neural Substrates of Coughing and Swallowing

A growing body of literature demonstrates considerable overlap in the peripheral and central neural control of coughing and swallowing (Mutolo, 2017; Pitts, 2014; Troche, Brandimore, Godoy, et al., 2014). This relationship is essential to the way in which both behaviours work in synchrony to defend the airway from foreign material.

2.3.1 Peripheral Neural Overlap

The vagus nerve (CN X) provides sensory and motor innervation to the pharynx and larynx, and therefore has an obvious role in coughing and swallowing (Mu & Sanders, 2000; Troche, Brandimore, Godoy, et al., 2014). CN X divides into two main branches, the superior laryngeal nerve (SLN) and the recurrent laryngeal nerve (RLN) (Sanders, Wu, Mu, Li, & Biller, 1993), both of which have key roles in sensorimotor control of coughing and swallowing. The internal (sensory) branch of the SLN (iSLN) carries sensory information from the pharynx and the supraglottic space, specifically, the posterior pharyngeal wall, epiglottis, aryepiglottic folds, laryngeal vestibule, cricoarytenoid region and the anterior wall of the larynx (Mu & Sanders, 2000). Electrical stimulation of the iSLN readily evokes swallowing and coughing in animal models (Bolser, 1991; Doty, 1951; Miller, 1972; Tsujimura, Udemgba, Inoue, & Canning, 2013). Tsujimura and colleagues (2013) found that swallowing and coughing were elicited at different stimulation intensities of the SLN (10 Hz versus > 20 Hz respectively) (Tsujimura et al., 2013). The greater level of stimulation required to elicit coughing supports the hierarchy of airway protective mechanisms proposed by Troche et al. (2014). Anaesthesia of the iSLN results in impaired coughing and swallowing in humans (Jafari, Prince, Kim, & Paydarfar, 2003). Significantly higher incidents of laryngeal penetration and aspiration were found in otherwise healthy participants with iSLN anaesthesia, compared to un-anaesthetized controls

(Jafari et al., 2003). In all cases of airway invasion under iSLN anaesthesia, coughing was only elicited after the entry of the bolus into the trachea (Jafari et al., 2003), from which sensation is carried by the sensory branch of the RLN. Unfortunately, no comment is made on the physiologic pattern of the cough elicited (i.e. an LER or reflexive cough pattern), or whether the cough was effective at expelling the aspirate from the airway. This information would have provided insight into the neural control of different types of coughing and whether impaired sensation also disrupts the effectiveness of coughing.

Motor innervation of the larynx is supplied by the RLN, with the exception of the cricothyroid muscle, which is innervated by the SLN (Sanders et al., 1993). With relevance to coughing and swallowing, the RLN innervates the lateral cricoarytenoid and the inter-arytenoid muscles that adduct the vocal folds (Sanders et al., 1993). These muscles are critical for airway protection during swallowing, and for building-up subglottic pressure prior to the expulsive phase of coughing (Shaker et al., 2002). The RLN also plays an important role in innervating the muscles that influence the pressure at the upper esophageal sphincter (UES) (Tsuji-mura et al., 2013), which may have implications on coughing and swallowing mechanisms. Pitts (2014) suggests the UES and larynx work as “dual valves” during coughing and swallowing (Pitts, 2014, p. 3). During swallowing the larynx is adducted, and UES resting pressure falls (via relaxation of the cricopharyngeus muscle) to allow the bolus to enter the esophagus (Pitts, 2014). During the compression phase of coughing, the larynx is adducted and the cricopharyngeus muscle is maximally contracted to provide a barrier against retrograde entry of gastric contents into the pharynx (Amaris, Dua, Naini, Samuel, & Shaker, 2012), and to maintain intra-thoracic pressure for cough effectiveness (Pitts, 2014; Pitts et al., 2013).

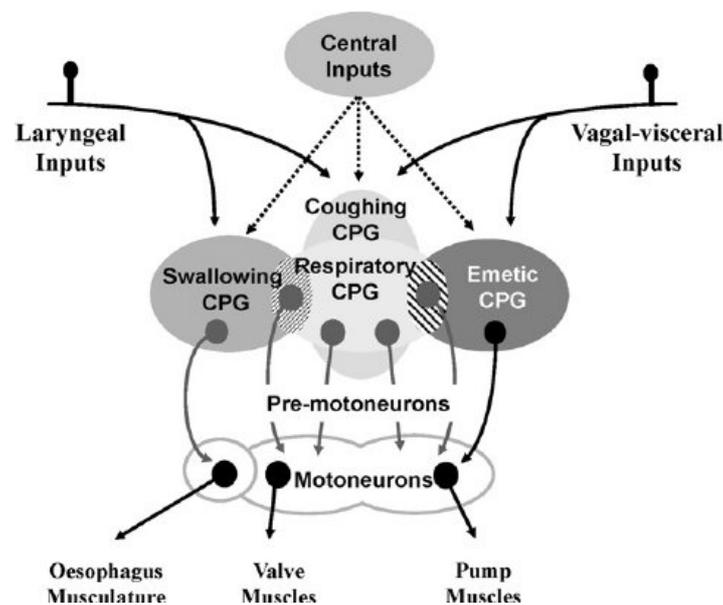
2.3.2 Central Neural Overlap

The neural control of coughing and swallowing also overlaps at a central level (Bianchi & Gestreau, 2009; Bolser, Gestreau, Morris, Davenport, & Pitts, 2013). The concept of a central pattern generator (CPG) is used to explain how relatively fixed motor patterns - such as breathing, swallowing and coughing - are generated by brainstem neuronal networks. In a landmark study by Doty and Bosma (1956), observations of the relatively fixed sequence of muscle activation in the pharyngeal phase of swallowing led to the concept of the swallowing CPG, which is fundamental to our current understanding of the neural control of swallowing. The swallowing CPG involves two important centres: the dorsal swallowing group (DSG) and the ventral swallowing group (VSG). The DSG houses the nucleus tractus solitarius (NTS) and the surrounding reticular formation (Jean & Car, 1979). It receives direct input from peripheral afferent nuclei, namely the facial (VII), glossopharyngeal (IX) and vagus (X) nerves, as well as supra-medullary input. The ventral swallowing group (VSG), houses the nucleus ambiguus (NA) and surrounding reticular formation (Jean & Car, 1979). The NA contains the motor neurons for the glossopharyngeal (IX) vagus (X) and spinal accessory (XI) nerves that activate muscles in the pharynx and larynx during swallowing. It receives direct input from the DSG.

Understanding of the coughing CPG is still incomplete (Haji, Kimura, & Ohi, 2013), but similar to the swallowing CPG, it is known to involve the NTS and the NA (Gestreau, Grelot, & Bianchi, 2000; Gestreau et al., 1996; Jordan, 2001; McGovern, Driessen, et al., 2015). It is suggested that coughing and swallowing form part of a multifunctional respiratory neuronal network in the brainstem (Bolser et al., 2013) (Figure 2). The concept of a behavioural control assembly (BCA) was proposed to explain how a single network of neurons exist for interrelated behaviours (Bolser et al., 2013). In theory, BCAs activate different CPGs (i.e. coughing, swallowing and breathing) by a process of reconfiguration that involves altering the excitability

of key elements of the neuronal network, presynaptic modulation, and/or recruitment of previously silent elements of the neuronal network in response to preceding sensory stimuli (Bolser et al., 2013). In essence, the BCA allows these behaviours to work in synchrony. However, the precise frequency and type of sensory stimuli that are necessary to excite differential components of the respiratory neuronal network remains unclear. While our understanding of this multi-functional neural network is still in its infancy, it supports clinical observations in which impairments of breathing, coughing and swallowing frequently co-occur (Clayton, Carnaby, Peters, & Ing, 2014; Martin-Harris, 2008; Terzi et al., 2007).

Figure 2: Interactions between the respiratory, coughing and swallowing CPGs From “The brainstem respiratory network: an overview of a half century of research” by Bianchi, A. L., & Gestreau, C. (2009), *Respir Physiol Neurobiol*, 168(1-2), p. 10. Reprinted with permission.



2.4 Dystussia and Dysphagia

The disadvantage of the neural overlap of coughing and swallowing is that impairment of both mechanisms can co-occur in multiple neurological disorders (Bolser et al., 2015; Pitts, Bolser, Rosenbek, Troche, & Sapienza, 2008; Pitts et al., 2010; Smith Hammond et al., 2001; Ward et

al., 2010). The term dysphagia refers to impairment in any or all phases of swallowing. Failed airway protective mechanisms result in aspiration and/or penetration of food, fluid or saliva, and is a common clinical sign observed in up to 30% of patients with dysphagia (Smith Hammond & Goldstein, 2006). Dystussia refers to impairment of the motor and/or sensory components of coughing (Ebihara, Sekiya, Miyagi, Ebihara, & Okazaki, 2016), the former resulting in impaired cough strength, and the latter resulting in a phenomenon known as silent aspiration, i.e. an absent cough response to aspiration (Ebihara et al., 2016).

The comorbidity of failed airway protective *and* airway clearance mechanisms has significant clinical consequences. Aspiration pneumonia - a respiratory infection secondary to aspiration - is associated with increased medical costs, longer hospital stays, and mortality (Hannawi, Hannawi, Rao, Suarez, & Bershada, 2013; Kidd, Lawson, Nesbitt, & MacMahon, 1995; Schmidt, Holas, Halvorson, & Reding, 1994). While the development of aspiration pneumonia is multi-factorial (Langmore et al., 1998), research has shown a robust relationship between dystussia and aspiration pneumonia in patients with dysphagia (Addington, Stephens, Gilliland, & Rodriguez, 1999; Bianchi, Baiardi, Khirani, & Cantarella, 2012; Fujiwara et al., 2017; Hegland, Okun, & Troche, 2014; Kimura, Takahashi, Wada, & Hachisuka, 2013; Nakazawa, Sekizawa, Ujiie, Sasaki, & Takishima, 1993; Pikus et al., 2003; Pitts et al., 2010; Plowman et al., 2016; Sekizawa, Ujiie, Itabashi, Sasaki, & Takishima, 1990). For example, among a heterogeneous cohort of patients with dysphagia, the relative risk of developing aspiration pneumonia was thirteen times higher for patients with silent aspiration, compared to those with unimpaired swallowing (Pikus et al., 2003). Among a cohort of stroke patients, the relative risk of developing aspiration pneumonia was 5.57 times greater for those with silent aspiration compared to those who aspirated with a cough response and those who did not aspirate (Holas et al., 1994). Other studies report that a reduction in cough peak flow (i.e. the peak airflow

achieved during the expiratory phase of coughing) is associated with higher risk of pulmonary morbidity. Bianchi and colleagues (2012) found that patients with aspiration pneumonia had lower voluntary cough peak flow than those without pulmonary complications. This was also true for the cough peak flow of citric acid induced coughing (Fujiwara et al., 2017). These data highlight an important relationship between the development of aspiration pneumonia and the integrity of the sensorimotor cough response in patients with dysphagia. As a result, the sensorimotor cough response is an important target for assessment and rehabilitation (Watts et al., 2016).

2.4.1 Assessment of Coughing in Patients with Dysphagia

Assessment of coughing in patients with dysphagia is not a new concept. Evaluation of volitional coughing (i.e. by asking a patient to cough on command), and/or coughing while eating and drinking has always been an integral part of the clinical swallowing evaluation as it provides insights into a patient's risk of aspiration (Mann, 2002; McCullough et al., 2005; Smith Hammond et al., 2001; Suiter & Leder, 2008; Watts et al., 2016). However, the clinical swallowing evaluation lacks adequate sensitivity for detecting patients at risk of silent aspiration (Ramsey, Smithard, & Kalra, 2003; Smithard et al., 1998). Asking a patient to cough on command does not provide information about the integrity of the sensorimotor cough response to airway invasion (Addington et al., 1999; Stephens, Addington, & Widdicombe, 2003). Furthermore, absence of coughing while eating and drinking does not reliably indicate absence of aspiration, as aspiration may be silent (Ramsay, Wright, Thompson, Hull, & Morice, 2008; Smithard et al., 1998). Thus, there is increasing clinical attention in evaluation of the sensorimotor cough response via cough reflex testing to make judgements about an individual's ability to protect their airway during swallowing (Watts et al., 2016).

2.4.1.1 The Cough Reflex Test

The cough reflex test (CRT) challenges the integrity of the sensorimotor cough response by introducing a tussive agent to the respiratory tract via inhalation, and observing for a cough response (Morice et al., 2007). As a test of laryngeal sensitivity to inhaled particles, the CRT provides a model for evaluating coughing in response to aspiration. This is evidenced by a number of studies demonstrating high sensitivity and specificity of the CRT in detecting silent aspiration on instrumental assessment. Wakasugi and colleagues (2008) were among the first to validate CRT as a screening tool for identifying risk of silent aspiration on instrumental assessments, video-fluoroscopic swallowing study (VFSS) and fiberoptic endoscopic evaluation of swallowing (FEES), in patients with cardiovascular disease (39%), head and neck cancer (24%), neuromuscular disease (17%), respiratory disease (15%), and other non-specified diseases (5%). Using 1% (w/v) citric acid, inhaled for one minute via ultrasonic nebulizer, the authors found high sensitivity and specificity for detecting silent aspiration (87% and 89% respectively). Greater than five coughs within one minute was considered a pass, while less than four coughs was considered a failed test. It is unclear why these cut off values were used, and how patients who coughed exactly four times were classified. Furthermore, rationale for the use of 1% w/v citric acid is not provided. Regardless, these data demonstrate that citric acid CRT can identify patients with silent aspiration with high accuracy. These findings have been replicated in later studies using a small portable vibrating mesh nebulizer, as opposed to an ultrasonic nebulizer, which improves the clinical feasibility of the method (Sato et al., 2012; Wakasugi et al., 2014).

More recently, Miles and colleagues evaluated the sensitivity and specificity of a different method of CRT on instrumental assessment in a cohort of patients with dysphagia secondary to stroke (38%), head and neck cancer (10%), respiratory disorders (17%), progressive

neurological disorders (10%), other neurological disorders (9%) and non-specified diagnoses (16%) (Miles, Moore, et al., 2013). The concentrations of citric acid used in the study (i.e. 0.4, 0.6 and 0.8 mol/L) were based on predetermined normative data in healthy adults (Monroe, Manco, Bennett, & Huckabee, 2014). Using 0.6 mol/L citric acid, inhaled for up to 15 seconds (tidal breathing) via facemask until C₂ cough thresholds (based on the European Respiratory Society guidelines for cough testing) were achieved (i.e. two consecutive coughs within 15 seconds), the authors found moderate sensitivity and specificity for detecting silent aspiration (71% and 60%, respectively). A higher concentration of citric acid (i.e. 0.8 mol/L) had less sensitivity but greater specificity (i.e. 58% and 84% respectively), while a lower concentration (i.e. 0.4 mol/L) had higher sensitivity, but lower specificity (77% and 35% respectively). Interestingly, patients with dysphagia following stroke were at higher risk of failing the CRT than those with a diagnosis of respiratory disorders (odds ratio = 16.7, 95% CI, 2.27, 122.21).

In a more recent study, the sensitivity and specificity of non-acidic tussigenic agents - capsaicin and ultrasonically nebulizer distilled water - were evaluated for detecting aspiration (defined as penetration-aspiration scale scores of > 5) versus no aspiration (defined as penetration-aspiration scale scores of < 4) in patients with Parkinson's Disease (Hegland, Troche, Brandimore, Okun, & Davenport, 2016). Using a C₂ criteria for classifying responders and non-responders, 1 minute tidal inhalations of distilled water yielded a high sensitivity and specificity (77.8% and 90.9% respectively) for differentiating between patients with Parkinson's disease with and without aspiration (Hegland et al., 2016). In contrast, low sensitivity (44.4%) and high specificity (100%), was found for single inhalations of capsaicin. These findings suggest that while capsaicin CRT can accurately determine those who are not aspirating, its use for identifying patients with aspiration appears limited. No study has evaluated the sensitivity and specificity of capsaicin CRT for differentiating between patients

with and without *silent* aspiration. Hegland and colleagues (2016) suggest the lack of sensitivity of capsaicin CRT in differentiating between patients with and without aspiration may reflect the use of a single inhalation, as opposed to a 1-minute inhalation of the aerosol that was used for distilled water CRT. It is unclear why different methods are used for both tussive agents in the study. An alternative hypothesis may relate to the underlying neurophysiological mechanisms of cough induction, that differ for capsaicin, compared to distilled water and citric acid (Canning et al., 2004; Mazzone & Udem, 2016; Morice, Higgins, & Yeo, 1992). This is discussed in more detail in Chapter 3, but studies suggest that capsaicin may induce coughing through different airway afferents and central pathways when compared with distilled water and citric acid (McGovern, Driessen, Simmons, Powell, et al., 2015, Mazzone & Udem, 2016, McGovern, Davis-Poynter, Simmons, Ferrell, et al., 2015). This may in part explain the discrepancies in the sensitivity and specificity of cough reflex testing with capsaicin, distilled water and citric acid. Along the same lines, differences in the underlying pathophysiology of dystussia and dysphagia (e.g. stroke, progressive neurological diseases, respiratory diseases) may also influence the sensitivity and specificity of the CRT, or the tussive agent that is most suitable, depending on neural cough pathway that is expected to be impaired (i.e. somato-sensory versus viscerosensory) (see Chapter 3, section 3.2.3 for further discussion).

2.4.2 Enhanced Long-Term Clinical Outcomes

A better understanding of assessment and management of dystussia in patients with dysphagia has been shown to have significant health care outcomes in terms of reduced medical costs, longer hospital stays and mortality. Addington and colleagues were among the first to evaluate whether inclusion of CRT to the clinical swallowing evaluation (CSE) would differentiate between stroke patients who did or did not develop aspiration pneumonia (Addington et al.,

1999). The CRT protocol involved a maximum of three deep inhalations of nebulized tartaric acid (20% solution of prescription grade *l*-tartaric acid dissolved in 2 ml saline). A weak or absent cough to all three inhalations was considered a failed test. The results revealed that 1% of patients in the CRT group developed aspiration pneumonia, compared to 13% in the non-CRT group ($p < 0.05$). These data suggest the inclusion of CRT may facilitate identification of patients at risk of silent aspiration, and provide information that has the potential to enhance management, although, management options for patients that fail the CRT were not provided by the authors. There are several methodological limitations. Firstly, CRT and non-CRT groups were from different hospitals. It is possible that different oral care practices or medical management may represent confounding variables. Furthermore, the authors note that patients in the CRT were required to achieve adequate lip seal around the mouthpiece for an “effective inhalation” (Addington et al., 1999, p. 1204). It is not mentioned how many patient were excluded due to inadequate lip seal, but it is possible that the patients in the CRT group were lower risk of aspiration pneumonia due to better oral motor control. Nevertheless, this study implies that inclusion of a CRT may enhance long-term clinical outcomes for patients with dysphagia.

More recently, implementation of a structured citric acid CRT protocol, that guided clinicians to optimal management decisions in acute stroke patients, reduced rates of pneumonia from 28% to 10% over a three year period in an acute hospital setting (Perry et al., 2018). Patients who failed the CRT were placed on non-oral methods of feeding and referred for VFSS. Interestingly, these findings were not replicated in a similar study by Field et al. (2018), who found a non-significant (2.2%) reduction in pneumonia following implementation of a citric acid CRT protocol. Differences in CRT protocols, or management decisions of patients who fail the CRT may explain the discrepancy in the results. Perry and colleagues (2018) evaluate

natural coughing ('cough if you need to') and suppressed coughing ('try not to cough') to 0.8 mol/L and 1.2 mol/L citric acid aerosols, respectively – which were based on pre-determined norms in healthy individuals (Monroe et al., 2014). Field et al. (2018) evaluated natural coughing to 0.6 mol/L only. The advantage of evaluating natural and suppressed coughing is that they may provide insights into different components or levels of impairment of the sensorimotor cough response (e.g. ascending sensory input and descending control of sensory processing, discussed further in Chapter 3) (McGovern, Ajayi, Farrell, & Mazzone, 2017). This raises an important issue regarding the most appropriate way to assess dystussia in patients with dysphagia, and suggests that methods of CRT may have important implications on the outcome of the test. The sensorimotor cough response is complex, and a number of factors - such as the concentration and type of tussigenic aerosol (e.g. capsaicin versus citric acid), the duration of aerosol inhalation, and instructions given to participants - are known to influence the afferents and central neural cough pathways that are targeted during CRT (Kollarik, Ru & Udem, 2007, Canning et al., 2004). An understanding of these factors is essential for developing appropriate methods of assessment of the sensorimotor cough response in patients with dysphagia.

Furthermore, enhanced long-term clinical outcomes are likely dependent on clinical management of patients with dysphagia and dystussia. Rehabilitation of the respiratory muscles involved in coughing has been shown to improve airway protection in patients with neurogenic dysphagia (Pitts et al., 2009; Troche et al., 2010). However, there are no rehabilitation approaches to address the sensory pathophysiology associated with silent aspiration. Pulmonary safety is likely to be compromised if oral intake occurs in the absence of laryngeal sensitivity that is required to elicit a sensorimotor cough response, regardless of respiratory muscle strength. Thus, rehabilitation of motor *and* sensory components of the sensorimotor

cough response would be advantageous to prevent adverse clinical outcomes associated with dystussia in patients with dysphagia. A comprehensive understanding of the neurophysiology of coughing is essential for developing appropriate methods of assessment and rehabilitation of the sensory and motor components of coughing, and is outlined in the subsequent chapter.

CHAPTER 3: Neurophysiology of Coughing

3.1 Afferent Pathway

Extensive studies in both animals and humans demonstrate that two subtypes of extrapulmonary afferents induce coughing when stimulated: jugular C-fibres and nodose A- δ fibres (Canning et al., 2014; Canning et al., 2004; Mazzone & Undem, 2016). These are distinct from intrapulmonary afferents, such as nodose C-fibres and nodose A β fibres (i.e. rapidly adapting receptors and slowly adapting receptors) that induce tachypnoea, bronchoconstriction, bronchodilation and the Hering-Breuer reflex (Mazzone & Undem, 2016). The characteristics of the cough afferents are summarized in Table 1. Jugular C-fibres and nodose A- δ fibres arise from distinct ganglionic origin, which reflect their different functions (Canning et al., 2004; Kollarik, Ru, & Undem, 2007; Mazzone & Undem, 2016). Cough afferents arising from the jugular ganglia are predominantly C-fibres (Canning et al., 2004; Kollarik et al., 2007). C-fibres are characterised by relatively slower action potential conduction velocity compared to A- δ fibres (Mazzone & Undem, 2016). C-fibres are broadly classified as chemoreceptors, reflecting their sensitivity to chemical stimuli (such as capsaicin, bradykinin, hypertonic saline and acid) and relative insensitivity to mechanical stimulation (Canning et al., 2004; Kollarik & Undem, 2002; Mazzone & Undem, 2016). Cough afferents arising from the nodose ganglia are exclusively A- δ fibres (Canning et al., 2004). They are commonly referred to as “cough receptors” in the literature (Canning et al., 2004). Nodose A- δ fibres (or cough receptors) are broadly classified as mechanoreceptors, due to their sensitivity to punctate mechanical stimulation. However, they are distinct from *intrapulmonary* mechanoreceptors (i.e. A β fibres - slowly adapting receptors and rapidly adapting receptors), in that they are insensitive to low threshold mechano-stimulation, such as airflow and changes in tracheal configuration

associated with breathing (Mazzone & Udem, 2016). Similar to jugular C-fibres, nodose A- δ fibres are readily activated by chemical stimuli, such as rapid reductions in pH (e.g. citric acid) and hypotonic solutions (e.g. distilled water) (Canning et al., 2004; Kollarik et al., 2007; Kollarik & Udem, 2002; Mazzone & Udem, 2016). In this respect, they represent a hybrid, chemo-mechanical type of airway afferent.

Table 1: Classification of Tracheal and Laryngeal Cough Afferents (adapted from Mazzone & Udem, 2016)

	Jugular Ganglia	Nodose Ganglia
Fibre Type	C-fibres	A- δ fibres (cough receptors)
Conduction Velocity (m/s)	~ 1	~ 5
Termination	Extrapulmonary	Extrapulmonary
Responsivity	Capsaicin, acid	Punctate mechanical stimulation, acid, hypotonic solutions
Physiological Responses	Apnoea, Cough	Cough
CNS Termination	Paratrigeminal nucleus	NTS

The relative contribution of both afferents to coughing in response to aspiration is still a matter of debate (Mazzone & Udem, 2016). It's unclear whether one afferent holds more importance than the other, or whether both are critical to adequate airway defence against aspiration. Research suggests that nodose A- δ fibres play an important role in mediating coughing to aspiration (Canning et al., 2004; Mazzone & Udem, 2016). This is based on their site of termination in the extrapulmonary bronchi, trachea and larynx, their fast action potential conduction velocity, their sensitivity to punctate mechanical stimulation of the laryngeal epithelium, and their sensitivity to rapid changes in pH (Canning et al., 2004). These

characteristics make A- δ fibres highly suitable to respond to aspiration of food, fluid, or reflux. A- δ fibre stimulation also evokes coughing under general anaesthesia, whereas stimulation of C-fibres is ineffective in doing so (Canning et al., 2004). This suggests that nodose A- δ fibres play a vital role in coughing to airway invasion.

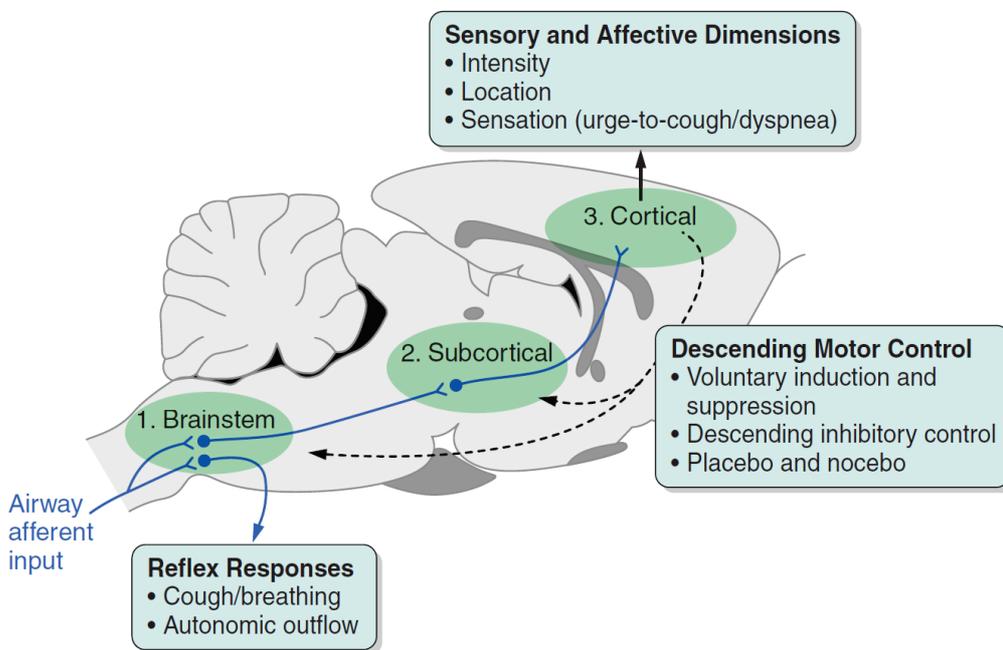
Jugular C-fibres greatly outnumber A- δ fibres in the extrapulmonary airway (Mei, Condamin, & Boyer, 1980; Ricco, Kummer, Biglari, Myers, & Udem, 1996), suggesting they too play an important role in upper airway sensation. C-fibre stimulants (such as capsaicin and bradykinin) effectively evoke coughing in conscious humans and animals (Dicpinigaitis, 2007; Karlsson, 1996). Blunted cough response to capsaicin (a C-fibre stimulant) has been linked with impaired airway protection in patient populations (Troche, Brandimore, Okun, Davenport, & Hegland, 2014). Based on these observations, C-fibres are also likely to play a role in airway protective coughing. Distinct central pathways are known to be involved for C-fibre versus A- δ fibre processing (Mazzone, Mori, & Canning, 2005). This may have implications on the cortical influences, and type of cough evoked from each afferent, and is outlined below.

3.2 Central Control of Coughing

It is now well accepted that at least two distinct central neural pathways exist for coughing (Mazzone & Udem, 2016; McGovern, Driessen, et al., 2015). Coughing can be elicited reflexively at the level of the brainstem, or via ascending pathways to subcortical and cortical regions (See Figure 3) (Mazzone & Udem, 2016). Evidence for a purely reflexive coughing pathway is derived from studies demonstrating that coughing can be elicited under general anaesthesia and in decerebrate animals (Canning et al., 2004; Haji, Ohi, & Kimura, 2012). This suggests, at its most primitive, coughing can be elicited by brainstem mediated process,

achieved via sensory input to the NTS, and subsequent activation of brainstem motor neurons (e.g. the nucleus ambiguus and rostral ventral respiratory group), innervating respiratory and laryngeal musculature (Ambalavanar, Tanaka, Selbie, & Ludlow, 2004; Gestreau et al., 1996; Tanaka, Yoshida, & Hirano, 1995).

Figure 3: Central pathway regulating airway afferent processing. From “Vagal Afferent Innervation of the Airways in Health and Disease” by Mazzone, S. B., & Udem, B. J. (2016), *Physiol Rev*, 96(3) p. 995. Reprinted with permission.



3.2.2 Cortical Control of Coughing

The role of higher subcortical and cortical brain centres in coughing in conscious, awake humans, is now well accepted and supported by numerous behavioural and brain imaging studies (Eccles, 2009; Hegland et al., 2012; Hutchings et al., 1993b; Hutchings et al., 1993a; Mazzone et al., 2011; Mazzone et al., 2007). Higher brain centres decode perceivable sensations, such as urge to cough, that arise upon airway irritation, and control voluntary

induction or suppression of coughing (Eccles, 2009; Hutchings et al., 1993b; Hutchings et al., 1993a; Mazzone et al., 2011).

Two landmark studies by Hutchings and colleagues were among the first to empirically demonstrate the role of higher brain centres in coughing (Hutchings et al., 1993b; Hutchings et al., 1993a). They found that cough thresholds were significantly higher when participants were instructed to consciously suppress their cough (Hutchings et al., 1993b; Hutchings et al., 1993a). The first study evaluated capsaicin-induced cough suppression in healthy volunteers (Hutchings et al., 1993a), while the latter examined suppression of spontaneous coughing as a result of an upper respiratory tract infection over a twenty-minute period (Hutchings et al., 1993b). Based on these findings it was hypothesized that at sub-threshold conditions, an urge to cough is perceived, and under voluntary control, a cough may (or may not) be elicited (Eccles, 2009; Hutchings et al., 1993b; Hutchings et al., 1993a). In contrast, when the threshold is reached and the individual can no longer suppress, reflexive coughing absent of cortical control is produced (Eccles, 2009; Hutchings et al., 1993b; Hutchings et al., 1993a).

The absence of cortical control in reflexive coughing was later questioned by Hegland and colleagues (2014). They found that healthy individuals can modulate (i.e. up and down-regulate) parameters (i.e. airflow and respiratory muscle activity) of *supra-threshold* concentrations of capsaicin induced coughing according to instructions. For example, when participants were instructed to produce a small cough (i.e. instructed to cough smaller or softer than normal), they tended to increase the compression phase duration (CPD) and decrease the post-peak phase duration (PPPD) and cough volume acceleration (CVA). CPD relates to sub-glottic pressure generation, while CVA and PPPD relate to cough airflow acceleration and velocity (Hegland et al., 2012). These studies suggest that coughing is subject to cortical

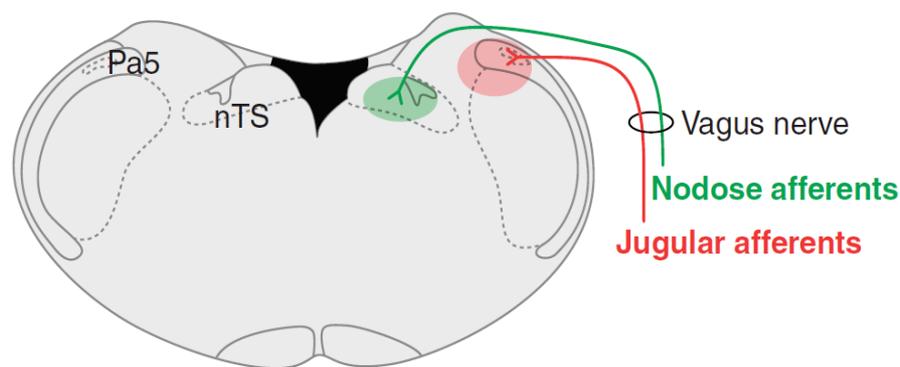
modulation – even at supra-threshold levels of tussigenic stimuli when the response is assumed to be reflexive. In light of these findings, coughing may be more aptly described as a sensorimotor response, as opposed to a reflex. These studies used capsaicin, which uniquely stimulates jugular C-fibres (Canning et al., 2004). It is unclear if the same capacity for suppression and modulation would be seen with tussigenic stimuli that stimulate other afferent pathways, such as in response to citric acid, which stimulates nodose A- δ fibres (Canning et al., 2004), or cough to aspiration.

3.2.3 Distinct Central Neural Pathways

Recent studies suggest that distinct ascending pathways exist for afferents arising from the jugular and nodose ganglia (i.e. C-fibres and A- δ fibres, respectively) (McGovern, Davis-Poynter, et al., 2015; McGovern, Driessen, et al., 2015). These afferents terminate in different anatomical locations in the brainstem (see Figure 4), and project to distinct higher brain regions (McGovern, Davis-Poynter, et al., 2015; McGovern, Driessen, et al., 2015). This may reflect their fundamentally different roles and different capacity for suppression and modulation. Nodose afferents terminate in the NTS, and project to several brainstem and hypothalamic nuclei that are well known for their role in viscerosensory processing and coordination of respiratory and autonomic responses (McGovern, Davis-Poynter, et al., 2015; McGovern, Driessen, et al., 2015). In contrast, tracheal jugular afferents terminate in the paratrigeminal nucleus and project to the ventrobasal and submedial nuclei, which play a role in somatosensation (i.e. encoding perceptual awareness of airway irritation and generating perceivable sensations, such as urge to cough) (McGovern, Driessen, et al., 2015). Based on these observations, nodose A- δ fibres are likely to play a key role in reflexive airway protective coughing via viscerosensory pathways, whereas jugular C-fibres are likely to evoke cortically

mediated coughing via perceived airway sensations that accompany coughing (i.e. urge-to-cough)(Mazzone & Udem, 2016).

Figure 4: Distinct terminations of jugular (C-fibres) and nodose (A- δ fibres) afferents in the brainstem, which give rise to distinct ascending central pathways. From “Vagal Afferent Innervation of the Airways in Health and Disease” by Mazzone, S. B., & Udem, B. J. (2016), *Physiol Rev*, 96(3) p. 995. Reprinted with permission.



However, notable overlap and interconnections between the two pathways have been identified at a central level. Previous studies show non-reciprocal projections of paratrigeminal neurons to the NTS (McGovern, Driessen, et al., 2015; Menetrey & Basbaum, 1987), suggesting jugular afferents may contribute to elicitation of autonomic reflexes through this pathway. This is evidenced by animal studies, in which capsaicin (jugular C-fibre stimulant) was shown to increase the sensitivity (i.e. reduce the threshold) of cough evoked by electrical stimulation and citric acid in anesthetized guinea pigs (Mazzone et al., 2005), alluding to central interactions between jugular C-fibres and nodose A- δ fibres. In addition, capsaicin desensitization (via TRPV1 receptor antagonist) did not prevent coughing, but significantly reduced the number of coughs elicited by citric acid (Mazzone et al., 2005). This cannot be attributed to peripheral sensitization of nodose A- δ fibres, as they do not express the capsaicin (TRPV1) receptor, thus

must arise from central interactions of the afferent nerve subtypes (Mazzone et al., 2005). Interestingly, sensitizing intrapulmonary C-fibres (via nebulized bradykinin to the lower airways) sensitized the cough reflex evoked by citric acid and mechanical stimulation of the trachea (Mazzone et al., 2005). The trachea was treated topically with bradykinin receptor antagonist, which eliminated any chance of peripheral sensitization of extrapulmonary cough receptors (Mazzone et al., 2005). This provides evidence for a central site of interaction between jugular and nodose afferents that exists throughout the respiratory tract, and suggests remote sensitization of the lower airways may have implications on upper airway sensitivity (Mazzone et al., 2005).

3.3 Efferent Pathway and Descending Motor Control

In the efferent (or descending motor) cough pathway, central impulses from the retroambiguus and nucleus ambiguus in the brainstem travel via the vagus, phrenic and spinal motor nerves to the diaphragm, abdominal wall and respiratory and laryngeal muscles to action a cough (Polverino et al., 2012). It was recently proposed that sensory information, processed at higher brain regions, also descends for volitional inhibition or facilitation of cough motor output (Hegland et al., 2012; McGovern et al., 2017). These pathways are believed to be analogous to the descending analgesia pathway that is described for somatosensory processing of noxious stimuli in the pain literature (McGovern et al., 2017). Midbrain nuclei, such as the periaqueductal grey, are believed to play a key role in these descending neural pathways that suppress or facilitate sensorimotor responses (McGovern et al., 2017). McGovern and colleagues (2017) questioned whether these descending pathways are impaired in disease, resulting in up and down-regulation of coughing that give rise to clinical impairments such as chronic cough or silent aspiration. This concept offers an alternative way of viewing downregulation of coughing in patients with dysphagia and suggests that the nature of silent

aspiration, or down-regulated coughing, may be partly attributed to enhanced inhibition of the descending motor cough pathway. This concept may hold most relevance for small amounts aspiration or, accumulation of saliva in the laryngeal vestibule, which may trigger a sensorimotor cough response through cortical processing of afferent input. The authors suggest that this pathway may represent a promising therapeutic target.

Interestingly, a recent study by Brandimore, Hegland, Okun, Davenport, and Troche (2017) demonstrated that patients with Parkinson's Disease and healthy elderly adults were able to upregulate capsaicin induced coughing using biofeedback. Participants were told to cough as hard as you can in response to capsaicin, to reach a grey target area on a computer screen, which was set to 25% above the participant's average baseline peak expiratory flow rate (PEFR) (Brandimore et al., 2017). Capsaicin induced cough expired volume (CEV) and PEFR were significantly increased from baseline when biofeedback was provided (Brandimore et al., 2017). Interestingly, PEFR of tartaric induced coughing has been shown to be reduced in patients with dysphagia with a history of aspiration pneumonia (Fujiwara et al., 2017), suggesting this may provide a therapeutic option for patients with dysphagia and dystussia.

While the precise underlying neural mechanisms of the observed increased in CEV and PEFR are unknown, it is possible that the effect of the biofeedback may represents enhanced facilitation of sensorimotor processing via the descending cough motor pathway. The use of biofeedback enables the cough response to be consciously monitored, facilitating on-line modification and enhancement (Athukorala, Jones, Sella, & Huckabee, 2014; Huckabee & Lamvik-Gozdzikowska, 2018). However, further research is necessary to clarify these hypotheses. The clinical implications of these findings are also unknown. The grey target on the screen was arbitrarily set to 25% above the participant's average baseline PEFR

(Brandimore et al., 2017). It remains unknown whether increasing PEFR to 25% above baseline would have any clinical significance in terms of more effective clearance of material from the airway or lower risk of aspiration pneumonia. In addition, it is unclear whether the observed improvements in cough motor output (i.e. CEV and PEFR) are accompanied by enhanced sensation. According to Mazzone and colleagues (2016), the afferent pathway is the “driving force” of the sensorimotor cough response (Mazzone, 2016, p. 1325), suggesting that an intact efferent pathway may be redundant if afferent input is impaired. Brandimore and colleagues (2017) evaluate the urge to cough (UTC), (i.e. the perceived intensity of the tussigenic stimulus), in response to 200 μ m capsaicin, and found that patients with Parkinson’s Disease had a lower UTC than healthy controls, suggestive of reduced laryngeal sensation, or central sensory processing of tussigenic stimuli at baseline. UTC was not evaluated post-therapy. However, it would be interesting to evaluate cough sensitivity, pre- and post-intervention in future studies to determine whether voluntary upregulation of cough motor output was accompanied by enhanced cough sensitivity.

**SECTION II. METHODOLOGICAL
STUDIES**

CHAPTER 4: Introduction to Methodological Studies on the Citric Acid Cough Reflex Test

The citric acid cough reflex test (CRT) has been used in the field of respiratory medicine for over 60 years, primarily as an outcome measure to evaluate the effects of antitussive medications. The earliest method was described by Bickerman and colleagues in 1954, and since then citric acid CRT has been used across a range of disciplines and populations (Bickerman & Barach, 1954). In the past 20 years, citric acid CRT has been adapted to the field of dysphagia, where it's used as a clinical test to evaluate laryngeal sensory deficits associated with silent aspiration (Lee et al., 2013; Miles, Moore, et al., 2013; Sato et al., 2012; Wakasugi et al., 2008).

4.1 Underlying Mechanisms of the Citric Acid Cough Reflex Test

The advantage of using citric acid as a tussigenic stimulus for patients with dysphagia is that it stimulates jugular C-fibres *and* nodose A- δ fibres (i.e. cough receptors) (Canning et al., 2004; Kollarik & Udem, 2002) which are hypothesized to play an important role in coughing to aspiration (Canning et al., 2004; Kollarik & Udem, 2002; Mazzone & Udem, 2016). However, it is important to acknowledge that the underlying mechanisms of how these airway afferents are stimulated are not fully understood. A number of studies suggest that pH plays an important role (Canning et al., 2004; Kollarik & Udem, 2002; Lowry, Wood, & Higenbottam, 1988; Rai, Fowles, Wright, Howard, & Morice, 2018; Wong, Matai, & Morice, 1999). Wong and colleagues (1999) report highly consistent tussive responses to aerosols of citric acid, acetic acid and phosphoric acid of the same pH in healthy individuals (Wong et al., 1999). More recently, citric acid aerosols of different pH (pH = 3.13, 5.05, 5.99) were found to evoke

inconsistent tussive responses in healthy individuals, with greater coughing rates at pH 3 (60%) compared with pH 6 (10%) (Rai et al., 2018).

Previous studies have also suggested that the rate at which the pH decreases in the respiratory tract has important implications on the airway afferents that are stimulated by citric acid. Electrophysiological studies in animal models demonstrate that A- δ fibres (or cough receptors) are sensitive to *rapid* decreases in pH, but are entirely insensitive to gradual drops in pH (Kollarik & Udem, 2002). For example, rapid transient (~ 3 s) administration of citric acid to the receptive field of airway afferents consistently evoked action potential discharge in nodose A- δ fibres, as well as jugular C-fibres (Kollarik & Udem, 2002). Whereas, gradual reductions in the pH of the receptive field of airway afferents evoked action potential discharge in C-fibres alone (Kollarik & Udem, 2002). These findings may have important implications on methods of citric acid CRT, in that, coughing may be induced by differential airway afferents depending on the method of citric acid administration. However, it is important to acknowledge that these findings are limited to animal models. Thus, the extent to which they apply to humans remains unknown.

4.2 Methods of Citric Acid Cough Reflex Testing

As outlined in Chapter 2, there is lack of consensus in the dysphagia literature on methods of citric acid cough reflex testing. Lack of standardization precludes comparison of cough sensitivity data across studies (Morice et al., 2007), and results in an inability to provide cohesive practice recommendations to clinicians (Watts et al., 2016). This may have implications for patient care, and have serious clinical consequences, given that citric acid CRT contributes to determining patient safety for oral intake and risk of silent aspiration (Miles, Moore, et al., 2013; Perry & Huckabee, 2017; Sato et al., 2012; Wakasugi et al., 2008).

Lack of standardization of the citric acid CRT is not unique to field of dysphagia. In reviewing the literature, it's apparent that no standardized method of citric acid CRT exists (Morice et al., 2007). In 2007, the European Respiratory Society (ERS) published guidelines for citric acid cough reflex testing (Morice et al., 2007). These guidelines aimed to standardize methods and optimize reproducibility. They included recommendations for preparation of citric acid solutions, methods of citric acid administration, nebulizer characteristics, inspiratory flow rates, end of test criteria, and methods of interpretation (Morice et al., 2007). A summary of these recommendations is provided in Table 2. While these guidelines provide a benchmark on which to base methods of citric acid CRT, there is a lack of empirical evidence on which many of the recommendations are based (discussed in detail below). This may go some way in explaining why they are poorly adhered to in the literature. It's also important to note that these guidelines were developed specifically for the respiratory physiology field, and predominantly focus on evaluation of chronic refractory cough or cough associated with respiratory diseases such as COPD or asthma (Morice et al., 2007). Thus, the extent to which these guidelines are relevant for evaluating coughing in response to aspiration are unknown. Critical analysis of the ERS guidelines for citric acid CRT, and other methodological factors that are known to influence citric acid cough thresholds are outlined below and form the basis on which the following studies in this chapter are based.

Table 2: ERS guidelines for citric acid CRT (Morice et al., 2007).

Method	ERS Recommendation
Preparation of Solutions	Serial dilution of 3M citric acid stock solution in sterile 0.9% saline solution is performed in order to obtain serial doubling concentrations ranging from 1.95 – 3,000 mM (Kastelik et al., 2002; Wong & Morice, 1999). In healthy volunteers, the lowest concentration prepared is 7.8 mM.
Methods of Administration	<p>Single-dose: Single concentration of citric acid (concentration unspecified).</p> <hr/> <p>Dose-response:</p> <ol style="list-style-type: none"> 1. Single vital capacity breaths of incremental concentrations of citric acid via dosimeter controlled nebulizer 2. Tidal breath inhalations of incremental concentrations of tussive agent, each over a fixed period, usually 15-60 seconds.
Nebulizer Characteristics	The exact output of the nebulizer in ml/min should be determined. This should be used to calculate exact output of aerosol per breath. The same nebulizer, or one with an identical output, should be used in studies incorporating serial cough challenges or when comparing populations.
Inspiratory Flow Rate/ Instrumentation	The ERS recommend the use of a compressed air driven nebulizer (model 646, De Vilbiss) controlled by a dosimeter (KoKo Digidoser) that is modified by the addition of an inspiratory flow regulator valve (RIFR, nSpire Health Inc.). The valve limits inspiratory flow rate to 0.5 L/s regardless of excessive inspiratory force (Morice et al., 2007).
Placebo	Inhalations of saline should be randomly interspersed to increase challenge blindness.
Instructions	Participants should be instruction not to attempt to suppress any coughs and not to talk immediately after inhalations as this may suppress coughing - “allow yourself to cough if you need to, and as much as you need to” (Morice et al., 2007, p. 1260).

Termination of tussive response to cough challenge	Only coughs occurring within 15 s of citric acid delivery should be counted when using a single-breath method, as the response should be immediate and brief.
Interpretation of the cough challenge	The concentration of citric acid causing two (C2) and five coughs (C5) are reported.

4.3 Citric Acid Solutions

An understanding of the different units of concentration and methods of dilution is important for interpreting studies using citric acid CRT. Citric acid concentrations are reported in molar (mol/L), millimolar (mM), mass concentration (g/L), or percentage weight over volume (% w/v). The relationship between these values is as follows: 1 mM = 0.001 mol/L = 0.2 g/L, = 0.02% w/v (PubChem Compound Database, 2019). There is no consensus on which unit of measurement is optimal. This creates confusion in comparing methods and interpreting results across studies. In studies where a range of citric acid concentrations are used (i.e. a dose-response method, described below), solutions of citric acid can be diluted in doubling concentrations (i.e. 0.4, 0.8, 1.6 mol/L), incremental concentrations (e.g. 0.1 mol/L, 5% w/v increments, etc.), or logarithmic dilutions. Logarithmic dilutions are non-linear and based on orders of magnitude, with a 10-fold dilution for a logarithmic dilution, and a 3.16-fold dilution for a half log scale.

The ERS guidelines recommend serial doubling concentrations of citric acid ranging from 1.95 – 3,000 mM (Morice et al., 2007). Highly specific starting points of 1.98 mM citric acid (for patient populations) and 7.8 mM citric acid (for healthy individuals) are recommended (Morice et al., 2007). However, there are no normative data on which these concentrations are based.

The ERS recommended concentrations are referenced to two studies on patients with COPD and chronic cough (Kastelik et al., 2002; Wong & Morice, 1999). However, these studies used half log concentrations of citric acid [i.e. 10, 30, 100, 300, 1000 mM] (Kastelik et al., 2002; Wong & Morice, 1999), inconsistent with the ERS recommendations. This creates confusion among researchers and clinicians in knowing which concentrations and increments to use. Only one published study has compiled normative data for citric acid CRT in healthy individuals (Monroe et al., 2014), with methods based, in part, on the ERS guidelines (Morice et al., 2007). Concentrations ranging from 0.1 mol/L to 2.6 mol/L (in 0.1 mol/L increments) were inhaled for up to 15 seconds (tidal breathing) via facemask, until C₂ cough thresholds were achieved. These concentrations translate to 100 mM – 2,600 mM. Both the natural cough threshold (NCT) and suppressed cough threshold (SCT) were evaluated. The study revealed that the majority of participants (92% and 70% respectively) triggered an NCT and SCT by 0.8 mol/L (i.e. 800 mM). While this is within the range of citric acid concentrations recommended by the ERS (i.e. 1.95 – 3,000 mM), the authors note that 5% of healthy individuals failed to trigger a C₂ response in the NCT condition, and 22% failed to trigger a C₂ response in the SCT condition (Morice et al., 2007), suggesting a ceiling effect of the test. No rationale is provided for the range or increments of citric acid concentrations used by Monroe and colleagues (2014), or the use of molar (mol/L), as opposed to millimolar (mM) units of concentration. It's also unclear why the authors did not test up to 3 mol/L (i.e. 3,000 mM), as recommended by the ERS. This would have extended the testing time, but may have reduced the percentage of non-responders observed in the study. Consistency in the units of concentrations of citric acid solutions would greatly facilitate comparison and interpretation of cough sensitivity data across studies.

4.3.1 Preparation of Citric Acid Solutions

The way in which citric acid solutions are made is a further consideration. The ERS guidelines recommend that 0.9% saline solution should be used to dilute citric acid solutions (Morice et al., 2007). However, the rationale for this is not specified. In the chemistry field, concentrations of citric acid are typically made with distilled water (PubChem Compound database, 2019). This may create confusion among researchers and clinicians as to why saline is added to citric acid for cough reflex testing. Careful analysis of previously published literature reveals that the absence of chloride ions (which are present in saline solution) has a pro-tussive effect on citric acid aerosols (Boggs & Bartlett, 1982; Eschenbacher, Boushey, & Sheppard, 1984; Lowry et al., 1988). This suggests that citric acid solutions diluted with or without saline will have different tussigenic properties (Lowry et al., 1988). Disparity in the solvent used to dilute citric acid solutions is likely to have crucial implications for the use of the citric acid CRT for clinical and research purposes and suggests that caution is warranted in comparing citric acid cough thresholds across studies where different solvents are used. For the dysphagia researcher and clinician, the validity of the citric acid CRT in identifying patients at risk of silent aspiration may be compromised if different solvents are used.

4.4 Nebulizer Characteristics

4.4.1 Nebulizer Output

According to the ERS guidelines, the nebulizer output (i.e. flow rate and/or dose of citric acid per inhalation) should be determined prior to CRT (Morice et al., 2007). However, the optimal nebulizer output or dose of citric acid per inhalation is unspecified. This offers little guidance to researchers and clinicians in determining methods of citric acid CRT for research and clinical practices. The nebulizer output may have important implications on the rate at which the pH of

the respiratory tract is reduced, by providing a greater volume of acid per inhalation. In this respect, it may influence the airway afferents that are targeted by citric acid inhalations.

The ERS guidelines recommend the use of a compressed air driven nebulizer, specifically the DeVilbiss 646 model (Morice et al., 2007). Previous studies have demonstrated significant variability in nebulizer output both across and within nebulizers of this make and model (Hollie, Malone, Skufca, & Nelson, 1991). These data suggest that, even when the ERS recommended nebulizer is used, under the same testing conditions, it is possible that the nebulizer output will vary substantially. This may have implications on the use of citric acid CRT as an outcome measure in cough research. The effects of the nebulizer output on citric acid cough thresholds has been evaluated in one previously published study in healthy individuals. Using the same order, increments and concentrations of citric acid, significantly lower cough thresholds were found with a higher nebulizer output (Barber et al., 2005). In this case, there was a 10-fold difference in nebulizer output (i.e. 8.4 μ L to 0.8 μ L per inhalation). These data suggest that caution must be made in comparing cough sensitivity data across studies where nebulizer outputs differ. These data also highlight that the nebulizer output, and the reliability of the nebulizer output, should be pre-determined, and monitored to ensure stability, as it may influence interpretation of study outcomes and the reliability of the test.

4.4.2 Particle Size

The produced nebulizer particle size may also account for discrepancies in cough thresholds (Barber et al., 2005). Jet nebulizers typically have a typical particle size of 2-3 μ m, while ultrasonic nebulizers theoretically have a larger particle size, of 5-7 μ m (Cohen et al., 2011). From the respiratory-physiology literature, it is well known that particle size influences the mechanisms of aerosol transport and deposition in the respiratory tract (Cheng, 2014; Heyder,

2004). The extra-thoracic region of the respiratory tract (mouth, pharynx, larynx), can be targeted with larger particle sizes (6 - 10 μm) (Bates, Fish, Hatch, Mercer, & Morrow, 1966; Cheng, 2014; Heyder, 2004). This is because the particles are heavier and more susceptible to gravitational forces (Cohen et al., 2011; Heyder, 2004). While particles of $< 5 \mu\text{m}$ are typically deposited in the lungs (Bates et al., 1966; Cheng, 2014; Heyder, 2004). No study has evaluated the effects of particle size, or different nebulizer types (e.g. jet versus ultrasonic) on citric acid cough thresholds. For capsaicin CRT, differences in cough thresholds have been seen when comparing larger and smaller particle sizes (Hansson, Wollmer, Dahlback, & Karlsson, 1992). These data suggest that particle size and nebulizer type (i.e. jet versus ultrasonic) may be an important factor in comparing and interpreting cough sensitivity data across studies.

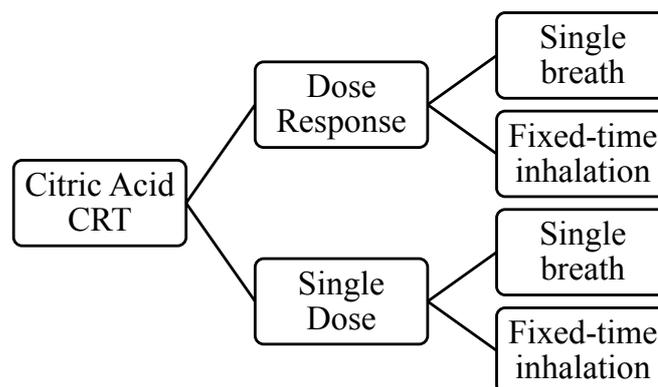
4.5 Methods of Citric Acid Administration

4.5.1 Single-Dose versus Dose-Response

A number of different methods of citric acid administration are reported in the ERS guidelines. These methods are outlined in Figure 5. A single-dose method involves inhalation of one concentration of citric acid only, and observing for a cough response (e.g. C_2 , C_5 , or cough frequency) (Morice et al., 2007). A dose-response method involves inhalations of increasing concentrations of citric acid until a cough response is achieved (Morice et al., 2007). The concentration at which an individual responds is referred to as the cough threshold. In the dysphagia literature, a single-dose method is often used as a clinical test to evaluate risk of silent aspiration (Lee, Kim, Seo, & Kang, 2014; Perry, Miles, Fink, & Huckabee, 2019; Sato et al., 2012; Wakasugi et al., 2008; Wakasugi et al., 2014). This is likely due to the clinical feasibility of the method. A dose-response method is used in dysphagia research to determine the most sensitive and specific citric acid concentrations in predicting a clinical outcome (e.g. silent aspiration or aspiration pneumonia) (Miles, Moore, et al., 2013; Nakajoh et al., 2000). A

dose-response method is also widely used as an outcome measure of cough sensitivity in response to pharmacological or behavioural therapies, where alterations in the citric acid cough threshold represents enhanced or diminished cough sensitivity (Faruqi et al., 2011; Janssens, Brepoels, Dupont, & Van den Bergh, 2015; Janssens et al., 2014; Smith, Owen, Earis, & Woodcock, 2006; Young et al., 2009). Although the ERS guidelines recommend a dose-response method, it would appear that the choice of method largely depends on the goals of the test.

Figure 5: Methods of citric acid administration. Adapted from “Cough challenge in the assessment of cough reflex” Morice, A. H., Kastelik, J. A., & Thompson, R. (2001), British Journal of Clinical Pharmacology, 52(4), p 367.



4.5.2 Single Breaths versus Fixed-time Inhalations

Citric acid can be inhaled in a single breath, or for a fixed time. For a single-dose method (i.e. one citric acid concentration inhaled), the ERS guidelines provide no recommendations on whether a single breath or fixed-time inhalation should be used. For a dose-response method, a single-breath, as opposed to a fixed-time inhalation method, is recommended due to the accuracy and reproducibility of the dose delivered (Morice et al., 2007). The ERS guidelines report that variations in respiratory frequency and tidal volume with tidal breathing over a fixed

inhalation time are likely to cause variation in the amount of aerosol inhaled and the outcome of the test across individuals (Morice et al., 2007). However, this hypothesis was not supported in a study using capsaicin CRT (Nejla, Fujimura, & Kamio, 2000). The coefficients of repeatability of the cough thresholds between the tidal breathing and single-breath methods were similar (i.e. 1.89 versus 2.71 doubling concentrations, respectively), suggesting little difference in test-retest variability between methods (Nejla et al., 2000). However, it is important to note that the volume of capsaicin inhaled in the tidal breathing method by Nejla and colleagues (2008) was carefully controlled. Capsaicin was nebulized into a 300-ml volume reservoir, connected to the nebulizer, prior to inhalation (Nejla et al., 2000). Thus, these findings may not apply to a tidal breathing method in which the dose of the aerosol is not controlled across and within participants. In situations where it may be advantageous to control the inhaled dose of citric acid across and within tests, a single breath method may be optimal.

4.6 End of Test Criteria

According to the ERS guidelines, both a C₂ and C₅ cough response are recommended as end of test criteria for citric acid CRT (Morice et al., 2007). There is lack of consensus as to which is superior (Morice et al., 2007). For capsaicin CRT, a C₅ response was more reproducible in a cohort of healthy volunteers in the short-term (i.e. test-retest interval of 14 days) (Dicpinigaitis, 2003). In this case, reproducibility was defined as a cough threshold within one doubling concentration (Dicpinigaitis, 2003). The nature of the variability of the C₂ response is unclear. The authors hypothesize it may be related to the “startle phenomenon” (Dicpinigaitis, 2003, p. 64). An individual undergoing their first cough challenge may produce a C₂ at a low concentration of capsaicin, but fail to cough, or cough less, at subsequent higher concentrations (Dicpinigaitis, 2003). According to the authors, the C₅ response may be less susceptible to this potential pitfall (Dicpinigaitis, 2003). However, the approach of using a C₂ response on two of

three trials of a citric acid or capsaicin may also overcome this pitfall and has been reported in numerous studies in the literature (Miles, Moore, et al., 2013; Miles, Zeng, McLauchlan, & Huckabee, 2013; Troche, Brandimore, Okun, et al., 2014).

4.6.1 Suppressed Cough Threshold (SCT)

The use of a SCT is not considered in the ERS guidelines. As previously mentioned, it aims to prevent individuals eliciting a volitional cough in response to a sub-threshold tussigenic stimuli (Eccles, 2009; Hutchings et al., 1993b; Hutchings et al., 1993a), and is thought to represent the point at which an individual can no longer suppress their cough response (Eccles, 2009; Monroe et al., 2014). In this sense, it theoretically more closely resembles a cough to aspiration (Monroe et al., 2014). On this basis, evaluation of the SCT may hold more appeal for the dysphagia researcher and clinician. However, there is limited data on the reliability of the SCT, which has implications on its use as an outcome measure in cough research and clinical practice. One study reports significant differences in the mean SCT on the first versus second CRT (i.e. 0.5 mol/L versus 0.6 mol/L), using a 15 second tidal breathing method (Perry & Huckabee, 2017). This order effect was present for females only but suggests lack of test-retest reliability of the SCT. Whether this effect is observed between the first and second test only, representing a “startle phenomenon” (Dicpingaitis, 2003, p. 64), or with every repeated test, is unclear. Previously studies have also demonstrated that 21 – 32% of healthy individuals may not achieve a C₂ response in a SCT condition, using a 15 second tidal breathing method via facemask (Mills, Jones, & Huckabee, 2017; Monroe et al., 2014; Perry & Huckabee, 2017). This may result in undetermined cough thresholds and missing data in a research setting. In the clinical setting, it makes it difficult to determine if the capacity to suppress reflects impaired cough sensitivity, or a normal response. It is possible that the concentrations of citric acid, or nebulizer output was too low in these studies, resulting in a ceiling effect of the test. Whether

altering the methods of citric acid CRT could minimize this ceiling effect is an important avenue for future research that may improve the use of SCT as an outcome measure in cough research and clinical practice.

4.6.2 Urge to Cough

Urge to cough is a measure of the intensity of perceived sensations elicited by tussigenic stimuli, and gives insight into the cognitive motivation to cough (Davenport, 2008). Given that coughing is recognized as a cortically modulated behaviour (Eccles, 2009; Mazzone et al., 2011; Mazzone et al., 2007), there is increasing attention in UTC as an outcome measure in cough research. Most research on UTC has been completed with capsaicin CRT (Davenport, 2008; Dicipinigaitis, Rhoton, Bhat, & Negassa, 2012). There are few studies characterising citric acid induced UTC. A small number of exploratory studies have evaluated citric acid induced UTC in healthy individuals in cross-sectional studies to evaluate the effects of mindfulness (Young et al., 2009), cough suppression (Young et al., 2009) and gender (Gui et al., 2010). Young and colleagues (2009) found that UTC ratings at C₅ cough thresholds were unchanged following cough suppression and a mindfulness intervention, compared to baseline (i.e. a natural cough threshold), despite the fact that cough thresholds increased (Young et al., 2009). These data suggest that UTC ratings at cough threshold may not be sensitive to changes in cough sensitivity. Gui and colleagues (2010) found that the dose of citric acid that induced an UTC of one (i.e. which they defined as the UTC threshold) was not significantly different between males and females, despite the fact that cough thresholds were.

Preliminary evidence suggests UTC is an important clinical outcome for patients with dysphagia. Yamanda and colleagues (2008) evaluated UTC in a cohort of elderly individuals with and without a history of aspiration pneumonia. The study revealed that there was no

difference in UTC at cough threshold (C_2) in patients with a history of aspiration pneumonia, when compared with aged-matched healthy controls. However, when they compared sub-threshold concentrations of citric acid (i.e. UTC at half the C_2 value), the patient group had significantly blunted UTC compared to the control group (Yamanda et al., 2008). This finding suggests that UTC at *sub-threshold* concentrations of citric acid may be more susceptible to blunting in patients at risk of airway protective deficits. Similar findings are reported by Troche and colleagues (2014) using capsaicin induced UTC in a cohort of patients with PD. Increasing levels of dysphagia severity (defined as a higher PAS score) resulted in significantly attenuated median UTC ratings at 200 μ m capsaicin (Troche, Brandimore, Okun, et al., 2014). These studies suggest that sub-threshold UTC may be an important clinical outcome for patients with dysphagia and dystussia. However, the clinical significance of these findings remains uncertain. Yamanda and colleagues (2008) found a mean difference of only one point on the UTC rating scale between patients with a history of aspiration pneumonia and healthy controls (i.e. 0.3 (SD = 0.7) versus 1.2 (SD = 0.8) points). It's also important to note that only eight patients were included in this study. One was excluded from the UTC analysis as they were an outlier (i.e. substantially higher UTC rating than the other seven). For the use of citric acid induced UTC as an outcome measure in cough research and clinical practice, important questions remain regarding the reliability of citric acid induced UTC across days.

4.7 Test-retest variability

4.7.1 Methods of citric acid CRT to optimize reproducibility

A number of recommendations are provided in the ERS guidelines to optimize repeatability of the CRT. Firstly, the ERS guidelines recommend the use of a dosimeter, which is a device that connects to the nebulizer, and ensures dose-to-dose reproducibility of the aerosol output across and within tests (Morice et al., 2007; Wright, Jackson, Thompson, & Morice, 2010). A further

addition is an inspiratory flow regulator valve (Morice et al., 2007). The valve is attached to the nebulizer and ensures the inspiratory flow is limited to 0.5 L/s despite excessive inspiratory force (Morice et al., 2007). However, it doesn't control inspiratory flow for individuals with an inspiratory flow rate lower than 0.5 L/s. It is also recommended that the same nebulizer – or a nebulizer with confirmed identical output - is used across and within participants if comparisons of cough sensitivity data are to be made (Morice et al., 2007). This is due to the known variation in nebulizer output, even in nebulizers of the same make/model (Hollie et al., 1991). The extent to which these recommendations are adhered to across studies are unknown. It's likely that these recommendations are more applicable to research settings, as increasing the complexity of the instrumentation for citric acid CRT may be prohibitive to clinical application. However, this creates a risk of introducing test-retest variability across and within tests, with implications on the reliability of the results.

4.7.2 Test re-test reliability

According to the ERS, large variation in cough thresholds exists *across* individuals, which diminishes the intrinsic significance of CRT outcomes (Morice et al., 2007). The nature of this variability is unknown, and is attributed to large variations in cough sensitivity in healthy individuals (Morice et al., 2007). No studies have explored the nature of this variation. It may relate to a number of physiological or psychosocial factors that are not controlled for in current methods of CRT. Test-retest reproducibility of citric acid CRT *within* individuals has been demonstrated by a number of studies using different methods of citric acid CRT (Morice et al., 2007). This facilitates the use of citric acid CRT as a measure of cough sensitivity in longitudinal cough research. A summary of these studies is provided in Table 4, at the end of this chapter. Of note, different instrumentation, methods of citric acid inhalation, test-retest

intervals and estimates of repeatability are used across these studies. Thus, it remains unclear how much variability in citric acid cough thresholds can be expected from repeating the test.

A number of other fundamental limitations also preclude the use of these data into research and clinical practices. For example, by report, it appears a somewhat biased sample of participants is used in some of the studies referenced by the ERS guidelines. Some early studies use “trained” individuals - who were known to demonstrate a fairly consistent response to citric acid CRT - to examine reliability (Bickerman & Barach, 1954, p. 157; Bickerman, German, Cohen, & Itkin, 1957, p. 192). Others report that only subjects with repeatable cough threshold were accepted into the study (Grattan, Marshall, Higgins, & Morice, 1995; Rostami-Hodjegan, Abdul-Manap, Wright, Tucker, & Morice, 2001), without comment on the number of participants excluded due to variable responses to citric acid CRT. These data will likely underestimate the test-retest variability that would be expected in populations with no prior experience of citric acid CRT. Many studies allude to a “learning effect” or “startle effect” (Bickerman et al., 1954, p. 157 Morice et al., 2007, p. 1260), in which cough thresholds or cough frequency is significantly higher on the first test, versus the second. However, quantification of this effect is not reported in previously published literature. These data are important for the use of citric acid CRT as an outcome measure in cough research to evaluate whether changes in cough thresholds can be attributed to true changes in cough sensitivity, compared to the artefact of repeating the test.

One study by Wright and colleagues (2010) evaluates test-retest variability of the ERS recommended method of CRT, referred to as the “KoKo Digidoser method” (Wright et al., 2010, p. 2). They compare this method to a commonly used “Mefar Dosimeter Cough Challenge Method” in a cohort of healthy volunteers (Wright et al., 2010, p. 2). Both methods

differ in terms of the instrumentation and protocol used (see Table 3). Intra-day (1, 2 4 hours post baseline) and inter-day (2 weeks post baseline) repeatability for both methods was evaluated. The results of the study demonstrated no difference in C_2 cough thresholds for the KoKo Digidoser method. In contrast, significant differences in C_2 cough thresholds were reported for the Mefar Dosimeter method. The nature of the poor repeatability for the Mefar Dosimeter method is unexplained by the authors. It cannot be attributed to the test-retest interval, suggesting that methods of citric acid CRT may have implications on test-retest variability. The authors speculate whether a single inhalation may be more repeatable than multiple inhalations, or whether a larger particle size, as seen with the Mefar dosimeter method, may contribute to greater downregulation of coughing with repeated tests. However, there is no empirical evidence to support or refute their speculations. These data suggest that the KoKo Digidiser method of CRT may serve as a more viable outcome measure in cough research. However, a notable limitation of incorporating these data into future research and clinical practices is the way in which test-retest variability of citric acid cough thresholds are reported. The authors log transform their data and report geometric mean cough thresholds. For example, an inter-day geometric mean difference of $-0.05 \log \text{ mM}$ is reported for the KoKo Digidoser method (Wright et al., 2010). From this data, it remains unclear how much variation one could expect upon repeating the test, or how this relates to their original citric acid cough thresholds. According to Feng et al. (2014), log transformation has the advantage of dealing with skewed data. However, the results of statistical tests performed on log-transformed data are difficult to relate back to the original non-log transformed data (Feng et al., 2014). This precludes application of these data to future clinical and research practices.

Table 3: Summary of the citric acid CRT protocols used by Wright et al. (2010)

	KoKo Digidoser Method	Mefar Dosimeter Method
Instrumentation	DeVilbiss 646 nebulizer controlled by a KoKo Digidoser	Mefar MB3 dosimeter
Citric acid concentrations	7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000 mM	1, 3, 10, 30, 100, 300, 1000 mM
Nebulizer output (ml/s)	0.89	0.1
Particle Size (MMAD)	<5 μm	5.4 μm
Number of inhalations	1	4
Duration of inhalations	1.2 s	1 s
End of test Criteria	C ₂	C ₂

Table 4: Studies reporting reproducibility of citric acid CRT, as referenced by the ERS guidelines (Morice et al., 2007)

Study (first author, year)	Protocol of citric acid inhalation	Test Interval	Estimate of Repeatability
Bickerman (1954)	Five successive inhalations of 5% or 10% citric acid.	Weekly to monthly for periods of 2-9 months.	The mean number of coughs per test was 7.4 (range: 2-19). Within-participant standard deviations ranged between 0.6 - 4.5 coughs per test.
Bickerman (1957)	Five inhalations of predetermined “threshold level” of citric acid.	1-2 minutes between each inhalation	The placebo drugs showed no significant mean percentage change in cough frequency. Estimate of reproducibility is not provided.
Schmidt (1997)	One inhalation from residual volume to total lung capacity of doubling concentrations of citric acid (0.625 – 320 mg/ml) were inhaled every 3 minutes.	Minimum of 7 days between challenges	“The mean (SD) difference between both challenges was 0.04 +/- 0.47 doubling concentrations (log transformed) of citric acid. Therefore, reproducibility was given within about 0.94 doubling concentrations” (p. 386).
Barber (2005)	Four single inhalations, separated by a 60 s interval of incremental doses of citric acid (0, 10, 30, 300, 1,000, 2,000, 3,000 mM).	Same time of day on consecutive days.	The standard deviation of the difference between the paired cough thresholds was 0.20 log mM, therefore the coefficient of repeatability was 0.40 log mM. Correlation coefficient $r = 0.96$ (95% CI = 0.84 – 0.99). 91% of cough thresholds were repeatable within one incremental dose.
Grattan (1995)	Five 1s inhalations of 5% citric acid. 60 s between each inhalation.	6 consecutive days.	“There was no significant difference between baseline cough response on each of the study

	Coughs were counted for 1 minute.		days” (p 262). Estimate of reproducibility is not provided.
Morice (1994)	Five 1s inhalations of 33 μ mol citric acid. 60 s between each inhalation. Coughs were counted for 1 minute.	5 tests. 1 hour intervals.	Placebo treatments (pine oil and air inhalations) showed no significant reduction in cough frequency to citric acid. Estimate of reproducibility is not provided.
Rostami-Hodjegan (2001)	Five 1 s inhalations of 10% (w/v) citric acid at 1 minute intervals, over 5 minutes.	1 minute intervals, over 5 minutes.	The cough response in the placebo and untreated condition was best characterized by a decrease in cough frequency, to a maximum of 1.6 (a decrease to 8.9 coughs from the baseline value of 10.5 coughs) at 4–4.5 h (tmax), followed by a non-linear increase in cough frequency and return to baseline.
Wright (2010)	KoKo Method Single inhalations of citric acid (7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1,000 mM) until C2 response was achieved.	Inter-day: Baseline, 1, 2 and 4 hours.	KoKo Method Intra-day: geometric mean C2 at baseline, and 1, 2 and 4 hours were not significantly different ($F = 602, p = 0.61$). Mean change from baseline was 1.57%, 3.15% and 2.08% respectively. Inter-day: geometric mean difference in C2 was -0.05 log mM (95% CI, 0.05 to -0.15). ICC = 0.70
	Mefar Method Four inhalations of citric acid (1, 3, 10, 30, 100, 300, 1,000 mM) until C2 response was achieved.	Intra-day: 2 weeks	Mefar Method Intra-day: geometric mean C2 showed a significant increase from baseline at 1, 2 and 4 hours ($F = 8.91, p < 0.001$). Mean change from baseline was 9.79%, 10.70% and 11.69% respectively.

			Inter-day: geometric mean difference in C2 was 0.127 log mM (95% CI, 0.25 to 0.0001). ICC = 0.41.
Morice (1992)	Citric acid (0.68% in 0.79% saline) were inhaled for 1 minute with normal respiration. Coughs were counted.	One week apart.	On the second test day (one week after the first), the number of coughs in the first 10 seconds was 3.1 (range 0-7) and in the last 10 seconds was 0 (range 0). The response to citric acid challenge on the first test was significantly greater than the second test ($p < 0.02$).

4.8 Gaps in the Literature

Despite publication of the ERS guidelines in 2007, there has been little progress in the last decade on standardization of methods of citric acid CRT. Methodological aspects of citric acid CRT are known to influence the outcome of the test. Thus, they are an important consideration in interpreting study outcomes. No study has systematically reviewed and compared methods of citric acid CRT reported in published literature. This information would facilitate comparison of citric acid cough thresholds across studies, and enhance interpretation of study outcomes.

Test-retest variability of citric acid CRT remains uncertain. This has implications on the use of citric acid CRT as an outcome measure in cough research. Test-retest variability of citric acid CRT across a multiple days has never been evaluated. Additionally, test-retest variability of suppressed cough thresholds and citric acid induced UTC are unknown, but are becoming increasingly important outcomes in longitudinal cough research.

CHAPTER 5: Methods Matter - Systematic Review of Methods of Citric Acid Cough Reflex Testing

5.1 Study Aims and Rationale

There are no accepted methodological standards for citric acid cough reflex testing (CRT). This precludes comparison and interpretation of cough sensitivity data across studies (Morice et al., 2007). Furthermore, lack of standardization results in an inability to provide cohesive practice recommendations to clinicians (Watts et al., 2016). The primary objective of this systematic literature review was to summarize and appraise methods of citric acid CRT used in published literature across disciplines. Data across studies were translated to standardized units of measurement to streamline comparison across studies. It is anticipated that this study will contribute towards the development of standards of methods of citric acid CRT, and highlight the potential implications of methods of citric acid CRT on the outcome of the test for researchers and clinicians.

5.2 Methods

This study was prospectively registered in the international prospective register of systematic review (PROSPERO), on 11th February 2018 (Registration number: CRD42017079055). For reporting, guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were followed.

5.2.1 Eligibility Criteria

Publications reporting a method of citric acid CRT in human participants (adults and paediatric populations) were included in the review. Eligibility for inclusion was restricted to publications in English and Spanish, due to availability of native speakers of these languages. Publications other than peer reviewed journal articles, such as conference abstracts, letters to the editor, review articles and guidelines were excluded. Full methods of CRT were required for this review, and the word limit of conference papers and abstracts often precluded this. Furthermore, by including peer reviewed journal articles only, it was expected that the methods of citric acid CRT would be of adequate standard for publication. There were no constraints regarding publication year.

5.2.2 Search Strategy

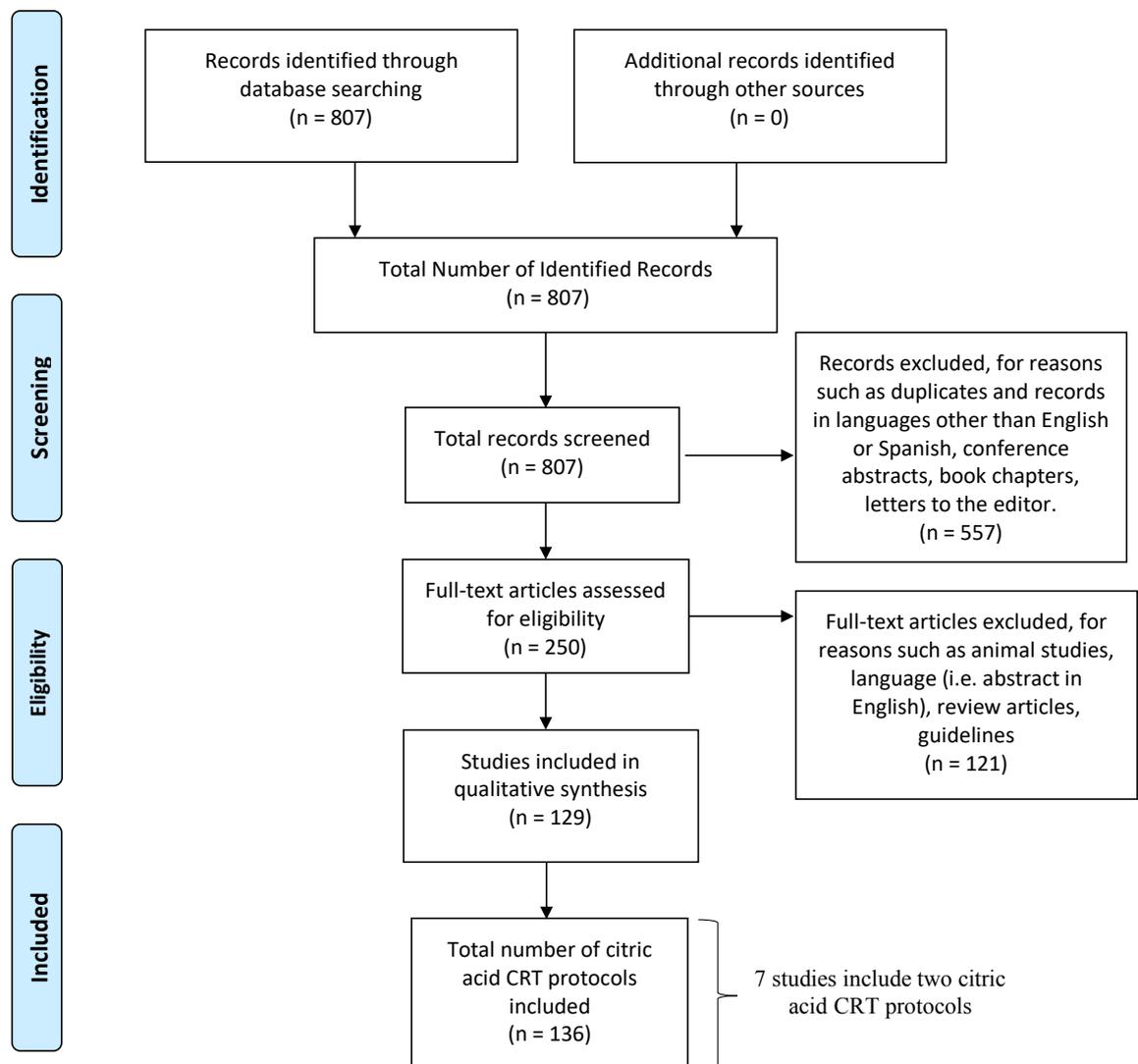
The complete search strategy is provided in Appendix 3. The term cough (including chronic cough, experimental coughing, or irritative coughing) as a medical subject heading (MeSH) and key word, was combined with the term citric acid as a medical subject heading (MeSH) and key word. The following databases were searched up to February 2018: MEDLINE, EMBASE, CINAHL, PsychINFO, Scopus.

5.2.3 Selection Procedures

Two researchers independently assessed the relevance of the studies retrieved from searching the electronic databases. The titles and abstracts were independently screened by both researchers for keywords: cough(ing) and citric acid. Any disagreement between the two researchers was resolved by consensus. The full texts of all included studies were retrieved and examined against the inclusion/exclusion criteria (detailed below). The reference list of all studies included in the qualitative synthesis were manually checked for further relevant or

missed studies using keywords: cough(ing) and citric acid. Figure 6 depicts the selection procedure.

Figure 6: Flow Diagram of Study Selection Procedures. Adapted from “ Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement”, Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). PLoS Med, 6(7), p. 3. CC BY-NC-ND.



5.3 Data Extraction and Analysis

Data was extracted and imported into an excel file, under two main headings:

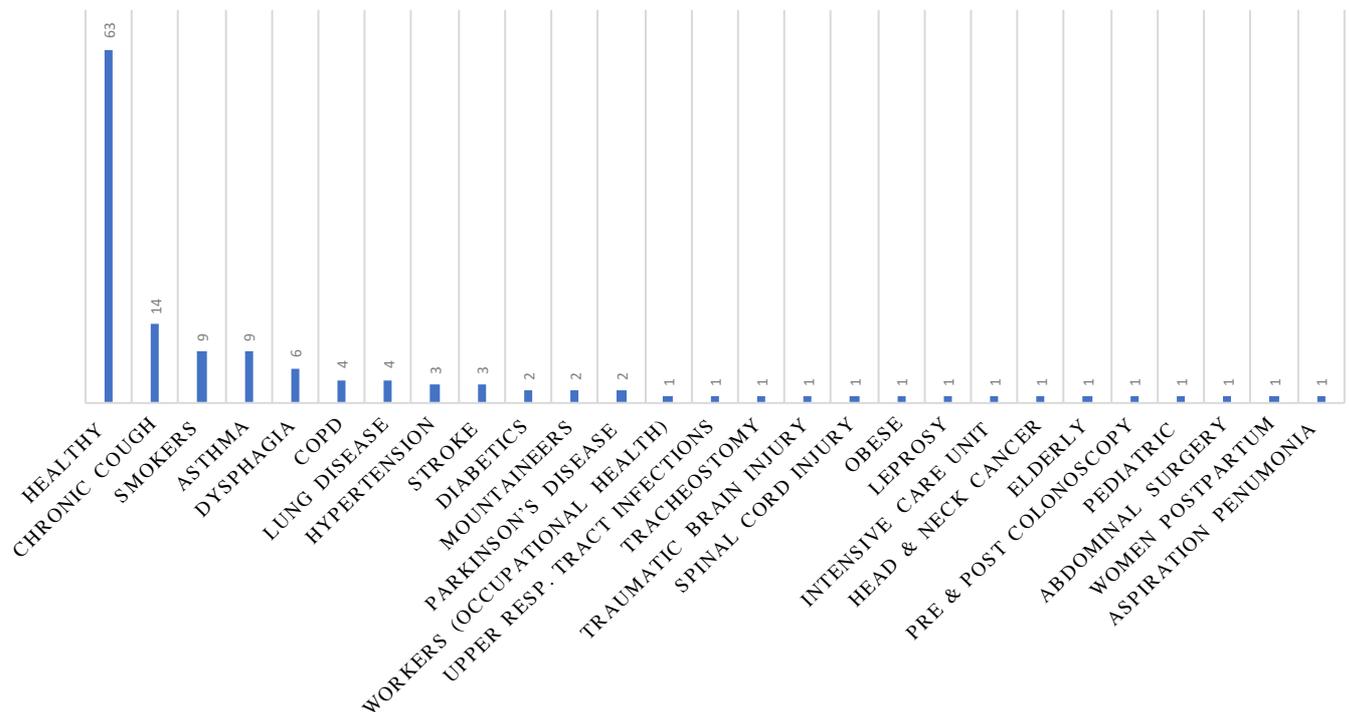
1. Instrumentation: including nebulizer model (i.e. brand), nebulizer type (i.e. ultrasonic, jet, mesh), dosimeter use and model, nebulizer output, nebulizer output testing and nebulizer particle size.
2. CRT Protocol: including methods of citric acid preparation, citric acid concentrations administered, methods of citric acid administration (i.e. single inhalations versus fixed-time inhalations), termination criteria for cough challenge and cough type investigated.

All data were independently extracted by two investigators from all studies that fulfilled the inclusion criteria. In total, four reviewers were involved in data collection. Agreement between raters was achieved by comparing data with the other investigator. Any disagreement between the two researchers was resolved by consensus, or by a third investigator. To streamline comparison across studies, the nebulizer flow rate and citric acid concentrations were standardized to mL/s and mol/L respectively.

5.4 Results

A total of 807 studies were retrieved from the electronic databases. On the basis of the inclusion criteria, 129 studies were retained. Seven studies (Barber et al., 2005; Hull et al., 2002; Karttunen, 1988b; Monroe et al., 2014; Morice et al., 1992; Winther, 1970; Wright et al., 2010) included two citric acid CRT protocols. Thus, a total of 136 citric acid CRT protocols are reported in the qualitative synthesis. Articles were published from 1954 to 2017. All articles were in English. Figure 7 shows the populations for which citric acid CRT is used across the retrieved studies.

Figure 7: Study populations for which citric acid CRT is used.



5.4.1 Instrumentation

5.4.1.1 Nebulizer

The nebulizer model was not reported in 47% of citric acid CRT protocols. Of the 72 protocols reporting nebulizer model, 25 different models were reported. Eleven (44%) were jet, eight (32%) were ultrasonic, one (4%) was vibrating mesh, and two (8%) were of unknown type (Table 5).

Table 5: Nebulizer model and type used across studies.

Nebulizer Model	Nebulizer Type	Studies (first author, year)
Micro Mist A7003	Jet	Leow, 2012
Acorn/Acorn 22	Jet	Barros, 1990, Barros, 1991, Kenia, 2008
CR 60 System 22	Jet	Vilardell, 2017
ATOMIZER NL11D®, La Diffusion Technique	Jet	Gayat, 2007
“ATOMIZER” La Diffusion Technique	Jet	Winther, 1970
DeVilbiss 646	Jet	Wright, 2010, Marsden, 2008, Katsumata, 1991, Lavorini, 2014, Schmidt, 1997, Faruqi, 2011, Di Franco, 2001
Pari Turboboy	Jet	Kondo, 1998, Rietveld, 2000
Salter Labs	Jet	Lin, 1999
Ganshorn ProvoJet	Jet	Pecova, 2007

Medipron FDC 88	Jet	Guglielminotti, 2005, Guglielminotti, 2007
Wright	Jet	Cross, 1976, Poundsford, 1985, Vianna, 1988, Riordan, 1994, Thompson, 1979, Costello, 1985, Dilworth, 1990, Empey, 1979, Stockwell, 1993, Winning, 1988, Winning, 1985
Hudson (MicroMist Nebulizer Model)	Jet	Rees, 1983, D'Souza, 1988, Cox, 1984, Belcher, 1986, Perry, 2017
DeVilbiss 35B	Ultrasonic	Behera, 1995
DeVilbiss 65	Ultrasonic	Morice, 1987, Morice, 1992
DeVilbiss 40	Ultrasonic	Barber, 2005, Karttunen, 1988, Karttunen, 1987, Karttunen, 1988b, Empey, 1976
Medix Easimist	Ultrasonic	Barry, 1997
Mistogen EN143	Ultrasonic	Van Meerhaeghe, 1986
Omron NE-U17	Ultrasonic	Gui, 2014, Sato, 2016, Kashiwazaki, 2013, Guillen- Sola, 2015, Wakasugi, 2008, Gui, 2012
Omron NE-UL1B	Ultrasonic	Ogihara, 1991
Omron MicroAir	Ultrasonic	Mincheva, 2014

Sharp MU-32	Ultrasonic	Gui, 2010, Yanamda, 2008, Kanezaki, 2010, Ebihara, 2011, Wantando, 2004, Ebihara, 2003
Soniz 200	Ultrasonic	Thompson, 2009, Mason, 1999, Mason, 2009
Soniclizer 305	Ultrasonic	Nishino, 2008
Omron NE-U22	Vibrating Mesh	Lee, 2014, Wakasugi, 2014, Sato, 2012
DeVilbiss (unknown model)	Unknown	Lavietes, 1988
Bird (asmastick, micro-nebulizer)	Unknown	Taylor, 1988, Midgren, 1992

5.4.1.2 Dosimeter

The use of a dosimeter was not reported in 72% of protocols. Of the 26% of studies reporting the use of a dosimeter, 17% did not specify the model. Table 6 outlines the dosimeter model used in the protocols in which it was reported.

Table 6: Dosimeter model used for citric acid CRT.

Dosimeter Brand	Studies (first author, year)
KoKo Digidoser	Young, 2009, Wright, 2010, Smith, 2010, Marsden, 2008, Faruqi, 2011, Kelsall, 2009, Smith, 2017
Mefar	Morice, 2007, Wright, 2010, Kastelik, 2002, Wong, 1999, Barber, 2005, Fahim, 2009, Gordon, 1997, Abdul Manap, 1999, Laude, 1993, Morice, 1992, Grattan, 1995, Rostami-Hodjegan, 2001, Morice, 1994, Smith, 2006, Smith, 2006, Decalmer, 2007, Wong, 2007

ATOMISER NL11D®, La Diffusion Technique	Gayat, 2007 *reported as a nebulizer dosimeter
Jaeger APS	Jassens, 2014, Ziora, 2005, Jassens, 2015
Medipron FDC 88	Guglielminotti, 2005, Guglielminotti, 2007

5.4.1.3 Nebulizer output testing

Only five studies (4%) reported testing their nebulizer output prior to its use in citric acid CRT. Of those five, only two studies (Barber et al., 2005; Empey, Laitinen, Jacobs, Gold, & Nadel, 1976) described how the nebulizer output was tested. Barber et al. (2005) report that the nebulizers were “calibrated by weight loss”, which was “checked by a more accurate fluoride tracer method” (Barber et al., 2005, p. 178). Empey et al. (1976) report the nebulizer output was determined “by filling it with solution and weighing it before and after allowing air to flow through it (for 1 minute)” (Empey et al., 1976, p. 132).

5.4.1.4 Nebulizer Output

The nebulizer output was not reported in 68% of protocols. Nebulizer output was reported as the flow rate (i.e. the volume of citric acid omitted from the nebulizer per unit time), or the nebulizer output per breath/ actuation, across studies. To streamline comparison across studies, nebulizer flow rate and output per breath were converted to mL/s and ml/breath, respectively (see Table 7).

Table 7: Flow Rate (mL/s) and Nebulizer Output (per breath/ actuation). Reported in ascending order.

Flow Rate (mL/s)	Studies (first author, year)
0.002	Auffarth (1991a), Auffarth (1991b)
0.0025	Katsumata (1991)
0.003	Lee (2013), Bossi (1988)
0.004	Wakasugi (2014)
0.005	Barros (1990)
0.0058	Lin (1999)
0.008	Midgren (1992)
0.011	Lavietes (1998)
0.015	Ziora (2005)
0.0225	Ogihara (1991)
0.0295	Stockwell (1995)
0.036	Gui (2010), Yamanda (2008), Kanezaki (2010), Watando (2004), Ebihara (2003)
0.033	Grattan (1995)
0.05	Guillen-Sola (2015), Wakasugi (2008)
0.1	Wright (2010) (Mefar protocol), Morice (1992) (Short term tachyphylaxis protocol), Nishino (2008), Wong (1999)
0.5	Empey (1976)
0.59	Karttunen (1987)
0.89	Wright (2010) (KoKo protocol)

133.3*	Monroe (2014), Kelly (2016) *This is reported as the nebulizer flow rate, but actually refers to the compressor.
167*	Poundsford (1985), Empey (1979) *This is reported as the flow rate the nebulizer was driven at, thus likely refers to the compressor.
0.0008 ml/breath	Barber (2005) (Yan Style Challenge)
0.008 ml/breath	Guglielminotti (2007), Gayat (2007)
0.0084 ml/breath	Barber (2005) (Mefar challenge)
0.012 ml/breath	West (2012), Smith (2006)
0.125 ml/breath	Kastelik (2002), Laude (1993), Morice (1992) (Long term tachyphylaxis protocol), Morice (1994)
0.1 ml/breath	Smith (2017)
2.5 mg/breath ^s	Griffin (1982) ^s cannot convert to ml as density of solutions are not known.
8 mg/breath ^s	Guglielminotti (2005) ^s cannot convert to ml as density of solutions are not known.

5.4.1.5 Particle Size of nebulizer output

Particle size was not reported in 82% of studies. The particle size reported in 18% of studies is listed in Table 8.

Table 8: Particle sizes reported across studies

Particle Size (µm)	Studies (first author, year)
1.6 (+/- 0.5)	D'Souza, 1988

2.5	Katsumata, 1991; Bickerman, 1956; 1954; Noel, 1962; Packman, 1977
2.65	Riordan, 1994
3	Calesnick 1967; Midgren, 1992
4	Van Meerhaeghe, 1986
5	Wakasugi, 2014; Sato, 2012; Auffarath, 1991a, 1991b
5.4	Yamanda 2008; Gui, 2010, Kanezaki, 2010; Watando 2004; Ebihara, 2004
5.6 (+/- 1.8)	Enright, 1980
1-5	Winther, 1970
0.5 - 5	Lin, 1999
1-8	Guillen-Sola, 2015; Wakasugi, 2008

5.4.2 Protocol of citric acid CRT used across studies

5.4.2.1 Methods of citric acid preparation reported across studies

Citric acid solutions were made with different solvents across studies. The solvents used to dilute citric acid are summarized in Table 9, with over half (53%) of the studies failing to report this methodological parameter.

Table 9: Solvent used to dilute citric acid solutions

Solvent	Percentage of Studies (%)
0.9% saline	3%
0.79% saline	1%
Saline (concentration not specified)	42%
Distilled water	1%
Not reported	53%

5.4.2.2 Citric acid concentrations used across studies

The citric acid concentrations were not reported in 6% of studies. A wide range of concentrations are used across the remaining studies. The studies were classified below into those that use single concentrations of citric acid, and those that use ranges of citric acid concentrations (i.e. dose-response method).

5.4.2.2.1 *Single Concentrations of Citric Acid*

Thirty percent of protocols used a single concentration of citric acid. A total of thirteen different single concentrations of citric acid are used, ranging from 0.04 mol/L - 1.3 mol/L. The most common single concentrations of citric acid were 0.5 mol/L, used in 26% of the protocols, and 0.3 mol/L, used in 15% of the protocols (see Appendix 4 for full list of single concentrations of citric acid used across studies).

5.4.2.2.2 *Range of Citric Acid Concentrations (i.e. dose-response method):*

Sixty-four percent of protocols used a dose-response method of citric acid CRT. Various increments were reported across studies: doubling, log incremental, incremental, 0.1 mol/L, multiples of 1.5, stepwise (0, 2, 4, 10, 20, 35, 50g per 100 ml), half log, quarter log, linear, percentage increments, or random increments. The range of citric acid concentrations varied widely across studies - with a total of 54 different reported ranges. The most common range of citric acid concentrations was doubling doses of 0.003 mol/L - 1.9 mol/L (reported as 0.7 - 360 g/L), used in 10% of the protocols (see appendix 4 for full list of the range of citric acid concentrations used across studies).

5.4.2.3 Methods of Citric Acid Administration

The method of citric acid administration was not reported in 12% protocols. Methods of citric acid administration were classified into those that reported the number of breaths per citric acid concentration, and those that reported a fixed-time of citric acid inhalation.

5.4.2.3.1 *Number of breaths*

Sixty-percent of protocols reported the number of breaths per citric acid concentration. This ranged from 1-16 across studies, with the majority of these protocols (52%) using a single inhalation method. The use of three inhalations, and five inhalations per citric acid concentration were the next most common methods, reported in 11% and 18% of studies, respectively.

5.4.2.3.2 *Fixed-time inhalation method*

Twenty-eight percent of protocols used a tidal breathing method and reported the duration of the citric acid inhalation. Of those using a fixed-time inhalation method, the durations of tidal breathing, and the percentage of protocols in which they are reported in brackets were: 15 seconds (28%), 20 seconds (3%), 30 seconds (4%) and 60 seconds (59%). The duration of tidal breathing was not reported in 6% of studies that use a tidal breathing method.

5.4.2.4 Termination of the citric acid CRT

The end of test criteria was defined differently across studies. Twenty-seven (20%) studies used a C₁ cough threshold. Twenty-seven (20%) studies used a C₂ cough threshold (2 of these also include a C₁ cough threshold). Eighteen studies (13%) used a C₂ and C₅ cough threshold. Three studies (2%) used a C₃ cough threshold. Fourteen (10%) studies use a C₄ or C₅ cough threshold. Twenty-nine percent of studies recorded cough frequency (i.e. number of coughs

elicited) following inhalation of a single concentration of citric acid (i.e. single inhalations and/or fixed dose methods) or in response to multiple doses (i.e. dose-response method). Seven (6%) studies did not report their end of test criteria. These studies measured the latency of the cough response, respiratory muscle EMG during cough, and spirometry.

5.4.2.5 Cough type

Ninety percent of protocols did not specify the cough type (i.e. natural cough threshold, suppressed cough threshold, laryngeal cough reflex) they evaluated in the study. The cough type is reported in 14 (10%) citric acid protocols. Four studies evaluated the NCT. Four studies evaluated the SCT. Five studies evaluated both NCT and SCT. One study evaluates the laryngeal cough reflex (LCR).

5.5 Discussion

Two important findings are derived from this study. This study provides the first empirical evidence of lack of standardization of methods of citric acid CRT across disciplines. This is evidenced by the wide range of instrumentation and protocols reported. Secondly, the study identifies that reporting methods of citric acid CRT is substandard in published literature. Many authors omit crucial elements of their citric acid CRT methods from their manuscripts. These findings, and the implications of these findings, are discussed in detail below.

5.5.1 Lack of standardization

There was lack of consensus in the instrumentation used across studies for citric acid CRT. For example, of the 72 protocols reporting nebulizer model, 25 different models were reported. The two most commonly reported nebulizers - the Wright nebulizer, and De Vilbiss 646 nebulizer - are both jet nebulizers. The following three most common - the Omron NE-U17, the Sharp-MU32, and the DeVilbiss 40 - are ultrasonic nebulizers. As outlined in Chapter 4, jet and ultrasonic nebulizers differ in terms of the particle size of aerosol they emit (Cohen et al., 2011). While the effects of particle size on citric acid cough thresholds have not been evaluated, previous studies using capsaicin CRT have demonstrated differences in cough thresholds with aerosols of different particle sizes (Hansson et al., 1992). This may be related to differences in the site of deposition in the respiratory tract with aerosols of different particle sizes, or the rate at which the pH in the extracellular fluid surrounding the airway afferents is reduced (i.e. larger particles, more rapid reduction). These findings suggest that caution may be warranted in comparing citric acid cough thresholds between studies using different types of nebulizers.

Few studies (28%) report using a dosimeter despite being a recommendation by the ERS guidelines (Morice et al., 2007), suggesting lack of compliance with ERS standards. All studies

that use a dosimeter are from the respiratory physiology literature, where citric acid CRT is used as an outcome measure to evaluate a cough therapy or antitussive medication. No studies from the dysphagia literature report using a dosimeter for citric acid CRT. The use of a dosimeter may be a prohibitive factor in the clinical application of methods of citric acid CRT. However, it is important to acknowledge that variability in the dose of citric acid delivered across and within tests may arise in the absence of a dosimeter (Morice et al., 2007). This is likely to be a crucial factor in studies using citric acid CRT as an outcome measure to evaluate the effects of cough therapies or antitussive medications, where dose-to-dose reproducibility is essential.

There was wide disparity in the output of nebulizers used across studies. Twenty-eight different nebulizer outputs are reported. Flow rates range from 0.002 ml/s to 0.89 ml/s, while nebulizer outputs per breath ranged from 0.0008 ml/breath to 0.1 ml/breath. The nebulizer output may have important implications for the rate at which citric acid is delivered to the respiratory tract, and, as a result, may influence the underlying mechanisms of cough induction. Rapid reduction of the pH in the respiratory tract – which may be achieved more readily by higher nebulizer outputs - activates nodose A- δ fibres, *and* jugular C-fibres (Kollarik & Udem, 2002). Whereas gradual reduction - which may be more readily achieved by lower nebulizer outputs - activates jugular C-fibres only (Kollarik & Udem, 2002). The capacity of alterations in the nebulizer output to induce differential activation of laryngeal afferents in humans is unknown. This is an important area for future investigations to clarify the optimal output for different populations. The optimal nebulizer output, and thus the rate at which citric acid is delivered to the airway, may differ for patients with chronic cough compared to patients with dysphagia, due to hypothesized differences in underlying cough pathophysiology. Thus, investigations specific to dystussia associated with dysphagia may improve the diagnostic accuracy of the test for

specific patient populations. However, differential activation of laryngeal afferents by citric acid CRT is also likely to be dependent on the citric acid concentrations and methods of citric acid administration. For example, single breaths to total lung capacity, and higher concentrations of citric acid, may be more effective at inducing rapid reduction in pH of the respiratory tract, compared to tidal breathing or lower citric acid concentrations. It is apparent that methodological factors, such as nebulizer output, method of administration and citric acid concentration cannot be considered mutually exclusive in determining the speed at which the pH of the airway is altered. However, it is important to acknowledge that these preconceptions lack empirical evidence. Further research is necessary to determine the capacity for different methods of citric acid CRT to target differential laryngeal afferents.

The results of the study revealed a wide range of citric acid concentrations and methods of citric acid inhalation reported across studies. A total of thirteen different single concentrations (for single-dose method), and fifty-four different ranges (for a dose-response method) of citric acid concentrations are reported. Only two studies (Vilardell et al., 2017; Wright et al., 2010) use the ERS recommended concentrations of citric acid, suggesting lack of compliance to these standards. This disparity makes it difficult to decide the optimal citric acid concentrations to use for clinical and research practices. Standardizing units of concentrations of citric acid to mol/L revealed a 40-fold difference in the highest and lowest single concentration of citric acid used across studies, i.e. 0.04 mol/L (Morice, Brown, Lowry, & Higenbottam, 1987; Morice et al., 1992; Ogihara, Mikami, Katahira, & Otsuka, 1991), versus 1.6 mol/L (Nishino, Isono, Shinozuka, & Ishikawa, 2008). Furthermore, there was a 1,000-fold difference between the highest and lowest ranges of concentrations of citric acid used across studies, i.e. 0.00001 – 0.0033 mol/L (Barros, Zammattio, & Rees, 1990; Barros, Zammattio, & Rees, 1991), versus 0.01 – 3.3 mol/L (Gayat et al., 2007; Guglielminotti et al., 2005; Guglielminotti et al., 2007).

The basis of this disparity is unclear. It is acknowledged that the populations across these studies differ. However, it is unlikely that a forty, or thousand-fold difference in the concentrations of citric acid to evoke coughing across these populations would be required.

Careful analysis of the methods across these studies suggest that the solvent used to dilute the citric acid may, in part, explain the nature of this disparity. For example, two of the three studies that use the lowest concentration of citric acid (i.e. 0.04 mol/L, single-dose method), dilute their citric acid with 0.79% saline (Morice et al., 1987; Morice et al., 1992). The solvent used to dilute citric acid is not reported in the third study (Ogihara et al., 1991). Previous studies suggest that solutions that are low or absent in chloride ions have a greater tussigenic potency (Lowry et al., 1988). It is possible that citric acid solutions made with 0.79% saline may have a greater tussigenic potency than studies using the ERS recommended 0.9% saline, due to the lower concentration of chloride ions. Although it appears that this is a small difference in saline (and thus, chloride ion) concentration, it is possible that airway afferents are sensitive to such small differences. Further research is required to clarify how the solvent used to dilute citric acid influences citric acid cough thresholds. This may have crucial clinical implications on the sensitivity and specificity of the citric acid CRT in predicting patients at risk of silent aspiration (Miles, Moore, et al., 2013; Wakasugi et al., 2008). For example, lower or absent concentration of chloride ions in citric acid solutions may be more likely to evoke a C₂ cough response in patients with laryngeal sensory deficits who are at risk of silent aspiration, diminishing the sensitivity of the test. Those preparing citric acid solutions for clinical use should be aware of the potential implications of using different solvents or different concentrations of solvents.

Methods of citric acid administration differed across studies. The majority of the studies used a fixed number of breaths, as recommended by the ERS (Morice et al., 2007), with one, three

and five inhalations being most commonly reported. No rationale is provided for the use of multiple inhalations over a single inhalation in any study. Twenty-eight percent of studies use a fixed-time inhalation method, which is discouraged by the ERS guidelines, due to lack of reproducibility of the dose delivered across tests (Morice et al., 2007). There is lack of consensus in the length of the inhalation. Most studies (59%) used a 1-minute inhalation time. However, others use 15 s, 20 s or 30 s. No rationale for these inhalation duration times is provided in any study. A shorter inhalation time may be advantageous to minimize the effects of tachyphylaxis (i.e. a rapidly diminishing cough response to citric acid in response to prolonged inhalation), which is reported over one-minute inhalations of citric acid (Morice et al., 1992).

5.5.2 Substandard reporting of methods of citric acid CRT

Many crucial components of the citric acid CRT protocol are omitted from a large proportion of published studies. This prevents full replication of CRT protocols. For example, the nebulizer model is not reported in almost half (47%) of CRT of protocols, and the nebulizer output is not reported in over half (68%) of protocols. It was noted that almost a third (27%) of studies who do not report the nebulizer output report the output of the compressor. The compressor is used to drive gas through the nebulizer chamber (Boe et al., 2001; O'Callaghan & Barry, 1997). However, nebulizer chambers have a resistance to flow (O'Callaghan & Barry, 1997). Thus, the flow rate of the compressor will not equate to the flow rate of the nebulizer once the compressed air has passed through its chamber (O'Callaghan & Barry, 1997). Furthermore, different nebulizing chambers will have different resistance (O'Callaghan & Barry, 1997). As a result, the use of the same compressor and compressor flow rate with different nebulizing chambers may result in variation in nebulizer output across these studies. It is suggested that the flow rate should be measured at the outlet of the nebulizer, rather than

from the compressor, to avoid over-estimating the aerosol available for inhalation (O'Callaghan & Barry, 1997). This would permit different nebulizers, of known output, to be used interchangeably (O'Callaghan & Barry, 1997), and facilitate universal replication of methods of citric acid CRT.

Few studies (5%) report testing their nebulizer output, or the reliability of their nebulizer output. Previous studies have demonstrated large variability in nebulizer outputs across and within nebulizers of the same make and model (Hollie et al., 1991; Ryan et al., 1981), suggesting that testing the nebulizer output is essential to ensure dose-to-dose reproducibility of the citric acid aerosol delivered across and within tests. Only one study (Young et al., 2009) alludes to testing the reliability of the nebulizer output. The authors report that the nebulizer was “re-calibrated at regular intervals” to ensure consistency across tests (Young et al., 2009, p. 995). However, the methods used to calibrate the nebulizer output were not reported in the study. In the ERS guidelines for bronchial challenge testing, it is recommended that the output of nebulizers used for bronchial challenge testing in clinical use have a test-retest coefficient of variation of less than 10% (Coates et al., 2017). This may be a useful standard to incorporate into future citric acid CRT guidelines. It is recommended that researchers evaluate their nebulizer output and the reliability of the output, in studies where this may be crucial to the outcome of the study, for example, when using across or within-subject repeated measures of citric acid CRT.

5.5.3 The nature and implications of these findings

It is important to consider the factors that may be precluding lack of standardization and inadequate reporting of methods of citric acid CRT. A possible explanation for the findings of this review may arise from the lack of a strong evidence base on which methods of citric acid

CRT are based. The first published protocol on citric acid CRT was over sixty years ago (Bickerman & Barach, 1954). However, there is still limited scientific understanding of the underlying neurophysiological mechanisms of citric acid CRT, and the parameters that influence citric acid cough thresholds. There is a paucity of methodological studies on citric acid CRT, and a number of fundamental questions remain regarding the effect of flow rate, particle size, inspiratory flow rate, preparation of citric acid solutions and methods of citric acid administration on citric acid cough thresholds in healthy and patient populations. Without a clear understanding of how these factors influence citric acid cough thresholds, interpretation of study outcomes and comparison of cough sensitivity data across studies is challenging. Furthermore, researchers and clinicians are forced to use methods of citric acid CRT without empirically-based rationale. To overcome these shortcomings, it is recommended that researchers and clinicians make themselves aware of methodological limitations that may have implications on the outcome of the test, and secondly, evaluate their method of citric acid CRT prior to its use to ensure reliability.

There is lack of consensus in the terminology used across studies. This hinders comparison of methods and results across studies. More importantly, it creates confusion, and may lead to misinterpretation of methods of citric acid CRT. An example of such confusion is evidenced in a recent study from the dysphagia literature. In describing methods of citric acid CRT, Holmes and colleagues (2016) report that according to a study by Monroe and colleagues, “92.5% of the normal population trigger a cough on 0.8 mmol of citric acid” (Holmes, 2016, p. 192). They also report that “0.6 mmol” of citric acid optimized sensitivity and specificity in detecting silent aspiration on VFSS in a study by Miles and colleagues (2013) (Holmes, 2016, p. 192). In reality, 0.6 *mol/L* (i.e. 600 mmol) and 0.8 *mol/L* (i.e. 800 mmol) were used in the above studies. This is an example of how lack of standardization in units of concentration

creates confusion among clinicians and researchers, which may have crucial implications on patient care and clinical decision making.

5.5.4 Potential biases in the review process

The search was limited to peer-reviewed, scientific studies, in English and Spanish only. This may have created a language bias (Higgins & Green, 2011) and resulted in some methods of citric acid CRT being omitted from the current review. Citation tracking was not completed, as reference checking yielded no additional studies, suggesting that all studies that met the inclusion criteria had been identified by the search. Lastly, there were no constraints regarding publication year in the current study. The results identify all methods of citric acid CRT used in published literature from 1954 to 2017. The ERS guidelines for CRT were developed in 2007. Thus, it is possible that less variability and better reporting of methods of citric acid CRT may have been observed if studies published prior to 2007 were excluded. However, as outlined above, few studies adhere to the ERS guidelines, suggesting that similar conclusions would be made for studies published after 2007.

5.5.5 Conclusions

This is the first study to evaluate methods of citric acid CRT in published literature. The results highlight lack of standardization and substandard reporting of methods of citric acid CRT. These findings suggest that caution is warranted in comparing citric acid cough thresholds across studies. Full replication of previously published methods of citric acid CRT may be limited due to crucial elements of the citric acid CRT protocol being omitted from the majority of published manuscripts. Further methodological studies on citric acid CRT are necessary to enhance understanding of factors that influence the outcome of the test.

CHAPTER 6: Quantifying Test-Retest Variability of the Citric Acid Cough Reflex Test

6.1 Study Aims and Rationale

Quantification of test-retest variability of citric acid CRT is poorly reported in the literature. Furthermore, no previous studies have quantified test-retest variability of citric acid suppressed cough thresholds (SCT) and urge to cough (UTC) ratings. These data are important for the use of citric acid CRT as a viable outcome measure in longitudinal cough research, as they enable the effects of an intervention to be compared to the artefact of repeating the test. The aims of this study were (1) to quantify test-retest variability of citric acid cough thresholds - both natural cough thresholds (NCTs) and suppressed cough thresholds (SCTs) - when citric acid CRT is repeated on three alternate days (i.e. Monday, Wednesday and Friday), and (2) to quantify test-retest variability of UTC at NCT, SCT, and at a sub-threshold citric acid concentration (0.05 mol/L), when citric acid CRT is repeated on three alternate days.

It was hypothesized that citric acid cough thresholds (NCT and SCT) would not change across the three alternate days, as methods of citric acid CRT used in the current study were chosen to optimize reproducibility of the test (Morice et al., 2007). Secondly, it was hypothesized that UTC at NCT, SCT and sub-threshold citric acid concentration would not change across days. It was expected that the perceived intensity of the same tussigenic stimulus would remain consistent with repeated tests.

6.2 Materials and Methods

6.2.1 Study Design

This was a prospective observational study. Participants citric acid cough thresholds were evaluated on three alternate days. Ethical approval was obtained by the local institutional review board (University of Canterbury, Human Ethics Committee, Reference Number: HEC 2017/15/LR-PS).

6.2.2 Participants

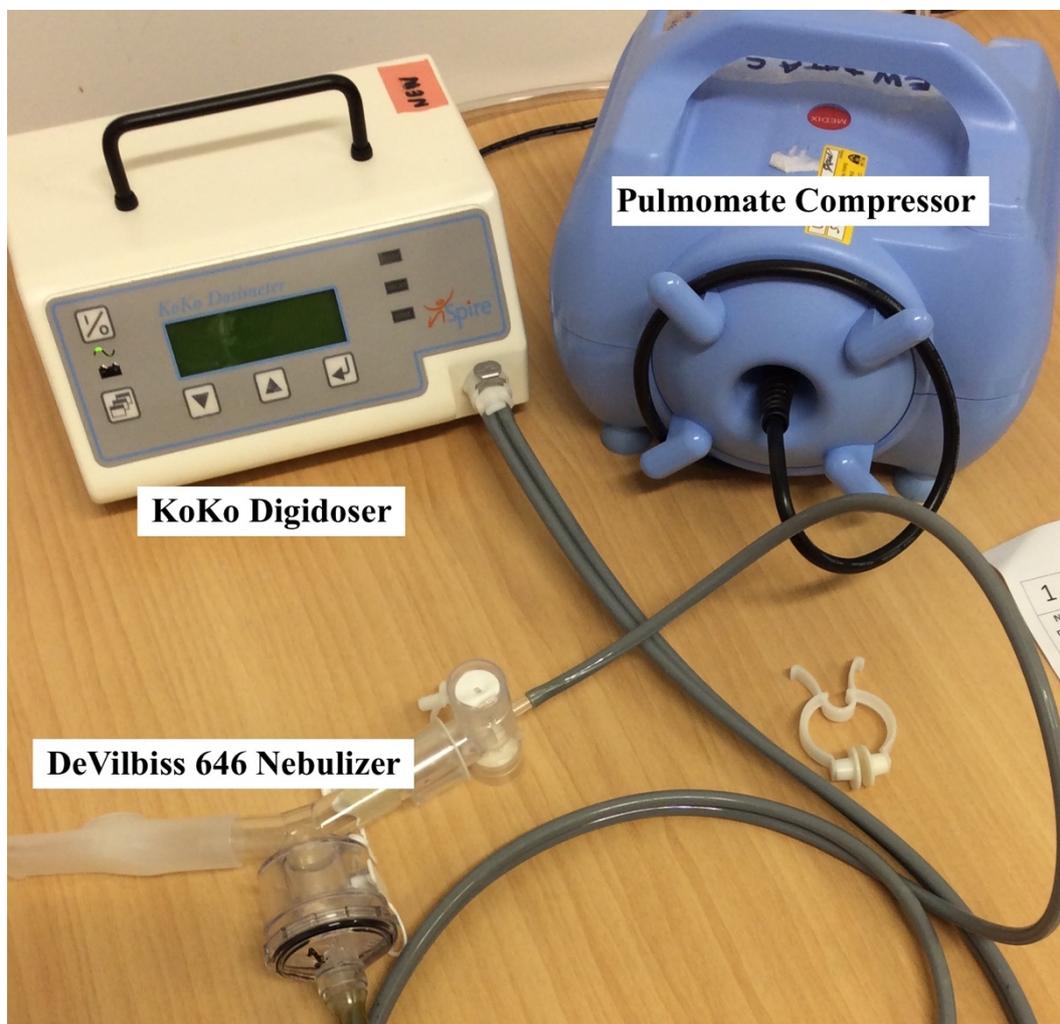
Sixteen healthy participants (7 males, 9 females) over the age of 18 years (mean 24 years, range 19-48 years) gave informed written consent prior to commencement of data collection. One participant (male, 21 years) withdrew from the study after Day 1 due to unforeseen university commitments that preventing him from returning to the research laboratory on Day 3 and 5. All participants were identified by self-report to be healthy. Participants were excluded from participating in the study if they had a history of any neurogenic disorder, a clinically significant respiratory disease (e.g. asthma, COPD, chronic bronchitis, emphysema), gastro-esophageal reflux, were taking ACE inhibitor or codeine-based drugs, were current, or previous smokers, or had a recent (< 2 weeks) acute upper respiratory tract infection (URTI), as these are factors that are known to influence cough sensitivity (Morice et al., 1987; Schmidt et al., 1997; Wong & Morice, 1999; Ziora et al., 2005).

6.2.3 Instrumentation & Materials

As recommended by the ERS Guidelines (Morice et al., 2007), citric acid was delivered using a compressed air-driven nebulizer (DeVilbiss 646; DeVilbiss Health Care, Inc.), controlled by a breath-activated dosimeter (KoKo Digidoser, nSpire health Inc.), connected to a compressor

(Pulmomate Compressor, model 46501). Compressed air was delivered at 40 psi, which was confirmed on the dosimeter at the start of each test. The nebulizer was modified with an inspiratory flow regulator valve (RIFR, nSpire health Inc.), which limited inspiratory flow rates to 0.5 L/s (Morice et al., 2007). Patients inhaled citric acid via a mouthpiece while wearing a nose clip. Citric acid, diluted in 0.9% sodium chloride, was prepared at 8 different concentrations: 0.01, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 mol/L.

Figure 8: Instrumentation used for citric acid cough reflex testing.



6.2.3.1.1 Nebulizer Output Testing

In order to ensure consistency and reproducibility of the dose of citric acid delivered across and within tests, the nebulizer output and the reliability of the nebulizer output was confirmed prior to data collection using a weigh-reweigh method (Tandon, Smaldone, & McPeck, 1997). The nebulizer was filled with saline and weighed before and after a series of ten actuations of 1.2 s duration each (the same duration used in the CRT). The nebulizer was triggered using a 3 L calibration syringe to ensure consistency of the volume of air pulled from the nebulizer across actuations. This process was repeated five times (i.e. five sets of ten actuations). Only one nebulizer was tested as the same nebulizer and instrumentation was used for all participants, across all tests, as per ERS guidelines (Morice et al., 2007). Adequate reliability of the nebulizer output was defined as a < 10% coefficient of variation across the five sets of ten actuations. This was based on the ERS guidelines for bronchial challenge testing (Coates et al., 2017).

6.2.4 Citric Acid Cough Threshold Testing

On each day, participants inhaled three successive 1.2 sec doses of incrementally increasing concentrations of citric acid, until C₂ cough thresholds (defined as two consecutive coughs within 3 seconds, on the same concentration of citric acid on two successive trials) were achieved. These criteria were based on the premise that the cough response to citric acid is immediate and brief (Dicpinigaitis, 2003; Morice et al., 2007). A C₂ response was required on the same concentration of citric acid on two trials to mitigate the “startle phenomenon” where individuals cough at a particular concentration of tussigenic aerosol, but then fail to cough at the same, or higher subsequent presentations of citric acid (Dicpinigaitis, 2003; Morice et al., 2007, p. 1260). Citric acid concentrations were administered in incrementally increasing order. This was because administration of a higher concentration of citric acid may influence the

response to a lower concentration of citric acid (Morice et al., 2007). Participants were blinded to the concentrations of citric acid they were inhaling. Saline placebos were randomly interspersed throughout the test to reduce the effects of participants anticipating progressively higher concentrations of citric acid (Morice et al., 2007). Participants' NCT and SCT were examined. In the NCT condition, participants were asked to "breathe in and out through your mouth and cough if you need to". Once the NCT was reached, participants were told to "breathe in and out through your mouth and try not to cough" to evaluate their SCT. Instructions were provided prior to each citric acid presentation. In the SCT condition, the concentration of citric acid immediately below the NCT was used as the starting point. This was based on prior research showing the SCT is always higher than the NCT (Leow, Beckert, Anderson, & Huckabee, 2012; Monroe et al., 2014), and therefore avoided unnecessarily extending the testing time.

6.2.4.1 Rationale for Methods of Citric Acid Cough Threshold Testing

The citric acid concentrations (i.e. 0.01, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 mol/L) and the number of inhalations (i.e. three inhalations per concentration) used in the current study were based on the outcome of a previous, unpublished study, that aimed to determine whether the dose (i.e. concentration *and* volume) of citric acid was appropriate to eliminate potential floor and ceiling effects in healthy individuals (Wallace, Ang, Guiu-Hernandez, & Macrae, in prep). In the study, a single inhalation of each concentration of citric acid was used. The use of a single inhalation was based on the KoKo Digidoser method of citric acid CRT used by Wright and colleagues (2010) and recommended by the ERS guidelines (Morice et al., 2007). The results of the study revealed that no participant produced a sensorimotor cough response at 0.01 mol/L. However, these data showed that a large number of healthy participants could suppress coughing up to 3.2 mol/L citric acid with a single inhalation (1.2 sec) of citric acid. This was

unanticipated given that 3 mol/L is the maximum dose of citric acid that is recommend by the ERS (Morice et al., 2007), but suggested that the volume of citric acid per inhalation may be too low to induce action potential discharge in the laryngeal afferents. To overcome this ceiling effect, three successive inhalations (3 x 1.2 sec) of increasing concentrations of citric acid were performed in the current study.

6.2.5 UTC Ratings

Participants were asked to rate their UTC following all citric acid inhalations, using a modified Borg Scale from 1-10, one representing no need to cough, and ten representing a maximum urge to cough (Hegland, Pitts, Bolser, & Davenport, 2011). No specific instructions were provided to participants on how to rate their UTC, but each number was accompanied by a written description (Table 10) (Hegland et al., 2011).

Table 10: Urge-to-Cough Scale (Hegland et al., 2011)

1	No need to cough
1.5	Just noticeable urge to cough
2	Slight urge to cough
3	Slight-to-moderate urge to cough
4	Moderate urge to cough
5	Moderate-to-strong urge to cough
6	Strong urge to cough
7	Strong-to-severe urge to cough
8	Severe urge to cough
9	Severe-to-maximum urge to cough
10	Maximum urge to cough

6.2.6 Sterilization of the Equipment

The nebulizer and component parts were sterilized between each subject as per local hospital and ERS guidelines (Morice et al., 2007). The nebulizer was washed in Medizyme[®] (6 ml per 1 L of cold water), a specialist enzymatic detergent solution, for 3 minutes. Afterwards, the nebulizer was placed in hospital grade disinfectant solution (Milton[®], anti-bacterial tablets, 1 tablet per 1 L of water) for 15 minutes. The nebulizer and component parts were allowed to air dry. A marker was placed on the base of the nebulizer bowl, to ensure consistency in the placement of the straw and baffle, as this is known to influence the nebulizer output (Morice et al., 2007).

6.3 Data Analysis

6.3.1 Nebulizer Output

The nebulizer weight (in grams), before and after five sets of ten actuations, was manually entered into Excel (version 16.16.7). The weight loss for each of the five sets of ten actuations

was calculated by subtracting the pre- and post-nebulizer weight. The mean and standard deviation of the nebulizer weight loss across the five sets of ten actuations was calculated. The reliability of the nebulizer output was determined by calculating the coefficient of variation of the nebulizer weight loss across the five sets of ten actuations.

6.3.2 Test Retest Variability of CRT

Test-retest variability of citric acid CRT is expressed in different ways. This was based on the recommendations by Barber et al. (2005), who advocate for expressing estimates of test-retest repeatability of citric acid CRT in a number of different ways to facilitate interpretation and comparison of cough sensitivity data across studies. For statistical analyses, citric acid cough thresholds were re-coded from 1-7, one representing 0.05 mol/L and seven representing 3.2 mol/L. No participant had a cough threshold at 0.01 mol/L. Participants who did not respond at the highest concentration of citric acid were coded as missing data so that variability was not skewed by assigning a false value. Linear mixed effects models were used to quantify (1) the effect of day, and the within-participant variability of citric acid cough thresholds (both NCT and SCT conditions) when citric acid CRT was repeated on three alternate days and (2) the effect of day on UTC ratings at NCT, SCT and at a subthreshold citric acid concentration (0.05 mol/L) when citric acid CRT was repeated on three alternate days. Data were analysed using R statistical package (version 3.5.2) (R Core Team, 2012) and a linear mixed effects models statistical package, lme4 (Bates, Mächler, Bolker, & Walker, 2015). Day was entered as a fixed effect, and intercepts for each subject were entered as random effects. A *p*-value of 0.05 was considered statistically significant.

6.4 Results

6.4.1 Nebuliser Output

Mean (SD) nebulizer output was 0.08 (0.004) ml per ten actuations (i.e. 0.008 ml per actuation). Based on these data, the volume of aerosol delivered per concentration of citric acid (i.e. 3 inhalations) was calculated as 0.024 ml (i.e. 0.008 ml x 3). The coefficient of variation of the nebulizer output across the five sets of ten actuations was 5.48%. This meets the ERS guidelines of < 10% coefficient of variation in nebulizer output for bronchial challenge testing (Coates et al., 2017).

6.4.2 Percentage of responders

The percentage of responders at each citric acid concentration on day 1-3 for NCT and SCT conditions are shown in Figures 9-11. Inhalation of citric acid concentrations (0.01 - 3.2 mol/L) induced a sensorimotor cough response in all participants (100%) in the NCT condition. In the SCT condition, two participants (8%) suppressed all citric acid concentrations on at least one day.

Figure 9: The percentage of responders at each citric acid concentrations for NCT and SCT on Day 1. Data is reported cumulatively, meaning that those who responded at 0.05 mol/L were assumed to respond at all higher concentrations.

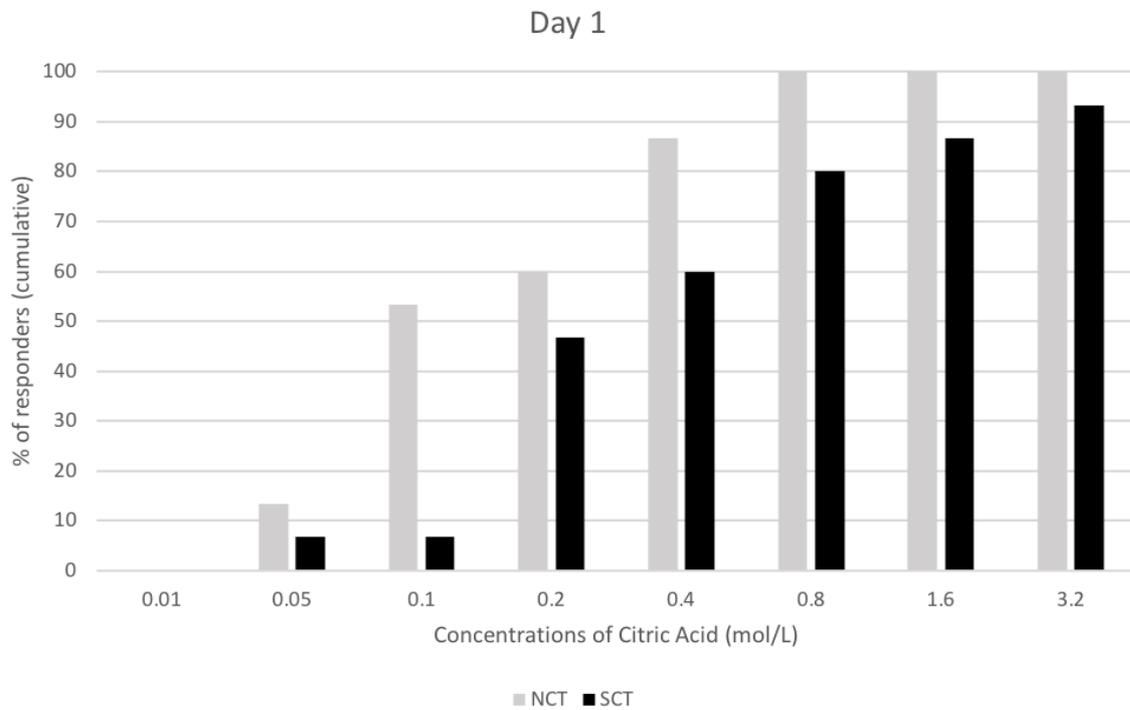


Figure 10: The percentage of responders (cumulative) at each citric acid concentrations for NCT and SCT on Day 2.

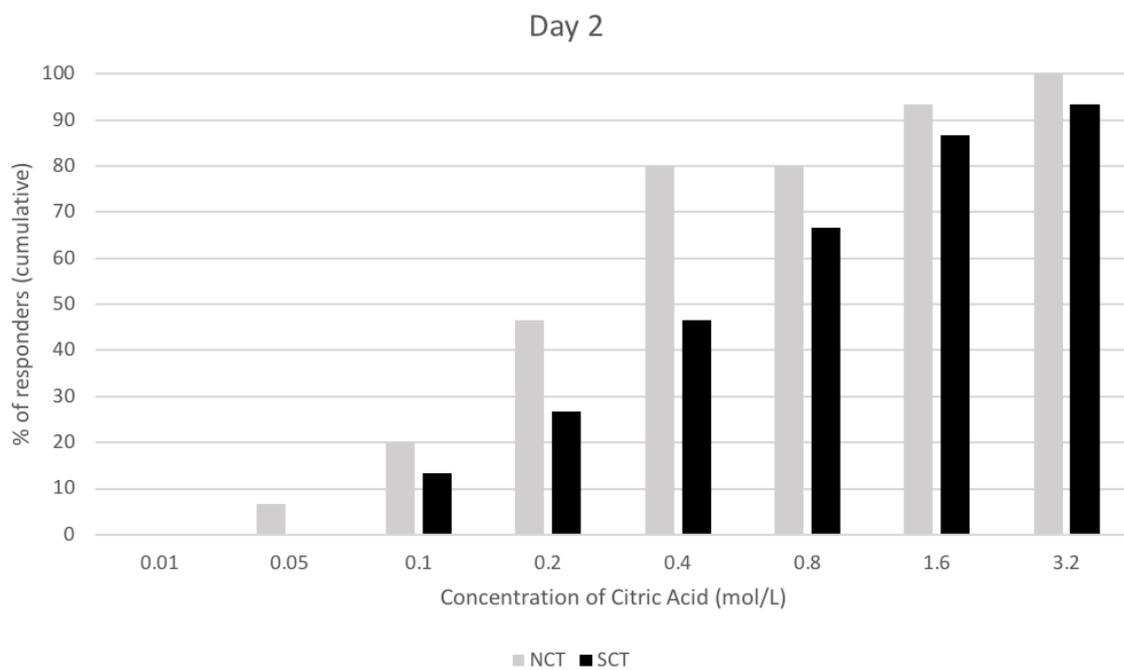
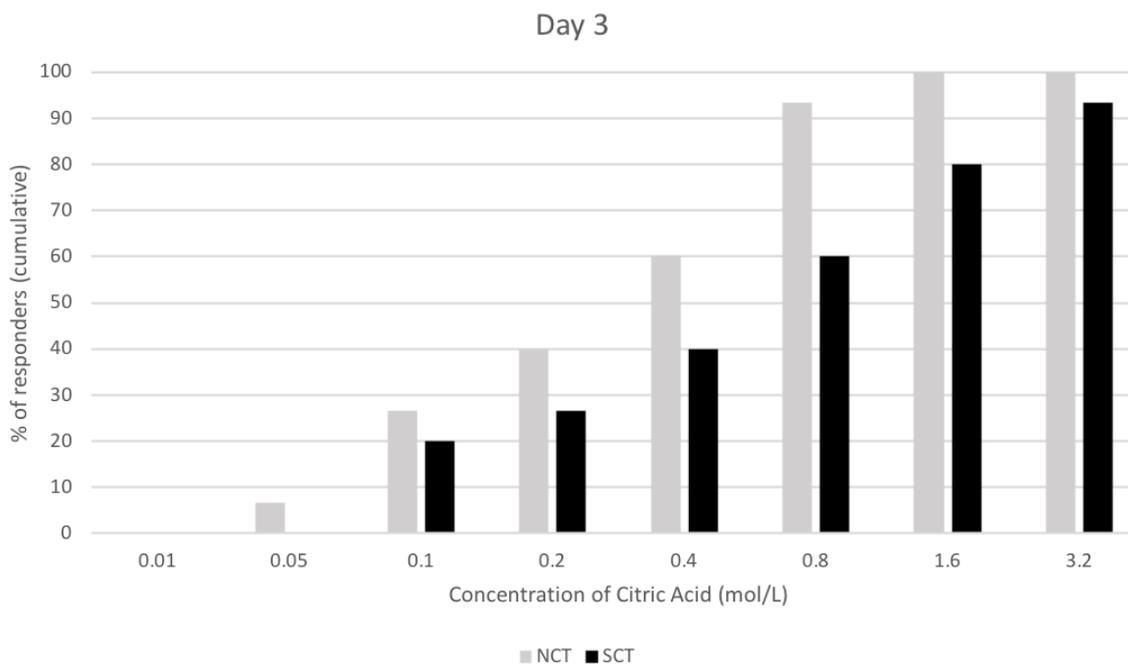


Figure 11: The percentage of responders (cumulative) at each citric acid concentrations for NCT and SCT on Day 3.



6.4.3 Reproducibility of citric acid cough thresholds

Table 11 shows the reproducibility of citric acid cough thresholds within one, two, or more than two doubling concentrations across the three days. In the NCT condition, one participant's cough threshold varied by more than two doubling concentrations. In the SCT condition, two participants did not respond to any concentration of citric acid on at least one day of testing. Thus, reproducibility across the three days was unable to be determined for these participants.

Table 11: Reproducibility of citric acid cough thresholds (NCT and SCT) across 3 days.

	100% Reproducible	Within one doubling concentration	Within two doubling concentrations	More than two doubling concentrations
NCT	1/15 (7%)	7/15 (46%)	6/15 (40%)	1/15 (7%)
SCT	1/15 (7%)	7/15 (46%)	5/15 (33%)	2/15 (14%)*

*both participants were non-responders.

6.4.4 Quantification of Test-retest Variability of Citric Acid Cough Thresholds

Table 12 shows the mean, standard deviation and range of NCT and SCT across days. Table 13 shows the estimated effects of day on NCT and SCT, and the variability of cough thresholds (NCT and SCT), expressed as the standard deviation, across day and participant.

Table 12: Mean, standard deviation (SD) and range of citric acid cough thresholds (citric acid concentrations recoded 1-7).

Day	Cough Type	Mean CT (recoded)	SD	Range (mol/L)
1	NCT	2.9	1.4	0.05 – 3.2
3	NCT	3.7	1.6	0.05 – 3.2
5	NCT	3.7	1.5	0.05 – 3.2
1	SCT	3.9	1.5	0.05 – 3.2
3	SCT	4.4	1.5	0.1 – 3.2
5	SCT	4.6	1.8	0.1 – 3.2

Table 13: Estimated day effects for NCT and SCT and variability across days.

	The Effect of Day			Variability Across Days	
	Estimated effect (doubling concentrations)	95% CI	<i>p</i>	Estimated standard deviation (doubling concentrations)	95% CI
NCT	0.43	0.16, 0.71	0.01*	0.78	0.58, 0.96
SCT	0.32	0.04, 0.59	0.04*	0.77	0.55, 0.98

*statistically significant

6.4.5 Quantification of Test-retest Variability of UTC

6.4.5.1 UTC at NCT and SCT

Table 14 shows the mean, standard deviation and range of UTC ratings at citric acid cough threshold (NCT and SCT) across individuals. There was no evidence of an effect of day in UTC ratings at participants' NCT ($p = 0.34$) or SCT ($p = 0.46$). (Note: NCT and SCT were not always the same for participants across days).

Table 14: Mean, standard deviation (SD) and range of UTC ratings at citric acid cough thresholds (NCT and SCT).

Day	Cough Type	Mean UTC	SD	Range
1	NCT	5.67	2.16	3-9
3	NCT	6.20	2.46	3-10
5	NCT	5.53	2.56	3-10
1	SCT	7.36	2.17	4-10
3	SCT	7.50	1.91	4-10
5	SCT	6.71	2.33	3-10

6.4.5.2 UTC at Subthreshold Concentration

Two participants were removed from the analysis of UTC at a subthreshold citric acid concentration, as their cough thresholds were 0.05 mol/L, thus, it did not represent a sub-threshold concentration. Table 15 shows the mean, standard deviation and range of UTC ratings at sub-threshold citric acid concentration (0.05 mol/L) across individuals. There were differences in UTC ratings at a subthreshold citric acid concentration (0.05 mol/L), between day 1 and 3 (1.4 UTC, 95% CI [0.5, 2.3], $p = 0.013$) and between day 1 and 5 (1.7, 95% CI [0.77, 2.63] $p = 0.003$), but not between day 3 and day 5.

Table 15: Mean, standard deviation (SD) and range of UTC ratings at sub-threshold citric acid concentration (0.05 mol/L).

Day	Cough Type	Mean UTC	SD	Range
1	Subthreshold	3.19	1.87	1-7
3	Subthreshold	1.78	0.99	1-4
5	Subthreshold	1.5	0.55	1-3

6.5 Discussion

6.5.1 Test-retest variability of NCT and SCT

The results of this study demonstrate that both NCT and SCT increase when citric acid CRT is repeated on alternate days, resembling a habituation effect to citric acid CRT over repeated testing. Estimated increases of 0.43 and 0.32 doubling concentrations per day are reported for NCT and SCT, respectively. These data are important for the use of citric acid CRT as an outcome measure in longitudinal cough research. They facilitate interpretation of whether changes in citric acid cough thresholds reflect *true* changes in cough sensitivity, rather than an artefact of repeating the test.

Unlike previously published literature (Barber et al., 2005; Schmidt et al., 1997; Wright et al., 2010), data in the current study are not log transformed, and thus, may be more easily interpreted and applied to future research practices (Feng et al., 2014). However, a recognized limitation of reporting test-retest variability in doubling concentrations of citric acid is that the increments between the citric acid concentrations are not equal. For example, there is a 0.05 mol/L difference between cough thresholds of 0.05 and 0.1 mol/L. Whereas, there is a 1.6 mol/L difference in cough thresholds of 1.6 and 3.2 mol/L. Caution must be made in assuming the test-retest variability of a doubling concentration equates to the same change in mol/L across individuals.

Statistical data are supported by descriptive results to facilitate interpretation and comparisons of results across studies (Barber et al., 2005). The results revealed that 93% and 86% of individuals' NCT and SCT were reproducible within two doubling concentrations (or two incremental doses) of citric acid, across the three days. Barber and colleagues report their data in a similar way and found that 91% of cough thresholds were reproducible within *one*

incremental dose. However, in this case, an incremental dose refers to a half-log (i.e. 3.16-fold) increments of citric acid, across two consecutive days. Thus, caution must be made in comparing these findings to studies using different increments and test-retest intervals.

As outlined in Chapter 4, the reproducibility of a similar method of citric acid CRT (i.e. the KoKo Digidoser method) was evaluated by Wright and colleagues (2010). In contrast to the findings of the current study, Wright and colleagues (2010) report no significant difference in citric acid cough thresholds from repeating the test with the KoKo Digidoser method. Different concentrations of citric acid, number of inhalations, and dose of citric acid per concentration are used in the current study, compared to the KoKo Digidoser method by Wright and colleagues (2010). For example, Wright and colleagues use doubling doses ranging from 7.8 to 1,000 mM (i.e. 0.0078 – 1 mol/L) citric acid. They use one, as opposed to three inhalations of citric acid, and the difference in the total dose per trial was 1.1 ml (Wright et al., 2010) versus 0.024 ml (current study). However, given these factors are kept consistent across tests, one might assume that test-retest variability would be similar. These discrepancies cannot be attributed to differences in test-retest intervals, as Wright and colleagues (2010) report significant differences in citric acid cough thresholds using the Mefar inhalation method over the same test-retest interval.

These findings raise questions regarding the parameters of citric acid CRT that are necessary to optimize reproducibility of the test, and whether disparity in the underlying mechanisms of cough induction may account for differences in test-retest variability. According to the nebulizer output documented by Wright and colleagues (2010), their participants received a greater dose of citric acid (i.e. 1.1 ml) over a single inhalation, compared to the current study (0.024 ml) over three inhalations. Electrophysiological studies in animal models demonstrate

that differences in how rapidly the pH of the airway afferents is reduced influences the afferents that are targeted by citric acid (Kollarik & Udem, 2002). Rapid reduction of the pH of airway afferents evokes action potential discharge in nodose A- δ fibres, whereas graduation reduction of the pH evokes action potential discharge in jugular C-fibres (Kollarik & Udem, 2002). The optimal stimulation parameters to target differential afferents during citric acid CRT in humans remains unknown. However, it is possible that differences in the rate at which the pH of the airway afferents is reduced (and thus, the underlying mechanisms of cough induction), may account for the discrepancy in test-retest variability across these methods.

Future studies should explore whether a single inhalation of citric acid or using a greater nebulizer output per breath (all other variables remaining the same), would reduce test-retest variability of the current method. It is hypothesized that these methods may be more suitable to targeting nodose A- δ fibres (or cough receptors) (Canning et al., 2004), that are processed by viscerosensory cough pathways in the central nervous system (McGovern, Driessen, et al., 2015) and assumed to play a greater role in airway protective coughing (Canning et al., 2004; Mazzone & Udem, 2016). This mechanism of cough induction may be less susceptible to variability than that evoked by jugular C-fibres, which are processed by somatosensory central neural pathways (McGovern, Driessen, et al., 2015), and are more likely to evoke cortically mediated coughing. However, further research is needed to substantiate this hypothesis.

It is also important to note that despite using the same nebulizer (i.e. DeVilbiss 646), dosimeter (i.e. KoKo Digidoser) and inhalation duration (i.e. 1.2 s) as Wright and colleagues, the nebulizer output in the current study was over 100 times lower than that reported by Wright and colleagues (i.e. 1.1 ml versus 0.008 ml). The reason for the lower nebulizer output in the current study is unclear. This finding highlights the variability in nebulizer output of the same

make and model, under similar testing conditions. It is hypothesized that the disparity in nebulizer output between the two studies may be attributed to the flow rate of the compressor, which is known to affect the nebulizer output (Smith, Denyer, & Kendrick, 1995). The compressor pressure, make or model is not reported by Wright and colleagues (2010) making it difficult to draw definitive conclusions on the nature of the disparity. This highlights the importance of documenting methods of citric acid CRT in order to fully replicate and make valid comparisons of citric acid cough thresholds across studies. In addition, it highlights the importance of determining and documenting the nebulizer output.

6.5.2 Test-retest variability of UTC

This the first study to characterize test-retest variability of citric acid induced UTC. Interestingly, the results of the study revealed that UTC at NCT and SCT did not change across days, despite the fact that the natural and suppressed cough thresholds themselves changed. These data suggest that the perceived intensity of a tussigenic stimulus that evokes a sensorimotor cough response remains stable across days, within participants. In other words, there is a reliable relationship between individuals' UTC and motor cough threshold, regardless of what concentration elicits that threshold.

There was a wide range of UTC ratings at NCT and SCT across participants. It is interesting that some participants rated their urge to cough at cough threshold as a three or four, while others rated it as a ten. This disparity may reflect the methods which were used to test UTC in the current study. Participants were not given any instructions on how to rate their UTC. Thus, it is unclear what sensory perceptions participants were rating, and whether these were the same across individuals. Gui et al. (2010) asked participants to rate the intensity of their UTC, but to ignore other sensations such as dyspnoea, burning, irritation and choking. This may facilitate

standardization of the sensations associated with an urge to cough rating. It is also possible that the wide range of UTC ratings may simply reflect the known variation in cough sensitivity across individuals (Morice et al., 2007). It is possible that despite eliciting a C₂ response, there are individual differences in the perceived intensity of that tussigenic stimulus. Regardless, these data provide insight into test-retest variability of UTC that can be used to interpret the outcomes of longitudinal cough studies, when UTC is used as an outcome measure.

UTC at a sub-threshold stimulus (i.e. 0.05 mol/L) behaved differently to UTC at cough threshold. The results of the current study revealed that UTC was higher on day one, compared to days three and five by 1.4 and 1.7 UTC increments, respectively. These data imply that individuals may “recalibrate” their UTC ratings of sub-threshold stimuli upon repeated presentations, based on their experience of CRT on day one. While progressing through concentrations on day one, participants had no prior experience on which to base their UTC ratings. However, after day one, participants had experienced the full range of citric acid concentrations (up to their cough threshold), and thus had something on which to base future UTC ratings. As the results show a reduction in UTC rating on days three and five, it is likely that the perception of irritation of 0.05 mol/L was less, after the maximum concentration had been experienced on day one. In the psychology literature, previous experiences are known to shape the perception of different sensory stimuli, such as tactile, visual and auditory stimuli (Snyder, Schwiedrzik, Vitela, & Melloni, 2015). These are known as “temporal contextual effects” in which prior experience of a stimulus can alter or stabilize the perception of a proceeding stimulus (Snyder et al., 2015). This may explain the difference in UTC ratings between day one and day three and five observed in the current study at 0.05 mol/L. However, it is important to note that whether the same pattern would be seen with all sub-threshold citric acid concentrations is unclear. As 0.05 mol/L is the second concentration that is presented to

participants, it may be more susceptible to modulation on consecutive testing. Given that this is the first study to evaluate test-retest variability of UTC in healthy individuals, further studies are necessary to validate or dispute these findings.

6.5.3 Within-participant variability

The use of linear mixed effects models to analyse the data revealed sources of *within* participant variability in NCT and SCT that are not explained by day. Table 13 shows that across the three alternate days, NCT and SCT varied by 0.78 and 0.77 doubling concentrations. This was an unexpected finding, and it is unclear what factors contribute to this within participant variability in NCT and SCT. In interpreting this finding, it is important to consider the underlying mechanisms of citric acid induced coughing, and how it may be influenced by day-to-day variability *within* participants. The pH of citric acid plays an important role in mediating coughing (Canning et al., 2004; Kollarik & Udem, 2002; Lowry et al., 1988; Rai et al., 2018; Wong et al., 1999). Thus, it is possible that day-to-day changes in the pH of the internal environment (i.e. the respiratory tract) in which the acid is inhaled may account for day-to-day variability within individuals. Salivary pH can be lowered by consumption of sugary beverages, coffee or acid reflux (Hans, Thomas, Garla, Dagli, & Hans, 2016; Loke, Lee, Sander, Mei, & Farella, 2016), an effect that can be sustained for up to 20 minutes (Johansson et al., 2004). Similarly, salivary volume can be altered by a number of variables such as degree of hydration, body position (i.e. seated versus standing), and circadian rhythms (Dawes, 1987). Increased salivary volume is known to alter the rate of swallowing, and thus, clearing and diluting acid in the mouth more effectively (Loke et al., 2016). Given the importance of pH as the primary mechanisms by which coughing is evoked by citric acid (Canning et al., 2004; Kollarik & Udem, 2002; Lowry et al., 1988; Rai et al., 2018; Wong et al., 1999), it is possible that alterations in salivary pH and volume may account for the unexplained variability in citric

acid cough thresholds *within* participants. In the current study, patients with acid reflux were excluded due to known differences in citric acid cough thresholds in individuals with acid reflux (Ziora et al., 2005). However, other factors that may influence the pH of the upper respiratory tract - such as prior consumption of sugary beverages, tea or coffee, or degree of hydration - were not controlled. It is possible that these factors may explain the observed within participant variability in the current study.

It is also possible that an imbalance in the pH of saliva or airway surface fluids may alter the response to citric acid CRT in disease populations. There is some evidence to suggest a relationship between oral bacteria levels and citric acid cough thresholds in elderly individuals (Watando et al., 2004). Wantando et al. (2002) found that citric acid cough thresholds were significantly decreased (i.e. increased sensitivity) following an intensive oral hygiene program. However, the nature of this relationship was unknown. From the dentistry literature, it is well known that bacteria in the oral cavity contribute to acidification, which is the main cause of dental caries (Forbes, Latimer, Sreenivasan, & McBain, 2016). Whether a relationship between intra oral pH and cough thresholds explain the findings by Wantando and colleagues (2002) requires further investigation.

Salivary or airway surface fluid pH may also account for the large variability *across* participants documented for citric acid CRT (Morice et al., 2007). Large inter-individual variability of citric acid cough thresholds prevents the development of parameters to accurately define hyper- or hypo- cough sensitivity (Morice et al., 2007). In line with previous findings in the literature (Monroe et al., 2014; Morice et al., 2007), the current study revealed a wide range of citric acid cough thresholds across healthy individuals, extending from 0.05 or 0.1 mol/L to 3.2 mol/L on all days of testing. To date, the nature of this variability across individuals is

unexplained in the respiratory physiology literature (Morice et al., 2007). It is possible that inter-individual variation in saliva pH or volume may explain this variation. Further research should explore whether monitoring intra-oral pH across and within individuals, prior to citric acid CRT, may explain the nature of test-retest variability across and within participations.

6.5.4 Limitations

It is acknowledged that the method of citric acid CRT used in the current study may not be suitable for clinical use due to the complexity of the instrumentation (i.e. compressor, dosimeter, nebulizer) and sterilization of the nebulizer that is required between individuals. The method of citric acid CRT used in the current study was designed to serve as a viable outcome measure to monitor changes in cough sensitivity in response to an intervention in Chapter 7. Thus, the desire to optimize test-retest reproducibility was prioritized at the expense of the clinical applicability of the method.

Secondly, the methods of citric acid CRT used in the current study were based on the ERS guidelines (Morice et al., 2007). However, there is lack empirical evidence to support many of these recommendations. As recommended by the ERS guidelines, the nebulizer in the current study was adapted with an inspiratory flow valve (RIFR, nSpire health Inc.) to limit inspiratory flow rate to 0.5 L/s (Morice et al., 2007). However, there is still lack of consensus regarding the optimal inspiratory flow rate for laryngeal deposition of an aerosol (Barros et al., 1990). It is also acknowledged that this valve would not standardize inspiratory flow rates for individuals who may inhale slower than this.

Lastly, the most appropriate end of test criterion for NCT and SCT remains unclear and may be dependent on the study population for which the test is used. Both non-responders in the

current study demonstrated a range of airway clearance behaviours such as single coughs, throat clearing and exhalations (Troche, Brandimore, Godoy, et al., 2014), in response to 1.6 and 3.2 mol/L citric acid. These behaviours are considered sensorimotor airway clearance mechanisms (Troche, Brandimore, Godoy, et al., 2014), but are considered failed responses to citric acid CRT under the current end of test criterion (i.e. a C₂ cough response). This end of test criterion was chosen due to the need for an outcome measure that is objective and reproducible. However, it is important to acknowledge that it is not known whether a C₂ response is the most likely response to aspiration – which in this context, the test is trying to replicate. Further research is required to determine the most appropriate end of test criteria. In the interim, clinicians and researchers should record all cough and non-cough behaviours (e.g. throat clears, exhalations) in response to citric acid CRT during testing protocols in order to make thorough judgements on cough sensitivity and airway protective mechanisms.

SECTION III. MODULATION OF THE SENSORIMOTOR COUGH RESPONSE

CHAPTER 7: Sensory Stimulation to Modulate Cough

Sensitivity - A Protocol Safety Study

7.1 Introduction

Silent aspiration is defined as aspiration without a sensorimotor cough response, or other overt signs of distress (Ramsay et al., 2008). Patients who silently aspirate are amongst the most vulnerable dysphagic patients. They are without vital means of airway protection *and* airway clearance mechanisms during swallowing, leaving them vulnerable to aspiration pneumonia and mortality (Nakashima et al., 2018; Nakazawa et al., 1993). The pathophysiology of silent aspiration can be attributed to reduced or absent laryngeal afferent input, or impaired central neural processing of laryngeal afferent input, from central and/or peripheral neurological damage (Garon et al., 2009; Holas et al., 1994; Horner & Massey, 1988). Damage to the brainstem, which houses the coughing and swallowing CPGs (Bianchi & Gestreau, 2009; Bonham, Sekizawa, & Joad, 2004; Haji et al., 2013), results in a high incidence of silent aspiration (Garon et al., 2009). A high incidence is also reported in patients following cortical stroke (Garon et al., 2009), reinforcing the role of the cerebral cortex in coughing to aspiration (Eccles, 2009; Hegland et al., 2012; Mazzone et al., 2011; Mazzone et al., 2007), which was historically considered reflexive in nature.

One of the greatest clinical challenges in assessing patients with dysphagia is that silent aspiration can occur in the absence of clinical signs of distress. The traditional clinical swallowing evaluation is inadequate for detecting silent aspiration (Ramsey et al., 2003; Smithard et al., 1998; Splaingard, Hutchins, Sulton, & Chaudhuri, 1988). Studies have shown that approximately 50% of patients who silently aspirate go undetected from CSE alone

(Smithard et al., 1998; Splaingard et al., 1988). This is likely due to the limited information available from asking patients to volitionally cough, or observing a lack of coughing on oral intake. Volitional coughing does not provide information about the sensorimotor cough response to aspiration, due to the disparity in the underlying neurophysiology of both cough types (Magni, Chellini, Lavorini, Fontana, & Widdicombe, 2011; Widdicombe et al., 2011). An absent cough during swallowing does not reliably indicate absence of aspiration, as aspiration may be silent.

The addition of the citric acid cough reflex test (CRT) to the clinical swallowing evaluation has provided a substantial contribution to the diagnosis of silent aspiration. When used alongside the clinical swallowing evaluation, the outcome of the CRT can be used to support clinical decision making and informed decisions about referral for instrumental assessment, and safety for oral intake. In a recently published clinical management protocol based on CRT outcomes, patients who passed the CRT proceeded to assessment of oral trials, and the clinical swallowing evaluation was used to guide further management decisions (Perry et al., 2019). This is based on the premise that these patients were at low risk of silent aspiration (Miles, Moore, et al., 2013) and would likely demonstrate overt signs of aspiration in response to misdirected food or fluid into the airway that can inform further management decisions. Patients who failed the CRT were considered at high risk of silent aspiration (Perry et al., 2019). These patients were recommended nil per orem (NPO) and referred for instrumental assessment (i.e. VFSS or FEES). Patients with confirmed silent aspiration on instrumental assessment remained NPO, and alternative nutrition/hydration was recommended (e.g. via nasogastric intubation) until spontaneous recovery of the sensorimotor cough response, or resolution of aspiration was confirmed. In theory, this mitigates the risk of pulmonary sequelae by negating the need for swallowing (and potential aspiration), while maintaining adequate

nutrition and hydration.–Although, studies suggest that patients on NG tube feeding may not have better outcomes against aspiration pneumonia than those who eat orally (Finucane & Bynum, 1996; Gomes, Pisani, Macedo, & Campos, 2003; Mamun & Lim, 2005).

There are no alternative recommendations in the literature for management of patients who fail to make spontaneous recovery and demonstrate long-term silent aspiration. Long-term use of non-oral methods of feeding (e.g. percutaneous endoscopic gastrostomy) in patients with severe dysphagia is associated with mortality, aspiration pneumonia and long-term swallowing impairment (James, Kapur, & Hawthorne, 1998). A number of management options, such as manipulation of bolus texture (Garcia & Chambers, 2010; Leonard, White, McKenzie, & Belafsky, 2014), thermal tactile stimulation (Regan, Walshe, & Tobin, 2010; Teismann et al., 2009), and/or postural changes (Ra, Hyun, Ko, & Lee, 2014; Saconato, Chiari, Lederman, & Gonçalves, 2015) are designed to prevent aspiration during swallowing. However, evidence for these treatments provides mixed results. There are no clear recommendations as to which swallowing deficits are best suited to their application, and there is lack of evidence to suggest that these treatments contribute to better long term clinical outcomes, such as reducing rates of aspiration pneumonia (O'Keeffe, 2018). Thermal-tactile stimulation and modification of bolus texture could be considered sensory-based treatments. However, they do not target specific sensory deficits, and are designed to enhance the general sensory experience during swallowing. This highlights the need for rehabilitation approaches that target specific laryngeal sensory deficits associated with silent aspiration.

7.1.1 Framework for Sensory Rehabilitation

Research in the limb literature suggests that sensory enhancement can occur, at a neural and behavioural level, in response to repetitive, passive exposure to sensory stimulation, without

the need for active attention from the subject (Beste & Dinse, 2013; Dinse & Tegenthoff, 2015; Godde, Spengler, & Dinse, 1996; Godde, Stauffenberg, Spengler, & Dinse, 2000; Pleger et al., 2001; Pleger et al., 2003). This passive approach challenges our traditional understanding and assumptions of learning and rehabilitation that is driven by intense training and active participation by the patient (Kleim & Jones, 2008; Robbins et al., 2008). The term “training independent sensory learning” (TISL) refers to learning induced by passive sensory stimulation that has the intention of changing perception and sensorimotor behaviours (Dinse & Tegenthoff, 2015, p.11). The advantage of not requiring active participation or attention by the participant, is that patients who are at risk of low cognitive functioning, receptive language impairments or fatigue may still benefit from such interventions (Dinse & Tegenthoff, 2015).

Dinse and Tegenthoff (2015) propose a conceptual framework outlining the factors that control learning as a result of passive sensory stimulation. According to their model, sensory learning occurs when sensory input passes a “learning threshold” (Dinse & Tegenthoff, 2015, p. 18). For TISL, “high frequency” or “burst-like features”, as well as heavy schedules of sensory stimulation must be present to drive learning past the learning threshold (Dinse & Tegenthoff, 2015, p 18). This aligns with the motor learning principals of “repetition matters” and “intensity matters” (Kleim & Jones, 2008, p. 227), and suggests that sufficient repetition and intensity are important components of both sensory and motor rehabilitation regimes.

7.1.2 The Efficacy of TISL in the limb literature

The efficacy of TISL for neural plasticity and enhanced sensory acuity in the limbs has been demonstrated in a number of studies (Godde et al., 1996; Godde et al., 2000; Pleger et al., 2001; Pleger et al., 2003). Enhanced sensory acuity and cortical reorganization following a tactile stimulation TISL protocol for the hand was reported by Pleger and colleagues (2001). Sensory

acuity was measured by a two-point discrimination task, in which participants had to decide if they felt the sensation of one or two needle tips on the index finger (Pleger et al., 2001). This was measured at the index finger of the right hand (test finger, that would be stimulated with TISL) and the index finger of the left hand (control finger). Cortical reorganization was measured by somatosensory evoked potential (SSEP) mapping of the digit representation in the primary somatosensory cortex, before and after the TISL protocol (Pleger et al., 2001). Tactile (electrical) stimulation involved a train of eight 1 Hz pulses, applied to the index finger, followed by a 15 second break (Godde et al., 1996; Pleger et al., 2001). After three trains of stimulation, there was a pause of 1 minute to minimize adaption and habituation to the stimulus (Godde et al., 1996; Pleger et al., 2001). The duration of the sensory stimulation was 3 hours, and was delivered during one session only (Pleger et al., 2001). The authors report enhanced sensory acuity and cortical reorganization following the TISL protocol (Pleger et al., 2001). However, the effects were short-lived and participants returned to baseline with 24 hours. Interestingly, sensory acuity enhancement was variable across participants, and was predicted by the extent of cortical reorganization (Pleger et al., 2001). Participants who demonstrated little gain in spatial discrimination showed small changes in the stimulated digit representation in the primary somatosensory cortex, suggesting that behavioural changes may align with cortical reorganization. The largest cortical reorganization and sensory acuity enhancement was observed for individuals with the lowest sensory acuity threshold (Pleger et al., 2001), suggesting that those with *better* sensory acuity at baseline yield the greatest benefit from TISL. It is possible that those with inferior sensory acuity may have required greater intensity or longer exposure (i.e. more than one session) of the TISL protocol to enhance sensory acuity.

The efficacy of TISL in enhancing sensorimotor performance has also been demonstrated in patient populations following stroke. Kattenstroth, Kalisch, Peters, Tegenthoff, and Dinse

(2012) conducted a series of case studies to examine the effects of a long term tactile TISL protocol in chronic (> 10 years) stroke patients with severe sensory deficits in the hand (n = 3). The stimulation sequence was more intense than that used for healthy individuals in Pleger and colleagues' study (2001), and consisted of 45 minutes of sensory stimulation per day on the paretic hand of the patients, for eight (n = 1), thirty-six (n = 1), and seventy-six weeks (n = 1). Stimulation intensity was delivered at the highest threshold each individual could tolerate, and differed for each subject. Beneficial effects were observed on tactile tasks (touch threshold, and two-point discrimination tasks) and sensorimotor behaviours (e.g. execution of fine motor movement) as well as functional tasks (such as moving and manipulating heavy, light and small objects). The impact of TISL on cortical activity in response to sensory stimulation was investigated in one patient, who received 36 weeks of sensory stimulation. No SEP was detectable prior to intervention. However, there were clear cortical SEP components (i.e. P50, N80 and P200) in response to sensory (pneumatic) stimulation following TISL treatment at 36 weeks (Kattenstroth et al., 2012), suggesting a partial restoration of processing of tactile information in the somatosensory cortex.

While the findings of this study are limited due to the small sample size, they were recently replicated in a randomized control trial in a cohort of sub-acute stroke patients (n = 46) using the same stimulation parameters and protocol as above (Kattenstroth et al., 2018). These patients had less severe sensory deficits than the previous study. After two weeks of intervention, patients in the group receiving standard therapy with TISL (n = 23) showed significant improvement in sensory, motor, proprioceptive and everyday tasks, compared to those receiving standard therapy alone (n = 23). The greatest measurable gains following the TISL protocol were in the sensory domain (i.e. touch threshold and two-point discrimination). However, the authors note large inter-individual variability, as demonstrated by large standard

deviations in pre- and post- test scores, which the authors attribute to the small sample size. In addition, patients with more severe sensory impairments were not included in the study, suggesting the positive effects of passive sensory stimulation may be limited to patients with less severe sensory deficits.

7.1.3 Sensory Stimulation of the Larynx

An issue of substantial relevance to the current research is the extent to which the principals of passive sensory stimulation of the skin could be applied to the larynx to enhance the sensitivity of the sensorimotor cough response. The sense of touch from the skin has distinct neural control to visceral senses arising from internal organs. However, comparisons have been made in the literature between central neural processing of chronic cough and chronic pain (Ando et al., 2016). This suggests that similar sensory neural mechanisms may underlie somatic and visceral sensations, and raises the possibility that passive sensory stimulation to the larynx may have the potential to enhance the sensorimotor cough response under the correct conditions. Based on the above studies, important factors of a sensory stimulation protocol include the repetitive nature of passive sensory stimulation (Kattenstroth et al., 2018; Pleger et al., 2001; Pleger et al., 2003), and preventing adaption to the stimulus (Godde et al., 1996).

7.1.4 Sensory Stimuli

From the principals of neurorehabilitation (Kleim & Jones, 2008), we know that the *specificity* of stimulation is an important factor for dictating the nature of the plasticity. Tussigenic aerosols (such as citric acid, capsaicin and distilled water) offer a means of activating afferents and central neural pathways involved in coughing (Morice et al., 1992), with relatively non-invasive methods. Thus, they offer an appealing possibility for enhancing the sensorimotor cough response. Previous studies have shown that citric acid, distilled water and capsaicin can

modulate coughing (Morice et al., 1992). For example, significant attenuation of the sensorimotor cough response has been demonstrated in response to prolonged (1 minute) inhalations of citric acid, capsaicin and distilled water aerosols - a phenomenon known as tachyphylaxis (Morice et al., 1992). Interestingly, both citric acid and distilled water induced rapid and substantial attenuation of the sensorimotor cough response (i.e. 84 – 100% reduction in cough frequency), compared to the more modest effects of capsaicin (i.e. 37-49% reduction in cough frequency) (Morice et al., 1992). The difference in modulation of the sensorimotor cough response was attributed to the distinct underlying afferents activated by each stimulus (Morice et al., 1992). Citric acid and distilled water are likely acting through the same neuronal pathway (i.e. laryngeal nodose A- δ fibres, or cough receptors), which is characterized by a rapidly adapting response (Canning et al., 2004; Mazzone & Udem, 2016; Morice et al., 1992). This is supported by studies demonstrating the nodose A- δ fibres are highly responsive to rapid alterations in pH, and hypotonic solutions (i.e. distilled water) (Anderson, Sant'Ambrogio, Mathew, & Sant'Ambrogio, 1990; Boushey, Richardson, Widdicombe, & Wise, 1974; Lee, Macglashan, & Udem, 2005; Mazzone & McGovern, 2006; Mazzone & Udem, 2016; Storey, 1968). In contrast, capsaicin-induced coughing is mediated through C-fibre activation, which is more slowly adapting (Canning et al., 2004; Mazzone & Udem, 2016; Morice et al., 1992). Given their underlying mechanisms of cough induction (i.e. via laryngeal nodose A- δ fibres), both citric acid and distilled water may target a greater number of afferents and central neural pathways involved in coughing to aspiration, compared with capsaicin.

7.1.5 Citric Acid as a Sensory Stimulus

Citric acid has been widely used in the literature for assessment of coughing without reported adverse effects (Miles, Moore, et al., 2013; Miles, Zeng, et al., 2013; Sato et al., 2012; Vilardell,

Rofes, & Clave, 2015; Wakasugi et al., 2008). However, this typically involves short inhalations of small quantities of citric acid. The safety of inhalations of citric acid in larger quantities and over a number of days – as required with a sensory stimulation protocol – must be carefully considered.

Acid induced bronchoconstriction is widely recognized in the literature (Ricciardolo, 2001). In animal models, an increase in respiratory resistance in response to citric acid inhalations was dose-dependent (Ricciardolo et al., 1999), suggesting the dose of citric acid is an important predictor of the development of bronchoconstriction. This finding is supported in a more recent study in humans. Wright and colleagues (2010) report a significant increase in respiratory resistance (as measured by impulse oscillometry), in healthy individuals in response to the KoKo Digidoser method of citric acid CRT (i.e. single inhalation of citric acid), but not on the Mefar method (i.e. four single inhalations of citric acid). The total volume of citric acid inhaled per concentration in the KoKo Digidoser method was much higher than that in the Mefar method (1.1 ml in one inhalation, versus 0.4 ml in four inhalations), suggesting that inhalations of larger quantities of citric acid may account for the development of bronchoconstriction in healthy individuals.

In the original studies by Bickerman and colleagues (1954), no signs of bronchoconstriction were reported in healthy individuals. However, the authors report a “slight degree of bronchospasm” in response to 10% citric acid aerosols (five successive inhalations) in asthmatic participants (Bickerman & Barach, 1954). It is not reported how this was evaluated or managed in the study. Other studies report no evidence of bronchoconstriction in asthmatics using 1-minute inhalations of citric acid (Auffarth, de Monchy, van der Mark, Postma, & Koeter, 1991a). However, the nebulizer output is not reported in either study, making it difficult

to determine whether the dose of citric acid inhaled may have accounted for the presence or absence of bronchoconstriction.

Prolonged inhalations of acidic aerosols are known to have adverse effects on the respiratory system (Folinsbee, 1989; Utell, 1985; Wyzga & Folinsbee, 1995). This has been studied specifically with airborne pollutants. For example, Spektor and colleagues (1989) demonstrated that inhalations of low concentrations of sulfuric acid aerosols ($100 \mu\text{g}/\text{M}^3 = \sim 1 \times 10^{-11} \text{ mol}/\text{L}$) for 1 hour and 2 hours on separate occasions, separated by one week, markedly reduced the rate of mucociliary clearance in a cohort of healthy adults. This may lead to enhanced bacterial colonization and upper respiratory tract infections (Antunes & Cohen, 2007). The longer the exposure to the acidic aerosol, the greater the prolongation of mucociliary clearance mechanisms (Spektor, Yen, & Lippmann, 1989), suggesting prolonged inhalations of acidic stimuli should be avoided. Holma and colleagues (1989) also suggested that prolonged inhalations of acidic aerosols may have adverse effects on the mucus in the respiratory tract. When mucus becomes acid-saturated (due to an excess of H^+ ions), these ions can access the surrounding tissues and gain access to tissues and cause intra and inter-cellular oedema and acid induced airway resistance (Holma, 1989). The authors suggest that the amount of acid an individual can tolerate is dependent on their buffer capacity and protein content of their mucus. Those with acidic mucus or mucus with a low protein concentration would be at higher risk of adverse effects from prolonged acid inhalation (Holma, 1989). These findings raise potential problems for repeated inhalations of citric acid as part of a sensory stimulation protocol.

Repeated ingestion of acidic substances is also associated with adverse health effects. In a published report for chemical and biosafety of citric acid (OECD, 2001), gastrointestinal

disturbances, such as diarrhoea, indigestion and nausea are reported following repeated ingestion of citric acid. Limited details are provided on the dose of citric acid that was ingested. However, this is an important consideration for repeated inhalations of citric acid, which would result in deposition of acid on the pharyngeal wall, that may be swallowed causing potential gastrointestinal disturbances. Overall, these risks preclude the use of citric acid as a potential stimulus to modify the sensorimotor cough response over prolonged durations.

7.1.6 Distilled Water as a Sensory Stimulus

There are numerous advantages of using distilled water. Distilled water is a relatively innocuous stimulus. It is purified by the process of distillation that removes salts and other compounds (Daintith, 2008) and is safe for ingestion. Distilled water can induce coughing when inhaled as an aerosol from an ultrasonic nebulizer, and has been used as a tussigenic stimulus in numerous studies for cough assessment in patients and healthy individuals, without reported adverse effects (Fontana, Pantaleo, Lavorini, Benvenuti, & Gangemi, 1998; Fontana, Pantaleo, Lavorini, Boddi, & Panuccio, 1997; Fontana et al., 1999; Hegland et al., 2016; Morice et al., 1992).

The safety of prolonged inhalations of distilled water in healthy individuals, without respiratory diseases, has been demonstrated in numerous studies that are described in detail below. However, these studies demonstrate that distilled water inhalation is associated with bronchoconstriction in asthmatics (Allegra & Bianco, 1980; Anderson, Schoeffel, & Finney, 1983; Chadha, Birch, Allegra, & Sackner, 1984; Cheney & Butler, 1968; Schoeffel, Anderson, & Altounyan, 1981). This observation gave rise to the original use of distilled water inhalations as a bronchial challenge test, to assess for bronchial hyperresponsiveness in asthmatics, and

warrants some caution in the use of distilled water inhalations as part of a sensory stimulation protocol.

Cheny and Butler (1968) were among the first to document a relationship between distilled water inhalations and bronchoconstriction. Ten healthy individuals and ten patients with pulmonary disease (asthma and bronchitis) inhaled distilled water for 15 minutes (nebulizer flow rate of 3.5 ml/min). Severe coughing was reported in all participants during distilled water inhalations. Bronchoconstriction (as measured by whole body plethysmography) was not evident in any of the healthy individuals. However, all patients with pulmonary disease experienced bronchoconstriction following distilled water inhalations (Cheny & Butler, 1968). These findings align with those by Chada, Birch, Allegra and Sackner (1984). Healthy individuals were exposed to 30 s, 1 minute, 2 minutes, 4 minutes and 8 minutes of distilled water, administered in 15-minute intervals, while the exposure times for asthmatics were 15 s, 30 s, 1 minute, 2 minutes, 4 minutes (nebulizer flow rate: 6 ml/min). The authors report that all participants experienced laryngeal irritation and coughing during distilled water inhalations, which increased in intensity with longer exposures to distilled water (Chadha et al., 1984). Interestingly, this contradicts the findings of Morice and colleagues (1992) above, who report *less* coughing with longer exposures of distilled water within one-minute. The reported nebulizer flow rates are the same in both studies (i.e. 6 ml/min). It is possible that initial exposure and adaption to distilled water, as reported by Morice and colleagues (1992), is mediated through rapidly adapting airway afferents (i.e. nodose A- δ). While subsequent increases in coughing to prolonged (> 1 minute) exposure to distilled water may be mediated through C-fibre activation, which are slowly adapting. In healthy participants, no signs of bronchoconstriction were reported during any of the inhalations (Chadha et al., 1984). Patients with asthma produced no changes in respiratory resistance or breathing patterns at 15 and 30 s

of distilled water inhalations. However, longer exposures (1, 2, and 4 minutes) produced a stepwise significant increase in mean respiratory resistance, suggesting a dose-response relationship between the dose of distilled water and bronchoconstriction in asthmatics (Chadha et al., 1984). A similar dose-response effect has also been demonstrated in subsequent studies, suggesting the volume of distilled water that is inhaled has important implications for the development of respiratory resistance. Schoeffel, Anderson and Altounyan (1981) examined bronchial hyper-responsiveness in asthmatics and healthy controls in response to distilled water inhalations. The authors evaluated the total ventilation (i.e. volume of air inhaled and exhaled from the lungs) required to induce a 20% fall in FEV₁. Distilled water was administered in total ventilation volumes of 10, 20, 40, 80, 80, 80 L. Of note, tidal volume is approximately 500 ml/ breath for a healthy adult (Landers, Barker, Wallentine, McWhorter, & Peel, 2003), suggesting that approximately 20, 40, 80, 160, 160, 160 inhalations of distilled water were administered. All healthy subjects inhaled all increments of distilled water, without registering a 20% fall in FEV₁. All patients with asthma registered a > 20% fall in FEV₁ in response to inhalations of distilled water. However, there was wide variation in total ventilation of distilled water that registered this response in the patients with asthma (ranging from a total ventilation of 1.3 L to 180 L) which is likely to reflect varying degrees of asthma severity. The output of the nebulizer is not reported by the authors, which is a limitation in applying these data to future studies.

In the above studies, all cases of bronchoconstriction induced by distilled water were reversed with a bronchodilator (Allegra & Bianco, 1980; Anderson et al., 1983; Chadha et al., 1984; Cheney & Butler, 1968; Schoeffel et al., 1981). However, one isolated case-study by Saetta et al. (1995) reports a fatal asthma attack following distilled water inhalation. In this case, a 22-year old man, who was reported to be taking regular treatment with inhaled steroids and B-

agonists to control his asthma, was referred for a distilled water inhalation challenge, as an alternative to a hypertonic saline bronchial challenge test. The dose or flow rate of distilled water inhalations was not reported in the study. The authors only reported that the patient was inhaling distilled water for one minute prior to his fatal asthma attack (Saetta et al., 1995). It is stated in the study that the patient's asthma was "well-controlled" (Saetta et al., 1995, p. 1285). However, evident oedema in the trachea and infiltration of mast cells and eosinophils in the peripheral airways observed during post mortem was suggestive of chronic inflammation (Murdoch & Lloyd, 2010), and potentially poorly controlled asthma. Although these findings have not been repeated in any additional studies in patients with asthma or healthy individuals, they highlight the importance of confirming the safety of distilled water inhalations.

7.1.7 Gaps in Knowledge

There are a number of gaps in knowledge that have potential safety implications in using inhalations of distilled water as part of a sensory stimulation protocol to enhance cough sensitivity. Firstly, the effect of distilled water inhalations over multiple days on the development of bronchoconstriction is not known. Longer exposures, or larger volumes of distilled water are known to increase risk of bronchoconstriction in patients with asthma (Chadha et al., 1984; Schoeffel et al., 1981). Thus, it is possible that repeated exposure to distilled water, over a number of days, may enhance the risk of developing bronchoconstriction.

Additionally, no previous studies have evaluated the effects of repeated, short inhalations of distilled water on the development of bronchoconstriction in healthy individuals. Repeated short inhalations of distilled water may activate different laryngeal afferents in the upper airway, than prolonged inhalations. Whether this method of inhalation would make individuals more or less susceptible to bronchoconstriction requires further investigation.

In previously published literature, prolonged (1-minute) inhalations of distilled water inhalations have been shown to *attenuate* the sensorimotor cough response (Morice et al., 1992). This is contrary to the objectives for patients with laryngeal sensory deficits and silent aspiration. Whether repeated, short inhalations of distilled water could enhance the sensorimotor cough response is not known. Given the risk of attenuating the sensorimotor cough response, the effects of a distilled water inhalation protocol on cough sensitivity should be first evaluated in healthy, non-dysphagic individuals.

The effects of different flow rates of distilled water on cough sensitivity modulation has never been evaluated. In the motor rehabilitation literature, different neuromuscular electrical stimulation frequencies have differential effects of either enhancing or inhibiting corticobulbar projections to muscles in the upper respiratory tract (e.g. muscles underlying the pharyngeal mucosa, faucial pillars, and submental muscles) (Doeltgen, Dalrymple-Alford, Ridding, & Huckabee, 2010; Fraser et al., 2002; Power et al., 2004). Thus, whether high versus low intensity distilled water inhalations have a differential effect on modulating cough sensitivity warrants investigation.

7.2 Study Aims and Hypothesis

7.2.1 Safety

The primary objective of this study was to evaluate the safety of a sensory stimulation protocol involving distilled water inhalations on pulmonary function in a cohort of healthy, non-asthmatic, adults. The percentage change in FEV₁ following distilled water inhalations was evaluated for each participant enrolled in the study. It was hypothesized that no participant would demonstrate bronchoconstriction (as defined by a > 20% fall in FEV₁ as measured on spirometry) in response to distilled water inhalations across four days.

7.2.2 Effects of Citric Acid Cough Thresholds

As a secondary objective, this study aimed to evaluate the effects of a high and low intensity distilled water inhalation protocol on citric acid cough thresholds, in a cohort of healthy adults, across days. Specifically, the study aimed to evaluate the effects of a high and low intensity distilled water inhalation protocol on the natural cough threshold (NCT) and the suppressed cough threshold (SCT) to citric acid, compared to control (0.9% saline inhalations). It was hypothesized that changes in NCT and SCT in the distilled water inhalation groups would differ to those in the control group. It was hypothesized that changes in the distilled water inhalation groups would be characterized by a decrease in citric acid cough thresholds, whereas changes in the control group would be characterized by an increase in citric acid cough thresholds.

As a further secondary objective, the study aimed to evaluate how potential changes in cough thresholds were reflected at a perceptual level. The study aimed to evaluate the effect of day and group on urge-to-cough (UTC) at NCT, SCT and subthreshold citric acid concentration (0.05 mol/L). It was hypothesized that changes in UTC in response to high and low intensity

distilled water inhalations would significantly differ to those in the control group receiving 0.9% saline inhalations.

7.3 Methods

This study was prospectively registered on the Australian New Zealand registry of clinical trials (ANZCRT) ON THE 28th October 2016 (Trial Id: ACTRN 12616001495415).

7.3.1 Participants

Twenty-eight healthy participants were recruited for the study. Participants were initially identified as healthy by self-report. A pre-participation medical questionnaire was then completed (Appendix 2). Participants were excluded from the study if they had a history of asthma (or any prior use of an inhaler), a clinically significant respiratory disease (e.g. COPD, cystic fibrosis, bronchitis) a history of neurogenic disorders, gastro-esophageal reflux, currently taking ACE inhibitor or codeine based drugs, current or prior smokers, individuals with a recent (< 2 weeks) upper respiratory tract infection (URTI), or abnormal baseline spirometry measures (based on the Global Lung Function Initiative (GLI) reference values) (Quanjer et al., 2013). Ethical approval was obtained from the New Zealand Health and Disability Ethics Committee (HDEC) (Reference number: 17/STH/2) and the local institutional review board. Informed, written consent was obtained from all participants, prior to the commencement of data collection.

7.3.2 Study Design

This study was a prospective quasi-randomized control trial. Participants were randomized into one of three groups, (1) high intensity distilled water inhalations, (2) low intensity distilled

water inhalations and (3) control (0.9% saline inhalation), using a computer-generated program (Urbaniak & Plous, 2013). Participants were blinded to their group assignment until after the treatment protocol. Each of the three groups underwent the same inhalation protocol (differing by the intensity and stimuli they were inhaling), completed over five days (Table 16). Citric acid cough threshold testing was performed at a research lab, and the distilled water inhalation protocols and spirometry were completed at a respiratory physiology laboratory at a local acute hospital. This was to ensure immediate access to trained respiratory physiologists and medical personnel in the event of any adverse respiratory events associated with the distilled water inhalations.

Table 16: Outline of the study design.

Day 1	Day 2	Day 3	Day 4	Day 5
	Baseline spirometry	Baseline spirometry	Baseline spirometry	Baseline spirometry
	Stimulation Protocol + Post-stimulation spirometry	Stimulation Protocol + Post-stimulation spirometry	Stimulation Protocol + Post-stimulation spirometry	Stimulation Protocol + Post-stimulation spirometry
Citric Acid CRT + UTC		Citric Acid CRT + UTC		Citric Acid CRT + UTC

7.3.3 Sensory Stimulation Protocol

Distilled water and saline aerosols were delivered using an ultrasonic nebulizer (Micro 801 U Suchatzki Ultrasonic Nebulizer) (see Figure 12). Distilled water inhalations were designed to

be below cough threshold to avoid participants expelling the aerosol during the sensory stimulation protocol. The high-intensity group received distilled water inhalations at the maximum flow rate of the nebulizer (1.6 ml/min). Based on prior research, this was not expected to induce coughing (Hegland et al., 2016; Lavorini et al., 2007). The low-intensity group received distilled water inhalations at the minimum flow rate of the nebulizer (0.5 ml/min). The control group received high-intensity 0.9% saline inhalations (1.6 ml/min). Saline was chosen as the control stimulus, as it does not evoke coughing or excite sensory receptors in the airway (Storey, 1968). The nebulizer outputs were confirmed prior to completing the study based on a weigh-reweigh method. An outline of the sensory stimulation protocol is provided in Figure 13. All groups underwent the same stimulation protocol, differing only by the solution, and flow rate of the inhalations. Participants completed a total of 300 inhalations of distilled water (at a flow rate of 1.6 or 0.5 ml/min) or saline (at a flow rate of 1.6 ml/min). Inhalations were divided into twelve cycles. Each cycle comprised twenty-five inhalations (5 inhalations, 10 seconds break, x 5). Spirometry was completed at baseline, and following every three cycles of distilled water inhalation. Participants received a one-minute break in between each cycle. The sensory stimulation protocol was designed based on previously described TISL protocols, which involved a high number of exposures to passive sensory stimuli, but incorporated short breaks into the stimulation cycles to avoid adaption and habituation to the stimulation (Godde et al., 1996; Godde et al., 2000; Pleger et al., 2001).

Figure 12: Instrumentation used for the distilled water sensory stimulation.

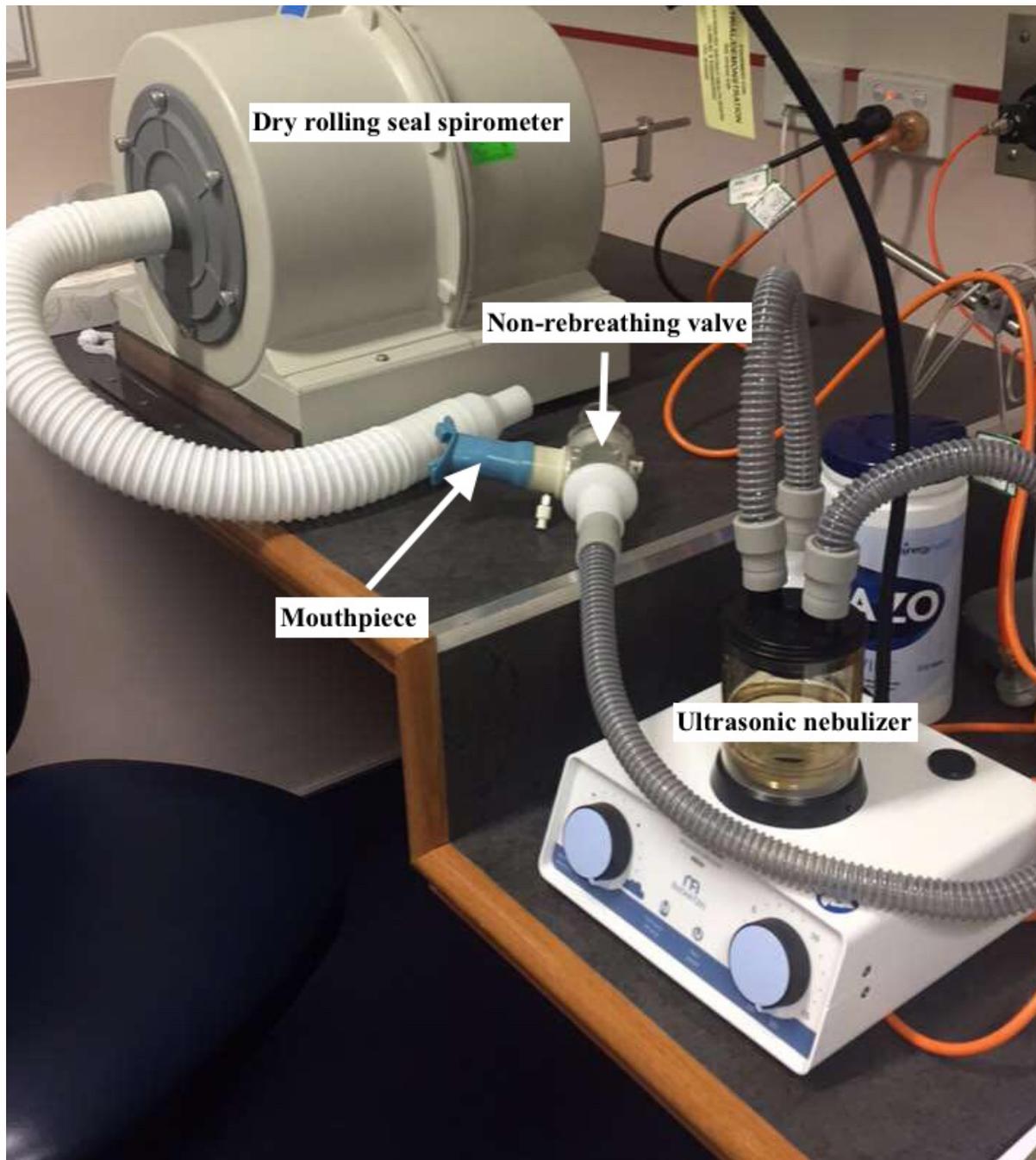
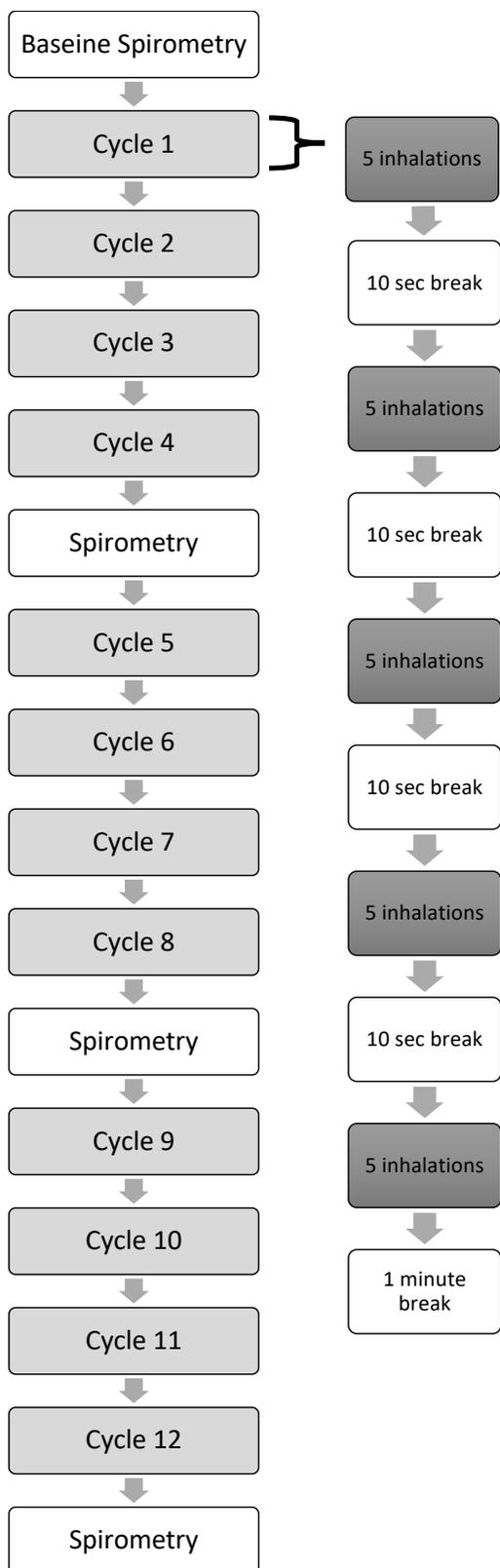


Figure 13: Outline of the sensory stimulation protocol



7.3.4 Outcome Measure 1 - Spirometry

Baseline spirometry was completed prior to the sensory stimulation protocol in all three groups, on days 2-5. All spirometry met the criteria for acceptable quality spirometry, as outlined by the ERS, and the American Thoracic Society (ATS) (Miller et al., 2005). On day two, baseline spirometry was compared to GLI reference values, which takes participants' weight, height, sex and ethnicity in account (Quanjer et al., 2012). Only participants with normal baseline spirometry (defined as > 80% of predicted values) were allowed to continue to the sensory stimulation protocol. Spirometry was performed throughout the sensory stimulation protocol, after every four stimulation cycles, to monitor for any signs of bronchoconstriction. Bronchoconstriction was defined as a > 20% fall in FEV₁, as per ERS guidelines for methacholine challenge testing (Coates et al., 2017). In the event any participant showed signs of bronchoconstriction, immediate treatment with a bronchodilator (i.e. 2 inhalations of salbutamol), under the guidance of a designated respiratory physician, was available for administration, with the a priori criteria to exclude any such participant from the remainder of the study. As per ERS recommendation for bronchial challenge testing, a bronchodilator and oxygen were available in the immediate vicinity (Joos & Connor, 2003). Medical help/resuscitation was available within two minutes, and an oximeter was available for monitoring oxygen saturation (Joos & Connor, 2003).

7.3.5 Outcome Measure 2 - Citric Acid Cough Threshold Testing

Citric acid cough threshold testing was completed as described in Chapter 6 (Section 6.2.4), using the same instrumentation. Citric acid was delivered using a compressed air-driven nebulizer (DeVilbiss 646; DeVilbiss Health Care), controlled by a breath-activated dosimeter (KoKo Digidoser, nSpire health Inc.), connected to a compressor (Pulmamate Compressor, model 46501). Compressed air was delivered at 40 psi, as measured on the dosimeter. The

nebulizer was modified with an inspiratory flow regulator valve (RIFR, nSpire health Inc.). Patients inhaled citric acid via a mouthpiece, wearing a nose clip. Citric acid, diluted in 0.9% sodium chloride, was prepared at 8 different concentrations: 0.01, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 mol/L. On each day, participants inhaled three successive 1.2 sec doses of each concentration of citric acid in ascending order until C2 cough thresholds (defined as two consecutive coughs within 3 seconds, on the same concentration of citric acid on two successive trials) were achieved. Participants were examined in a natural cough threshold (NCT) condition and a suppressed cough threshold (SCT) condition. In the NCT condition, participants were asked to “breathe in and out through your mouth and cough if you need to”. Once the NCT was reached, participants were told to “breathe in and out through your mouth and try not to cough” to evaluate their SCT. Instructions were provided prior to each citric acid presentation. In the SCT condition, the concentration of citric acid immediately below the NCT was used as the starting point. This was based on prior research showing the SCT is always higher than the NCT (Leow et al., 2012; Monroe et al., 2014), and therefore avoided unnecessarily extending the testing time. Saline placebos were randomly interspersed throughout the test to reduce the effects of participants anticipating progressively higher concentrations of citric acid (Morice et al., 2007).

7.3.6 Outcome Measure 3: Urge-to-cough Ratings

Following each citric acid inhalation, participants were asked to rate their urge to cough (UTC) according to methods reported by Hegland, Pitts, Bolser & Davenport (2012). For this, a modified Borg Scale from one to ten was used, with one representing no need to cough and ten representing a maximum urge to cough (Figure 17). No specific instructions were provided to participants on how to rate their UTC, but the scale was visually available throughout all

inhalations, with each number including a written description (e.g. 3 - slight to moderate urge to cough).

Table 17: Urge-to-Cough Scale (Hegland et al., 2011).

1	No need to cough
1.5	Just noticeable urge to cough
2	Slight urge to cough
3	Slight-to-moderate urge to cough
4	Moderate urge to cough
5	Moderate-to-strong urge to cough
6	Strong urge to cough
7	Strong-to-severe urge to cough
8	Severe urge to cough
9	Severe-to-maximum urge to cough
10	Maximum urge to cough

7.4 Data Analysis

7.4.1 Safety Data

FEV₁ was analysed for all participants throughout the sensory stimulation protocol, and dictated whether the stimulation protocol was continued or ceased. The maximum, range and mean change in FEV₁ across all participants and tests is reported below. Statistical analysis was not completed for the safety data as it was not intended to compare safety data across groups. Rather, safety data was evaluated on an individual basis for all participants.

7.4.2 Citric Acid Cough Thresholds and Urge-to-Cough

Citric acid cough thresholds were re-coded from 1-7, one representing 0.05 mol/L and seven representing 3.2 mol/L. No participants' cough threshold was 0.01 mol/L. Participants who did not respond at the highest concentration of citric acid were coded as missing data, so that variability was not skewed by assigning a false value. Linear mixed effects models were used to estimate the interaction effect between day and group on NCT and SCT. In the linear mixed-effects analysis, the interaction between day and group was entered as a fixed effect into the model and intercepts for each participant were included as a random effect. The inclusion of the interaction effect was firstly evaluated by comparing the model with and without the interaction effect using a likelihood ratio test. If the likelihood ratio test was significant (i.e. p -value < 0.05) the analysis was continued and the coefficient estimates, 95% confidence intervals and p -values of the model coefficients are reported. If the likelihood ratio test was non-significant (i.e. p -value > 0.05) no further analyses were completed.

For UTC data, linear mixed effects models were used to estimate the interaction effect between day and group on UTC at NCT, SCT and subthreshold citric acid concentration (i.e. 0.05 mol/L). The interaction between day and group was entered as a fixed effect into the model,

and intercepts for each participant were included as a random effect. As above, the inclusion of the interaction effect was firstly evaluated by comparing the model with and without the interaction effect using a likelihood ratio test. If the likelihood ratio test was significant (i.e. p -value <0.05) the analysis was continued and the coefficient estimates, 95% confidence intervals and p -values of the model coefficients are reported. If the likelihood ratio test was non-significant (i.e. p -value >0.05) no further analyses were completed. Data were analysed using R statistical package (R Core Team, 2017) and a linear mixed effects models statistical package, lme4 (Bates et al., 2015). A p -value of 0.05, or less, was considered statistically significant.

7.5 Results

7.5.1 Participants

A total of 28 healthy individuals (6 males, mean age, 30 years, range 19-74) were recruited for the study. Data from four participants were excluded. Three participants were excluded from the sensory stimulation protocol on day two, based on their baseline spirometry data. One participant (male, 24 years) was unable to perform acceptable quality spirometry, as per ERS, and ATS guidelines (Miller et al., 2005). Two participants had obstructive spirometric patterns at baseline (1 female, 22 years, 1 male, 24 years), and were excluded from continuing to the sensory stimulation protocol. These participants were offered a consultation by the respiratory physician on the research team, and were provided with a copy of their spirometry report for their GP.

One participant (female, 56 years) could not tolerate the high intensity distilled water inhalations without coughing. Despite continuous coughing, this participant showed no signs of bronchoconstriction when spirometry was completed after the first cycle of distilled water inhalations. As the sensory stimulation protocol was designed to be sub-threshold, it was decided to continue with distilled water inhalations inhalation at a lower flow rate that did not evoke coughing in the participant, with the primary aim of evaluating the safety of the distilled water inhalations. A flow rate of approximately 1 ml/min, based on a weight, re-weigh method, was provided. The participant demonstrated no signs of bronchoconstriction on spirometry throughout the distilled water inhalation protocol. The maximum change in FEV₁ for this participant was -6% across all tests (range: +4% to -6%). This participant's data was excluded from the citric acid cough threshold and UTC analysis, as the intensity of the inhalations was customized.

A total of 24 healthy individuals were included in the final analysis. A summary of the participants included in each group is provided below (Table 18).

Table 18: Participant demographics across groups

	High Intensity (n = 8)	Low Intensity (n = 8)	Control (n = 8)
Gender (females)	6	6	8
Mean Age (range)	23 (19 – 30)	38 (21 – 74)	28 (19 – 70)

7.5.2 Outcome Measure 1 – Spirometry

There were no adverse events during the study. All spirometry met acceptability and repeatability criteria as per ERS and ATS guidelines (Moore, 2012). All baseline spirometry was within normal limits, as per GLI reference standards, for all participants who received the sensory stimulation protocol (Quanjer et al., 2012). No participant demonstrated a > 20% fall in FEV₁ in response to distilled water inhalations. The maximum fall in FEV₁ in response to distilled water inhalation across all participants and days was 8%. Changes in FEV₁ across participants ranged from +4% to -8%. The mean change across all tests was -2%.

7.5.3 Outcome Measure 2 - Citric Acid Cough Thresholds

7.5.3.1 NCT

Descriptive statistics for NCT across days and groups is provided in Table 19. There was no significant interaction effect between day and group on NCT ($X^2(4) = 9.14, p = 0.058$).

Table 19: NCT (recoded) across the days and groups.

Group	Day	Mean NCT (recoded)	SD (recoded)	Range (mol/L)
Control Group	1	3.43	0.98	0.1 – 0.4
	3	3.63	1.41	0.1 – 0.8
	5	4.00	1.07	0.1 – 0.8
High Intensity	1	3.38	1.51	0.05 – 1.6
	3	2.88	1.13	0.1 – 0.8
	5	4.00	1.00	0.1 – 0.8
Low Intensity	1	3.86	1.46	0.1 – 1.6
	3	3.13	1.55	0.1 – 1.6
	5	3.83	1.17	0.2 – 0.8

7.5.3.2 SCT

Descriptive statistics for SCT across days and groups are provided in Table 20. There was an interaction effect between day and group on SCT ($X^2(4) = 11.32, p = 0.023$). At baseline, there was no significant difference in SCT between the control group and high intensity group ($p = 0.54$) and the control group and low intensity group ($p = 0.52$). There were no significant difference in SCT in the high intensity group, or low intensity group across days ($p > 0.05$). No significant difference in SCT was revealed in the control group between day 1 and day 3 ($p = 0.11$). In contrast, SCT was higher on day 5 compared to baseline in the control group (1.2, 95% CI [2.0, 0.4], $p = 0.008$). The estimated differences in the change in SCT between day 1 and 3 and 1 and 5, comparing high and low intensity groups to the control group, are provided in table 21. The change in SCT was different across days 1-3 and 1-5 in the high intensity group compared to the control group. SCT changed differently across days 1-5 in the low intensity group compared to the control group (Table 21 and Figure 14).

Table 20: Descriptive statistics of SCT (recoded) across the days and groups.

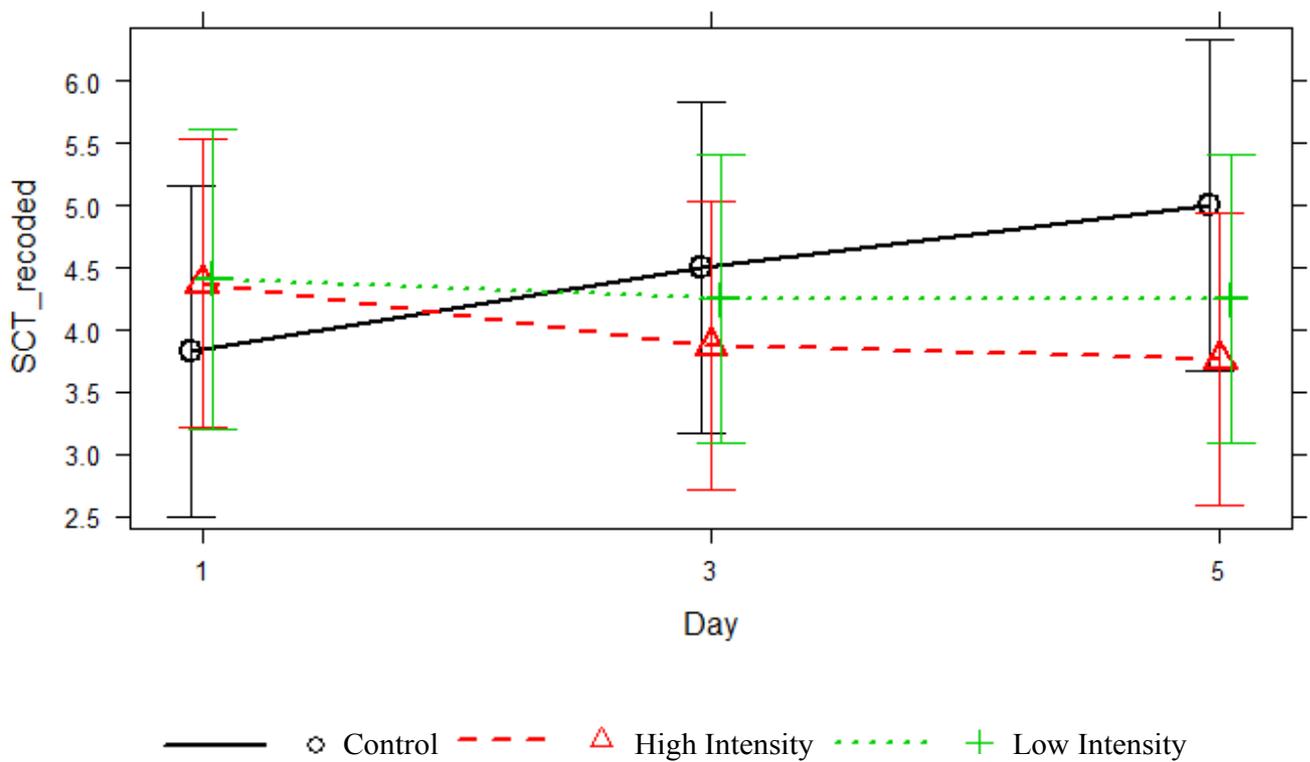
Group	Day	Mean SCT (recoded)	SD (recoded)	Range (mol/L)
Control Group	1	3.83	1.72	0.05 - 1.6
	3	4.50	1.38	0.2 - 1.6
	5	5.00	1.27	0.2 - 1.6
High Intensity	1	4.38	1.41	0.1 - 1.6
	3	3.88	1.25	0.1 - 1.6
	5	3.71	1.25	0.1 - 0.8
Low Intensity Group	1	3.67	1.63	0.1 - 1.6
	3	4.25	1.75	0.1 - 1.6
	5	4.25	2.19	0.05 - 3.2

Table 21: Estimated differences of the change in SCT for the high intensity and low intensity groups, compared to the control group.

Day	Group	Estimated differential change in SCT (doubling concentrations)	95% CI	<i>p</i> value
1-3	High vs. Control	-1.2	-2.26, -0.14	0.04*
1-5	High vs. Control	-1.8	-2.88, -0.72	0.01*
1-3	Low vs Control	-0.8	-1.98, 0.38	0.16
1-5	Low vs Control	-1.3	-2.4, -0.2	0.03*

* significant at $p = <0.05$

Figure 14: Estimated effects of day and group on SCT (error bars reflect the confidence intervals of the mean SCT for each group, across days).



7.5.4 Outcome Measure 3 – Urge to Cough

7.5.4.1 NCT

Descriptive statistics for UTC in the NCT condition across days and groups are provided in Table 22. There was no significant interaction effect between day and group on UTC in the NCT condition ($X^2(4) = 8.68, p = 0.07$).

Table 22: Descriptive statistics for UTC in the NCT condition across the days and groups.

Day	Group	Mean UTC	SD	Max UTC	Min UTC
1	Control	6.43	2.47	9	1.5
3	Control	6.63	1.60	9	5
5	Control	6.13	1.46	8	4
1	High	8.13	1.72	10	5
3	High	6.25	2.38	10	3
5	High	8.14	2.34	10	4
1	Low	6.63	2.93	10	3
3	Low	5.75	2.71	10	2
5	Low	6.13	2.10	9	3

7.5.4.2 SCT

Descriptive statistics for UTC in the SCT condition across days and groups are provided in Table 23. There was no significant interaction effect between day and group on UTC in the SCT condition ($X^2(4) = 3.57, p = 0.47$).

Table 23: Descriptive statistics for UTC in the SCT condition across the days and groups.

Day	Group	Mean UTC	SD	Max UTC	Min UTC
1	Control	6.83	1.72	9	4
3	Control	6.33	0.82	7	5
5	Control	7.33	1.37	9	6
1	High	8.38	1.72	10	2

3	High	7.38	2.45	10	3
5	High	7.86	2.80	10	3
1	Low	7.00	2.37	10	3
3	Low	7.25	2.49	10	3
5	Low	7.38	2.13	10	4

7.5.4.3 UTC at subthreshold (0.05 mol/L)

Descriptive statistics for UTC at subthreshold (0.05 mol/L) condition across days and groups are provided in Table 24. There was no significant interaction effect between day and group for UTC at 0.05 mol/L ($X^2(4) = 7.39, p = 0.117$).

Table 24: Descriptive statistics for UTC at 0.05 mol/L across the days and groups.

Day	Group	Mean UTC	SD	Max UTC	Min UTC
1	Control	2.4	1.4	5	1
3	Control	1.9	0.6	3	1
5	Control	1.7	0.6	3	1
1	High	4.5	2.8	8	1.5
3	High	3.4	2.5	9	1.5
5	High	3.6	2.8	7	1.7
1	Low	1.46	0.4	2	1
3	Low	3.0	3.0	10	1
5	Low	1.5	0.3	2	1

7.6 Discussion

In this study, the safety of a proposed sensory stimulation protocol to modulate citric acid cough thresholds was evaluated in healthy individuals. The results of the study revealed that distilled water inhalations did not induce bronchoconstriction (defined as a $> 20\%$ fall in FEV₁) in any participant enrolled in the study across the five days. These data suggest that the distilled water inhalation protocol utilized in this study is safe in healthy, non-asthmatic adults.

The results also revealed that SCTs changed differently in the distilled water inhalation groups, compared to the control group. In evaluating the changes in SCT in the stimulation and control groups, it is apparent that while SCTs increased across days in the control group, SCTs did not change across days in the high or low intensity distilled water inhalation groups. It is important to reflect on the variability of CRT as an outcome measure, when considering the implications of this result. Chapter 6 demonstrated that citric acid cough thresholds significantly increased across three alternate days in healthy individuals in the absence of sensory stimulation, resembling a habituation effect to repeated exposure to CRT. In light of this, the current data suggests that the observed increase in SCT across days in the control group may be attributed to the artefact of repeating the citric acid CRT. In contrast, the lack of change in SCT in the high and low intensity distilled water inhalations suggest that the distilled water inhalation protocol may inhibit the expected increase in citric acid cough thresholds associated with this habituation. It is therefore possible that cough sensitivity is enhanced by the distilled water inhalation protocols, but this effect is masked, or diminished, by the use of citric acid CRT as an outcome measure. This is a notable limitation of using citric acid CRT as an outcome measure to observe for decreases in cough sensitivity.

There are few alternative valid methods for evaluating cough or laryngeal sensitivity. One possibility is the use of laryngeal air puff stimulation, which has been used in a recent study to detect cough thresholds (i.e. the minimum air pulse intensity that elicited a cough response) in patients with and without dysphagia (Giraldo-Cadavid et al., 2017). The instrumentation included an endoscopic laser range finder, to overcome some of the known limitations in the reliability of laryngeal air puff stimulation that arise from inconsistencies in the distance and angle of the tip of the endoscope in the upper airway across tests (Cunningham, Halum, Butler, & Postma, 2007; Giraldo-Cadavid et al., 2017). The authors reported excellent intra- and interrater agreement when cough thresholds were determined eight times by an experienced and novice clinician (i.e. four times each), during the same endoscopic procedure. However, quantification of test-retest variability of air puff cough thresholds across repeated tests is not reported in the study. It is possible that individuals would also habituate to air puff stimulation following repeated tests, similar to citric acid cough threshold testing.

Interestingly, SCTs in the control group increased by an estimated 1.2 doubling concentrations on day five compared to baseline. This is almost double the estimated increase in SCT across three alternate days with no intermittent sensory stimulation, as reported in Chapter 6 (i.e. 0.32 doubling concentrations per day, $\times 2$ days = 0.64 doubling concentrations). This suggests that while habituation to CRT may somewhat explain the increase in thresholds seen in the control group, it is likely that saline inhalations enhanced the effect of increased thresholds across days. Saline does not evoke cough or activate airway afferents (Storey, 1968). However, it is possible that a tactile stimulation effect from repeated inhalations of an aerosol contributed to the observed increase in citric acid cough thresholds. These findings raise the question of whether saline inhalations may be beneficial for patients with cough hypersensitivity, where it would be advantageous to increase their cough sensitivity. However, as this study has identified this

effect in only a small number of healthy individuals, more thorough investigations in patient populations are required prior to any clinical conclusions being drawn about saline stimulation for hypersensitive airway afferents.

It is possible that the high intensity distilled water inhalation protocol used in the current study was not of adequate intensity to induce a significant reduction in SCT across days. A sub-threshold flow rate was chosen to avoid excessive coughing, which would result in expelling the aerosol during the sensory stimulation protocol, and possibly contribute to lowered participant tolerance. As this is the first study to use distilled water in an attempt to modulate sensitivity of airway afferents, the protocol parameters were the first to be investigated for such purposes. It is possible that alternative durations or number of inhalation cycles could increase the differences between the groups. It is also important to acknowledge that the distilled water inhalation protocol was not tailored to the sensory threshold of each individual. The results of the current study, and previous studies in the literature, suggest that cough thresholds differ widely across individuals (Monroe et al., 2014; Morice et al., 2007). As a result, the high and low intensity distilled water inhalation protocols may have represented different intensities for different participants. An individual with a low cough threshold may have experienced more laryngeal irritation and urge to cough during the distilled water inhalations than that of an individual with a high cough threshold. This may have contributed to different magnitudes of change in SCT across days, as demonstrated by the wide confidence intervals in the estimates of change in SCT across days and groups (Table 20).

Sensory stimulation of the hand in sub-acute and chronic stroke patients was administered at an intensity that was individualized for each patient (Kattenstroth et al., 2012). This was found to enhance tactile tasks, sensorimotor behaviours, functional tasks and cortical activity from

baseline (Kattenstroth et al., 2012). Originally, it was intended to determine participants' distilled water cough threshold - defined in the literature as the flow rate of distilled water evoking a cough response during two distinct cough challenges separated by 30 minutes (Fontana et al., 1998; Fontana et al., 1997; Fontana et al., 1999) - and administer distilled water at flow rates of minus one increment (high intensity stimulation) and minus three increments (low intensity stimulation) below that threshold. However, it was anticipated that this may cause problems for potential future application of the distilled water inhalation protocol for patients with silent aspiration, where baseline cough thresholds may be impossible to determine due to laryngeal sensory deficits. Monitoring urge to cough during the distilled water inhalation protocol in future studies may provide an opportunity to gauge, and regulate, the intensity of the distilled water inhalations across participants, although this measure was characterised by large variation across participants in Chapter 6.

It is of interest that differential changes in cough thresholds between the stimulation groups and the control group was only identified in the SCT condition, and not in the NCT condition. There are a number of possible explanations for this observation. Brain imaging studies in humans have demonstrated distinct central neural networks involved in natural and suppressed coughing to capsaicin (Mazzone et al., 2011). Although capsaicin has distinct neural networks to citric acid, these data imply that NCT and SCT conditions may reflect distinct cough types and neural pathways. The current results, and those of previous studies (Monroe et al., 2014) highlight that SCTs are elicited at higher citric acid concentrations than NCTs. Higher concentrations of citric acid may evoke rapid reduction of pH in the respiratory tract. This has been shown to evoke action potential discharge in nodose A- δ fibres (Kollarik & Undem, 2002), which mediate coughing through viscerosensory cough pathways in animal models (McGovern, Davis-Poynter, et al., 2015; McGovern, Driessen, et al., 2015). Distilled water is

known to activate nodose A- δ fibres (Canning et al., 2004; Mazzone & Udem, 2016). Thus, it is possible that the distilled water inhalation protocol has a greater effect on the suppressed cough pathway.

It also bears mentioning that in the NCT condition, mean NCTs in the high and low intensity groups non-significantly *decreased* on day 5 compared to baseline, and mean NCTs in the control group non-significantly *increased* on day 5 compared to baseline. Thus, NCTs were observed to change differently in the stimulation groups compared to the control group. This differential change did not reach statistical significance (although it approached significance, $p = 0.058$). It is possible that the sample size in the current study was too low to accurately characterize this effect. Given that this is the first study to evaluate the effects of the distilled water inhalation protocol on citric acid cough thresholds, further studies, with large sample sizes, are necessary to validate or refute these findings.

7.6.1 Implications for rehabilitation of silent aspiration

The finding that the distilled water inhalation protocol was safe in healthy adults provides the basis to evaluate the safety and potential efficacy of the protocol in patients with laryngeal sensory deficits following neurological injury. The extent to which these findings would be replicated in patients with silent aspiration is unknown. Patients with silent aspiration secondary to stroke would differ to those in the current study. They would be expected to have relatively high baseline citric acid cough thresholds, given that an absent C₂ cough response to 0.6 mol/L citric acid had high sensitivity and specificity for detecting silent aspirators using a 15 second tidal-breathing method (Miles, Moore, et al., 2013). In the limb literature, Pleger and colleagues (2003) report that the largest cortical reorganization and sensory enhancement following TISL was observed in healthy individuals with the lowest sensory acuity threshold

(i.e. better sensory discrimination abilities) (Pleger et al. 2001). This suggests that those with better sensory acuity at baseline yield the greatest benefit from TISL. This questions whether the observed inhibition of habituation to CRT in the current study is limited to healthy individuals with adequate sensory perception. However, using a similar, but more intense (i.e. 45 minutes per day for two weeks) stimulation protocol for patients with reduced sensation in the hand following cortical stroke, Kattenstroth and colleagues (2018) reported greatest enhancement of sensorimotor performances in patients with more severe sensory deficits. These data imply that patients with reduced cough sensitivity may require a longer, more intense sensory stimulation protocol than that used in the current study. Further research is required to explore these hypotheses. Longer (1-minute) inhalations of distilled water have been shown to decrease cough sensitivity, as demonstrated by a significant reduction in cough frequency over the one-minute inhalation (Morice et al., 1992). This suggests that different distilled water stimulation parameters may have different effects on cough sensitivity, with longer inhalations potentially decreasing cough sensitivity. This should be carefully considered before altering the current stimulation parameters for patients with silent aspiration.

A greater estimated differential change in SCT across the five days was demonstrated for the high intensity group versus the low intensity group, when compared to the control group (i.e. -1.8, [95% CI, -2.88, -0.72] versus -1.3, [95% CI, -2.88, -0.2] respectively). This suggests that a greater flow rate and output of distilled water may be more effective at changing SCTs compared to the control group, across days. This aligns with recommendations of “high frequency” and “heavy schedules” of sensory stimulation as part of a TISL protocol (Dinse & Tegenthoff, 2015, p. 18), as well as the motor learning principal of “intensity matters” (Kleim & Jones, 2008, p. 227). This observation suggests that the high intensity distilled water inhalation protocol may be the most appropriate sensory stimulation protocol to evaluate in

further studies for patients with laryngeal sensory deficits and subsequent impairment of airway clearance mechanisms.

7.6.2 Limitations

It is acknowledged that the findings of the current study must be interpreted with caution due to the small sample size. Furthermore, the participants recruited in the current study were predominantly female. Females are known to be at higher risk of chronic cough than males (Kavalcikova-Bogdanova, Buday, Plevkova, & Song, 2016), which raises the question of whether females are more susceptible to cough sensitivity modulation. It is possible that a predominantly male cohort may be less likely to demonstrate the observed differential changes in cough sensitivity across days. Additionally, the age range in the high intensity groups was less than that in the low intensity and control groups. Age is known to have implications on SCTs, with younger individuals demonstrating a greater capacity for suppression (i.e. higher SCTs) than older individuals (Leow et al., 2012; Monroe et al., 2014). However, the mean age across the groups was similar, and baseline citric acid cough thresholds were not significantly different across groups.

There was no assessment of maintenance of effects in the current study design. Thus, it remains to be seen whether the differential changes in SCT between the groups would be maintained over time. The dynamic nature of cough sensitivity has been demonstrated in previous studies (Dicpinigaitis et al., 2006; Morice et al., 1992). For example, tachyphylaxis resolves 3.5 hours following exposure to tussigenic aerosols (Morice et al., 1992). Therefore, it is possible that the effects of the sensory stimulation protocol may be short-lived. Citric acid cough reflex testing was completed within one hour of the sensory stimulation protocol on days 3 and five.

The longer-term effects of the sensory stimulation protocol should be explored in future research.

It is also important to acknowledge that patients with respiratory diseases and asthma were excluded from the current investigation. However, there is a high prevalence of respiratory diseases, such as COPD and asthma in patients with stroke (Lekoubou & Ovbiagele, 2016; Wen et al., 2016), who are at high risk of laryngeal sensory deficits. If this sensory stimulation protocol is to be evaluated in patients with laryngeal sensory deficits following neurological injury, these patients should be excluded, or carefully monitored for risk of bronchoconstriction. Previous studies have demonstrated that the underlying mechanisms of coughing and bronchoconstriction induced by distilled water are distinct (Eschenbacher et al., 1984; Lavorini et al., 2001; Sheppard, Rizk, Boushey, & Bethel, 1983). It was hypothesized that pre-treatment with a bronchodilator may mitigate risk of bronchoconstriction in individuals with respiratory diseases, while enabling them to derive potential benefits from the sensory stimulation. However, further studies may be necessary to determine the efficacy of the sensory stimulation protocol in patients with laryngeal sensory deficits *without* respiratory diseases, before warranting the risk associated with evaluating this hypothesis in patients with respiratory diseases.

In conclusion, this study evaluates the safety and efficacy of a novel sensory stimulation protocol, that aimed to enhance cough sensitivity in a cohort of healthy adults. Results indicate that the sensory stimulation protocol is safe, evidenced by no signs of bronchoconstriction following distilled water inhalations in any participant. Secondly, evidence of no change in citric acid cough thresholds in the high and low intensity groups suggests that the sensory stimulation protocol mitigates the habituation effect of repeating the citric acid CRT across

alternate days, implying a sensitization effect. These data provide the basis to evaluate the safety and efficacy of the sensory stimulation protocol in patients with laryngeal sensory deficits and silent aspiration.

SECTION IV. COUGH STRENGTH STUDIES

CHAPTER 8: Introduction to Cough Strength Studies

8.1 What is cough strength?

Coughing is considered as the mechanism by which the airways are cleared of secretions and/or foreign materials (Gauld, 2009; Laciuga, Brandimore, Troche, & Hegland, 2016; Watts et al., 2016). A ‘strong cough’ may be considered as one which *effectively* expels secretions and/or foreign materials from the airway, while a ‘weak cough’ may be considered as one which *ineffectively* expels secretions and/or foreign materials from the airway. Assessment of “cough strength” is challenging in dysphagia research and clinical practice. There is lack of agreement on what constitutes “cough strength” and the measurement that best reflects this. For example, a “strong cough” has been defined as one with a peak flow of > 60 L/min, and has been shown to predict successful extubation in patients with tracheostomy (Salam, Tilluckdharry, Amoateng-Adjepong, & Manthous, 2004; Smina et al., 2003). A “strong cough” has also been defined as a cough volume acceleration of > 46 L/s and has been related to lower penetration-aspiration scale scores (PASS) in patients with dysphagia (Plowman et al., 2016; Smith Hammond et al., 2009). Others report a “strong cough” as voluntary cough peak flow of > 242 L/min and have linked this to lower risk of developing aspiration pneumonia following stroke (Bianchi et al., 2012). This highlights the arbitrary nature of cough strength and the range of contexts and methods in which it can be measured. Objective measurement of “cough strength” and its relationship to efficacy of *clearance* of penetration and/or aspiration has never been reported in previously published literature. This gap in the literature forms the basis of the current research, which attempts to validate the use of acoustic intensity as an objective measure of cough strength in patients with dysphagia.

8.2 Evaluation of Cough Strength

There are numerous ways to evaluate effective/ineffective clearance of penetration and aspiration from the airway. Subjective evaluation of voluntary and/or reflexive coughing is often used by clinicians to guide clinical decision making during the clinical swallowing evaluation (CSE) (Laciuga et al., 2016; Mann, 2002; McCullough & Martino, 2013; Smith Hammond, 2008; Watts et al., 2016). Subjective evaluation of cough strength is appealing in the clinical setting due to its ease of use. However, numerous studies dispute its reliability (Laciuga et al., 2016; Miles & Huckabee, 2013; Miles, McFarlane, & Huckabee, 2014). Laciuga and colleagues (2016) found lack of agreement among clinicians (i.e. speech-language therapists, otolaryngologists and neurologists) on ratings of perceived voluntary cough strength (i.e. strong versus weak) in healthy individuals. In addition, coughs rated as ‘strong’ and ‘weak’ represented a wide range of aerodynamic measures of coughing (i.e. peak cough flow, expiratory phase rise time, cough volume acceleration and compression phase duration) (Laciuga et al., 2016). This highlights lack of agreement between subjective ratings and objective measures of cough motor output. The nature of this lack of agreement is attributed to lack of training and uniform terminology by the authors (Laciuga et al., 2016). Clinicians were not provided with a definition of cough strength, or criteria on which to make judgements of strong or weak coughing. It’s also important to note that the cough epochs evaluated by clinicians in this study were produced by healthy individuals mimicking different types of coughing. Thus, it’s unknown how these results translate to reliability in cough ratings from patient populations.

Similar to these findings, Miles and colleagues (2013) found fair-to-moderate agreement among speech-language therapists’ ratings of perceived strength of citric acid induced coughing. In this case, coughing was elicited from patients with dysphagia of heterogeneous

aetiology, as well as two non-dysphagic patients. This ensured that cough samples were representative of a typical patient population that clinicians may evaluate during CSE. Clinicians were given no specific definition of cough strength, and were asked to rate coughs as “strong”, “weak” or “absent”. Qualitative analysis of discussions between clinicians during the study revealed confusion regarding the definitions of a cough and cough strength. This suggests that lack of training and uniform terminology may explain the nature of this poor agreement, as hypothesized by Laciuga and colleagues (2016). In a follow up study, clinicians were given two hours of training on cough physiology and cough strength judgement (Miles et al., 2014). As part of this training, clinicians observed videotaped examples of “strong”, “weak” and absent coughs during citric acid CRT. A weak cough was defined by the authors as “a cough that does not appear strong enough to clear aspiration and is substantially weaker than your own reflexive cough” (Miles et al., 2014, p. 205). However, perceptual characteristics of coughing to aid this judgement were not provided by the authors. Regardless of training, no improvement in inter-rater agreement of cough strength was found, even among experienced clinicians, and qualitative comments still reflected lack of certainty in ratings of perceived cough strength. These data suggest that subjective evaluation is not a reliable tool for assessing cough strength in patients with dysphagia, regardless of the amount of training received by raters.

More recently, attention has shifted to *objective* evaluation of voluntary and reflexive coughing, to overcome the limitations of subjective evaluation (Watts et al., 2016). Numerous assessment tools are available for objective measurement of different components of cough motor output. These include aerodynamic measures, respiratory muscle activity, intra-abdominal pressure, and acoustic intensity. The advantages, disadvantages and clinical applicability of these measures are discussed below.

8.2.1 Aerodynamic Measures

Aerodynamic measures are the most commonly evaluated parameters of cough motor output in patients with dysphagia. They have been shown to predict risk of aspiration and aspiration pneumonia in patients with dysphagia with high sensitivity and specificity (Pitts et al., 2008; Pitts et al., 2010; Plowman et al., 2016; Silverman et al., 2016; Smith Hammond et al., 2009). Smith Hammond and colleagues (2009) were among the first to report cut-off values for voluntary coughing for differentiating patients with dysphagia at high risk of aspiration (PAS of > 5) versus no aspiration (PAS of < 4) on instrumental assessment following stroke. Cut-off values of < 2.9 L/s for peak cough flow (PCF), > 65 ms for expulsive phase rise time (EPRT) and < 50 L/s for cough volume acceleration (CVA), had excellent sensitivity (i.e. 91%, 91% and 82% respectively) and specificity (81%, 92% and 83% respectively) for predicting risk of aspiration on instrumental assessment. The PCF (also referred to as the peak expiratory flow rate) is the peak airflow achieved during the expiratory phase of cough (Plowman et al., 2016; Tabor-Gray, Gallestaggi, Vasilopoulos, & Plowman, 2019). EPRT refers to the duration from the beginning of expiration to the greatest peak of the expiratory phase (Plowman et al., 2016; Tabor-Gray et al., 2019). CVA is calculated by dividing PCF by EPRT, and provides an index of the volume and speed of air expelled from the lungs (Plowman et al., 2016; Tabor-Gray et al., 2019).

Similar findings are reported in patients with progressive neurological disorders. Aerodynamic measures of voluntary coughing were sensitive to the presence or absence of aspiration in patients with Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) (Pitts et al., 2008; Pitts et al., 2010; Plowman et al., 2016). A summary of these cut-off values are outlined in Table 25. One of the notable limitations of these studies is the cut-off values that are used.

Reducing the PAS to a binary variable has limitations, as a higher score is not always indicative of greater clinical risk (Steele & Grace-Martin, 2017). For example, a patient who penetrates and does not eject the material from the laryngeal vestibule (i.e. PASS = 3) may be at greater risk of aspiration pneumonia than a patient who aspirates, but ejects the aspiration from the laryngeal vestibule (i.e. PASS = 6). Furthermore, the cut-off values in the above studies are only based on depth of airway invasion, but offer little insight into patients' ability to effectively expel aspirate from the airway. This is an important differentiation in the management of these patients in clinical practice. It is also important to acknowledge that evaluating voluntary coughing provides limited insight into the sensorimotor cough response to aspiration (Widdicombe et al., 2011). As a result, caution is warranted in drawing conclusions about airway protective mechanisms during swallowing from evaluation of voluntary coughing.

Table 25: Summary of cut-off values of aerodynamic measures of voluntary coughing that predict aspiration in patients with dysphagia.

Study	Population	PASS groups	PCF (L/s)	EPRT (ms)	CVA L/s/s
Smith-Hammond (2009)	Stroke	PASS < 4 versus PASS > 5	< 2.9	> 65	< 50
Pitts et al. (2010)	Parkinson's Disease	PASS 1-5 versus PASS 6-8	< 5.24	NA	NA
Pitts et al. (2010)	Parkinson's Disease	PASS 1 versus PASS 2-8	< 7.49	NA	NA
Plowman et al (2016)	Amyotrophic Lateral Sclerosis	PASS 1-2 versus PASS 3-8	< 3.97	> 75	< 45.28

PASS = Penetration- aspiration scale score. NA = not evaluated in the study. PASS = penetration-aspiration scale score.

Other authors have evaluated the relationship between aerodynamic measures of coughing and risk of pulmonary morbidity. Bianchi et al (2012) measured the PCF of voluntary coughing in patients with dysphagia, all of whom had confirmed aspiration on VFSS. PCF was significantly lower in patients who developed aspiration pneumonia versus those who did not (3.37 (1.12) L/s versus 5.06 (1.35) L/s). Based on these findings, a cut-off value of 4 L/s (242 L/min) for voluntary PCF had high sensitivity and specificity (77% and 83% respectively) for predicting pulmonary morbidity (Bianchi et al., 2012). More recently, a cut-off value of PCF for citric acid induced coughing to predict aspiration pneumonia was evaluated in a cohort of patients with neurological dysphagia (Sohn et al., 2018). A PCF of <0.98 L/s had high sensitivity and specificity (81% and 83% respectively) for predicting risk of aspiration pneumonia. Interestingly, the lower PCF for citric acid induced coughing, compared to voluntary coughing provides an example of the limitations in evaluating voluntary coughing to draw conclusions about the sensorimotor cough response. These data also raise a number of questions – do patients with higher PCF more effectively expel aspirate from the laryngeal vestibule, mitigating their risk of aspiration pneumonia? Or does a higher PCF represent better overall pulmonary health, which mitigates the risk of aspiration pneumonia? Bianchi et al. (2012) note that silent aspiration was more prevalent in patients who developed aspiration pneumonia than those who did not (i.e. 39% versus 19% respectively), suggesting that the absence of a sensorimotor cough response to airway invasion is a risk factor for aspiration pneumonia. However, no comment is made on the *effectiveness* of coughing in clearing airway invasion in patients with and without aspiration pneumonia. Thus, it's difficult to directly determine whether effective clearance of aspirate from the airway is a protective factor against risk of aspiration pneumonia – although, it seems likely that it would be.

A number of tools can be used evaluate aerodynamic measures of cough motor output. Different tools are used across the aforementioned studies, and are outlined in Table 26. Pneumotachographs are highly sensitive and accurate instruments for measuring air flow and volume of cough motor output (Mandal, 2006). They have the advantage of being able to evaluate numerous aerodynamic measures of coughing simultaneously (see Figure 15) (Mandal, 2006; Plowman et al., 2016). This may facilitate differential diagnosis of the nature of weak coughing. For example, an impaired compression phase may signal poor glottic closure (Britton et al., 2014; Tabor-Gray et al., 2019). While impaired peak cough flow may indicate weak expiratory musculature (Plowman et al., 2016; Tabor-Gray et al., 2019).

However, pneumotachographs are expensive, not easily transportable and require training by the user for correct operation (Kulnik et al., 2015; Plowman et al., 2016). This renders them inaccessible to many clinicians, and unsuitable for measuring cough strength at bedside. Portable spirometers and peak flow meters are less expensive and more conveniently applied in the clinical setting. These devices have been used to measure the PCF of voluntary (Kimura et al., 2013; Kulnik et al., 2016; Silverman et al., 2014) and/or induced coughing (Fujiwara et al., 2017; Lee et al., 2013; Sohn et al., 2018). However, poor inter-instrument reliability and accuracy of these devices, compared to laboratory based pneumotachographs, have been demonstrated by a number of studies (Brouwer, Roorda, & Brand, 2007; Kulnik et al., 2015; Miller, Dickinson, & Hitchings, 1992; Takara et al., 2010). For example, significant differences in PCFs across different peak flow meters and portable spirometers have been demonstrated (Takara et al., 2010). This would preclude the use of cut-off values of PCF to identify patients at risk aspiration pneumonia or ineffective clearance of aspirate, due to variability across instrumentation. Coughs with lower PCFs (i.e. between 0.97-3.95 L/s, as measured on the pneumotachograph) were not always registered on portable peak flow meters and spirometers

(Kulnik et al., 2015). This may be problematic for measuring coughing in patients with dysphagia, who are likely to have a lower PCFs (Bianchi et al., 2012; Plowman et al., 2016; Sohn et al., 2018). In contrast to these findings, Silverman and colleagues reported concordance between digital and analog peak airflow meters and pneumotachograph measures of peak cough airflow in healthy individuals and patients with PD. However, correlation analysis of peak cough flow measurement between age and gender within groups revealed poor correlation in certain groups, such as healthy younger (60-70 years) and older (71-80 years) females ($r = 0.60, 0.56$ respectively), and healthy younger males ($r = 0.64$). Interestingly, correlation between cough peak flow measurement was higher in patients with PD compared to healthy individuals.

Of relevance to the current research, it is important to note that aerodynamic evaluation of coughing requires the use of a facemask or mouthpiece. This would preclude evaluation of effective/ineffective clearance of aspiration by coughing during eating and drinking. Fontana and colleagues (1997) also highlight that for (citric acid or capsaicin) induced coughing, having a pneumotachograph and respiratory valve between the nebulizer and the mouthpiece may affect the particle size and or output of the tussigenic aerosol. The complexity of this instrumentation is also likely to be prohibitive to clinical application.

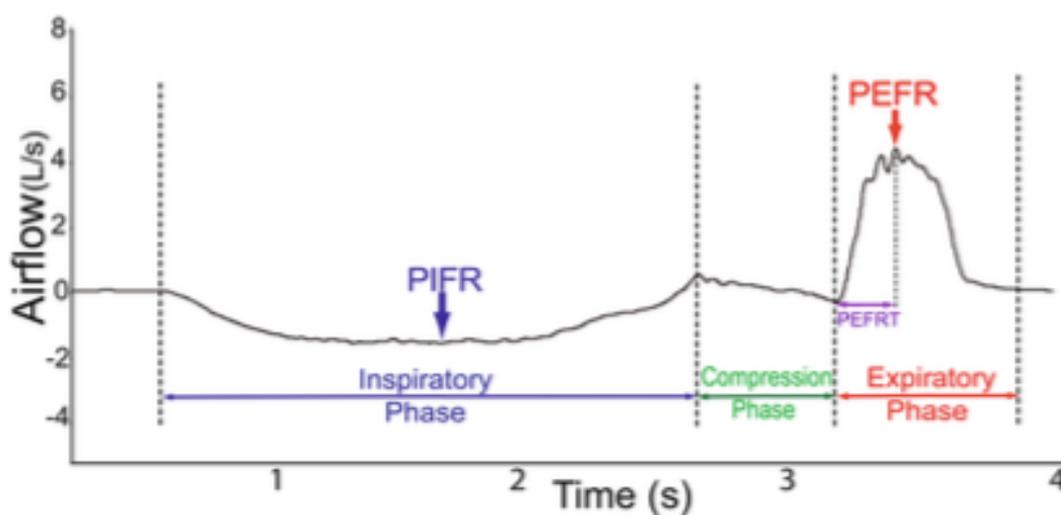
Table 26: Instrumentation used to measure voluntary and reflexive cough strength in patients with dysphagia.

Study	Cough Type	Instrumentation
Hegland et al (2014)	RC	Pneumotachograph
Troche et al (2014)	VC	Pneumotachograph
Troche et al., (2016)	RC	Pneumotachograph

Pitts et al. (2008)	VC	Pneumotachograph
Pitts et al. (2010)	VC	Pneumotachograph
Smith-Hammond et al. (2001)	VC	Pneumotachograph
Smith-Hammond et al. (2009)	VC	Pneumotachograph
Kulnik et al. (2016)	VC & RC	Pneumotachograph
Plowman et al. (2016)	VC	Pneumotachograph
Fujiwrara et al. (2016)	RC	Portable Spirometer
Kimura et al. (2013)	VC	Portable Spirometer
Silverman et al. (2014)	VC	Peak flow meter & pneumotachograph
Silverman et al. (2016)	VC	Peak flow meter
Bianchi et al. (2012)	VC	Peak flow meter.
Sohn et al. (2018)	VC & RC	Peak flow meter.
Hutcheson et al (2018)	VC	Peak flow meter

VC = Voluntary coughing, RC = Reflexive coughing

Figure 15: Schematic of a voluntary cough spirometry waveform depicting the range of aerodynamic measures of cough motor output. From “Voluntary Cough Airflow Differentiates Safe Versus Unsafe Swallowing in Amyotrophic Lateral Sclerosis”, Plowman et al. (2016), *Dysphagia*, 31(3), p. 387.



PEFR (PCF) = peak expiratory flow rate (peak cough flow). PIFR = peak inspiratory flow rate. PEFRT = peak expiratory flow rise time. CVA = =PEFR/PEFRT.

8.2.2 Respiratory Muscle Activation

Respiratory muscle activity (as measured by surface electromyography of abdominal/respiratory muscles) during voluntary and reflexive coughing (Fontana et al., 1997; Vovk et al., 2007) has also been evaluated as an objective measure of cough strength. Fontana et al. (1997) used surface electromyography (EMG) of abdominal muscles (the obliquus external muscles and the transversus muscles) as a measure of cough intensity in response to ultrasonically nebulised distilled water (UNDW) in healthy individuals. Cough intensity (as measured by EMG of the abdominal muscles) was highly repeatable at each individuals' cough threshold and increased with inhalation of progressively higher ultrasonically nebulised distilled water flow rates, suggesting a dose-response relationship (Fontana et al., 1997). Intra-abdominal muscle EMG has also been used to evaluate cough strength in patients with PD compared to healthy controls (Fontana et al., 1998). Patients with PD had lower intra-abdominal muscle EMG compared to aged-matched healthy controls during volitional and UNDW induced coughing (Fontana et al., 1998). The authors attribute this observation to impaired central neural mechanisms involved in the recruitment of motor units during coughing in patient with PD (Fontana et al., 1998). It is also possible that this weakness may be attributed to the peripheral changes in muscles and nerves. Muscle biopsies from patients with PD demonstrated changes (i.e. atrophy and hypertrophy) in the muscle fibres in the limbs, resulting in weakness and spasticity (Edstrom, 1970; Rossi et al., 1996). This may explain observations of reduced intra-abdominal muscle EMG (Fontana et al., 1998). However, studies demonstrate that respiratory muscle strength is improved (determined by increased maximum inspiratory and expiratory flow-volume curves) following antiparkinsonian meditations (apomorphine)

which acts centrally (de Bruin, de Bruin, Lees, & Pride, 1993), suggesting that respiratory muscle weakness is likely of central origin.

Vovk et al. (2007) evaluated respiratory muscle activation (as measured by EMG of the rectus abdominis, external abdominals obliquus and the 8th intercostal space) as a measure of the intensity of the LER and induced coughing to capsaicin. The LER and induced coughing were elicited simultaneously (via single inhalations of capsaicin), but were differentiated using the airflow tracing by presence or absence of a preceding inspiratory phase (Vovk et al., 2007). Similar to Fontana et al. (1997), a dose-response trend between respiratory muscle EMG and concentration of capsaicin was found (Vovk et al., 2007). The authors also note that EMG activation decreased with each successive expulsive event in the cough epoch (Vovk et al., 2007), suggesting a decline in ‘cough strength’ with each successive cough.

Abdominal and respiratory muscle EMG may be a valuable tool for measuring cough strength in patient populations, as it mitigates the need for a facemask or mouthpiece, enabling coughing during eating and drinking to be evaluated. However, numerous limitations are associated with measuring surface EMG of respiratory and abdominal muscles in a clinical setting. Lack of consistency in the placement of the electrodes across and within participants can account for large intra- and inter- participant variability (Vovk et al., 2007). For example, placing EMG electrodes in slightly different positions can affect the signal amplitude, due to variation in abdominal fat content, skin resistance and muscle size (Vovk et al., 2007). In addition, measuring respiratory muscle activity as a surrogate measure of cough strength to aspiration may be unsuitable for patients with dysphagia, where deficits at the laryngeal level may not be captured (Fontana & Widdicombe, 2007).

8.2.3 Intra-Abdominal Pressures

Cough strength can also be assessed by measuring changes in intra-abdominal pressures using rectal or urethral catheters (Addington, Stephens, Phelipa, Widdicombe, & Ockey, 2008). Addington et al. (2008) used this method to evaluate voluntary coughing and tartaric induced coughing in eleven heterogeneous female patients. Rationale for the use of intra-abdominal pressure recordings was based on prior VFSS observations during which coughing was observed to increase displacement of the diaphragm (Stephens, Addington, Miller, & Anderson, 2003). The authors hypothesized that this diaphragmatic displacement generates the expiratory forces that are required for airway clearance, and is synchronized with urethral and rectal closure to prevent incontinence during coughing (Addington et al., 2008). The results of the study reveal that area under the curve (AUC) pressure was higher for induced versus voluntary coughing (Addington et al., 2008). It is important to note that in this case, the entire cough epoch is compared, and differences in the number of coughs between voluntary [mean = 6 (+/- 0.94)] and induced coughing [mean = 1.8 (+/- 0.28)] are not taken into consideration. Interestingly, longer sustained elevated intra-abdominal pressures was noted for induced coughing compared to voluntary coughing (Addington et al., 2008). The authors suggest this may be an important factor in airway protection, reflecting sustained closure of the glottis (Addington et al., 2008). Addington and colleagues conclude that measurement of intra-abdominal pressure changes during coughing may be a useful clinical tool for determining airway protection deficits and quantitative assessment of changes in a patient's functional recovery or decline (Addington et al., 2008). However, the cost and invasive nature of this method of cough evaluation is likely to make it poorly accepted by patients, and unsuitable for daily clinical application.

8.2.4 Acoustic Analysis

There is growing interest in acoustic analysis of coughing as a measure of cough strength (Lee et al., 2017; Umayahara et al., 2018a, 2018b). Measurement of acoustic intensity is non-invasive, relatively simple and accessible through the use of smartphone technology (Umayahara et al., 2018a, 2018b). This makes it an appealing option for clinical application. Acoustic analysis of coughing is not a new phenomenon, and has been used for over 60 years as a valid measure of cough frequency (Woolf & Rosenberg, 1964). Acoustic analysis of coughing is used in a number of automated ambulatory cough monitoring systems, such as the Hull Automated Cough Counter, The Leicester Cough Monitor, and The VitaloJAK™, for patients with chronic cough or asthma (Shi, Liu, Wang, Cai, & Xu, 2018; Smith, 2007). These systems measure the frequency of coughing as an estimate of cough severity (Smith, 2007).

Numerous studies have also used acoustic analysis to measure the intensity of voluntary and induced coughing across patient population and healthy individuals (Lee et al., 2017; Mills et al., 2017; Smith Hammond, 2008; Smith Hammond et al., 2001). The cough sound is recognised as a function of airflow turbulence in the respiratory tract, and is influenced by the magnitude of the expiratory airflow (Sarkar, Madabhavi, Niranjana, & Dogra, 2015; Von & Isshiki, 1965). In this sense, it is a logical estimate of cough strength, as airflow turbulence is considered the process by which the airway is cleared of secretions and foreign materials (Sarkar et al., 2015; Von & Isshiki, 1965). Smith-Hammond and colleagues (2001) were among the first to measure acoustic intensity of voluntary coughing - specifically sound pressure levels (SPLs) - in patients with dysphagia. Using a calibrated microphone attached to the pneumotachograph, the authors found a significant difference in the SPLs between patients with severe (i.e. all consistencies administered aspirated) and no aspiration, as seen on VFSS. The authors suggest that the loudness of coughing may have limited utility as an indicator of

aspiration severity, as there was no difference in the decibels of severe and mild aspirators (i.e. think fluids and Ensure drinks aspirated). This may be due to the influence of secretions, which can affect airflow turbulence in the respiratory tract, and introduce considerable variability across participants (Smith Hammond et al., 2001). A notable limitation of this study is that the relationship between acoustic intensity and *clearance* of aspirate on VFSS is not evaluated.

In patients with chronic cough, voluntary cough sounds were repeatable and strongly correlated with other physiological measures of coughing (i.e. airflow and esophageal pressures) (Lee et al., 2017). Specifically, measurement of the sound power (i.e. the area under the curve of the power spectral density) and the peak energy (i.e. the maximum value of the root mean square [RMS]) of the expulsive phase of coughing had the strongest and most consistent correlation with esophageal pressures [median (IQR) correlation coefficient for sound power and peak energy = 0.89 (0.84-0.95), 0.89 (0.82-0.95)] and cough flow [median (IQR) correlation coefficient for sound power and peak energy = 0.88 (0.78-0.93), 0.87 (0.78-0.92)] (Lee et al., 2017). Other parameters (e.g. rise time, duration, bandwidth, and centroid frequency) were poorly correlated and had wide inter-individual variations. In contrast to speculation by Smith Hammond and colleagues (2001), the influence of secretions in the airway was not identified by the authors as a potential factor that may influence the cough sound (Lee et al., 2017). However, it is possible that presence of secretions in the respiratory tract may not be an issue for patients with chronic cough who were evaluated in this study. Although not specifically stated in the study, patients with chronic cough are typically characterized by a non-productive cough type (McGarvey et al., 1998). The authors note that the factors influencing cough sounds are poorly understood (Lee et al., 2017). Differences in sound power and energy were observed between gender, with moderate associations between height ($r = 0.4$, $p = 0.03$) and lung function, specifically FEV₁ ($r = 0.4$, $p = 0.03$) (Lee et al., 2017). This may be attributed to

differences in airway geometry which influences airflow turbulence (Sarkar et al., 2015). However, more research is needed to fully substantiate the influence of these variables. Of note, standardization of the position of the microphone relative to the mouth is an important consideration for measuring cough sounds, as the power and energy of the acoustic signal can fluctuate with varying positions of the microphone (Lee et al., 2017; Subburaj, Parvez, & Rajagopalan, 1996).

Mills and colleagues (2016) evaluated acoustic intensity, in addition to measures of flow and pressure, of voluntary and citric acid induced coughing in healthy volunteers. This is the first study to evaluate the acoustic intensity of coughing during CRT, and specifically suppressed coughing. The benefit of using a suppressed cough as opposed to a natural cough is that it aims to prevent elicitation of a volitional cough in response to sub-threshold tussigenic stimuli (Eccles, 2009; Hutchings et al., 1993a). It is thought to represent the point at which an individual can no longer suppress their cough response (Eccles, 2009; Monroe et al., 2014), and in this sense, it may more closely resemble a cough to aspiration (Monroe et al., 2014). An objective measure of citric acid induced coughing would likely overcome some of the limitations of subjective judgements of cough strength that have been documented by Miles and colleagues (Miles & Huckabee, 2013; Miles et al., 2014).

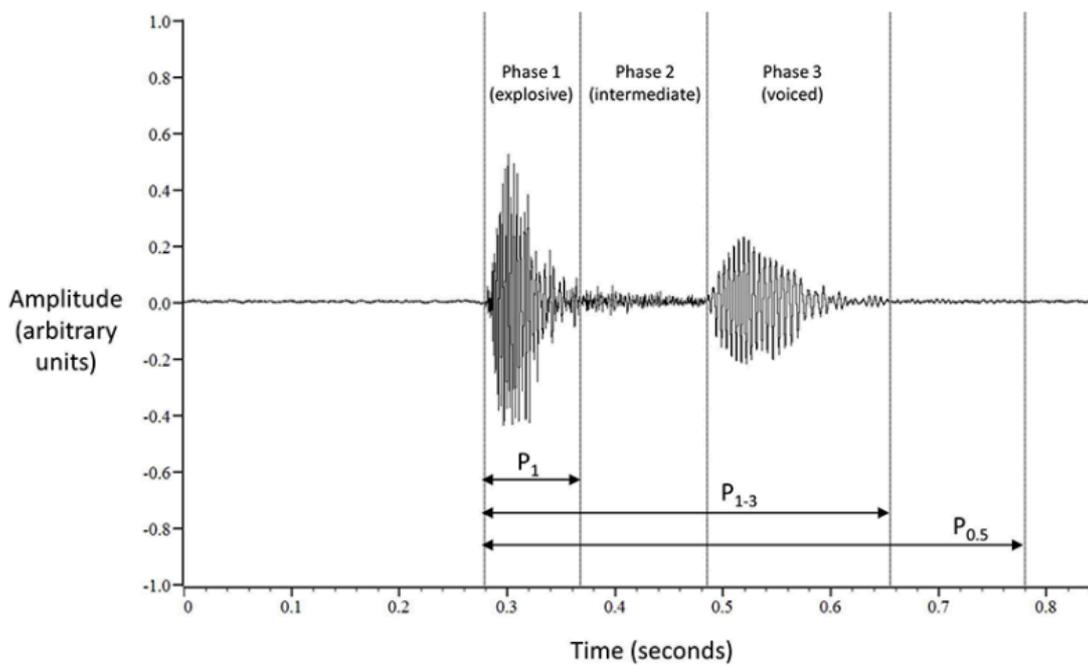
The results of the study reveal that acoustic intensity, flow and pressure of voluntary coughing were strongly correlated in healthy individuals (Mills et al., 2017). Furthermore, all measures were sensitive to differences in volitionally modulated strong and weak coughing (Mills et al., 2017). In contrast, poor correlations between acoustic intensity, flow and pressure of citric acid induced suppressed coughing were found (Mills et al., 2017). There was no dose-response relationship between increasing concentrations of citric acid and the magnitude of the cough

response (Mills et al., 2017). Based on correlation and effect sizes, the authors note that peak flow and AUC pressure appear to provide optimal measurement and the greatest potential for clinical application, while acoustic intensity was found to be the least accurate and sensitive (Mills et al., 2017). However, there are a number of methodological limitations that must be considered in interpreting these findings. Firstly, Mills et al. (2017) use an impedance microphone, connected to a stethoscope (i.e. contact microphone) to record acoustic intensity. According to Lee et al. (2017) free field microphones are regarded as superior to contact microphones, and yield higher correlations with objective measures of cough flow (Lee et al., 2017). Thus, it's possible that the use of a free field microphone by Mills and colleagues (2017) would have provided better correlation between acoustic intensity and flow for citric acid induced coughing.

Secondly, Mills and colleagues evaluate the entire acoustic signal of the cough (i.e. the expulsive, compressive and voiced phase). Lee et al. (2017) evaluate the expulsive phase (also referred to as the “explosive” phase) (Lee et al., 2017, p. 2) of the cough signal only (see Figure 16). This is because the expulsive phase of coughing is the most easily identifiable and always present on the acoustic waveform in healthy individuals (Lee et al., 2017). The voiced phase is not always present, and can make identification of the start and end of the cough signal difficult (Lee et al., 2017; Thorpe, Toop, & Dawson, 1992). This may have introduced variability in the automated macro applied to the acoustic waveforms to identify the beginning and end of the cough activity by Mills and colleagues (2017). These factors may explain the lack of correlation between acoustic intensity, flow and pressure for citric acid induced coughing observed by Mills et al (2016). It is also important to note that while measures of acoustic intensity, flow and pressure for citric acid induced coughing were not found to be correlated by Mills and colleagues (2017), their relationship to clearance of aspiration remains unknown.

Acoustic analysis of coughing is likely to have the greatest usability in the clinical setting. Whether acoustic intensity could differentiate between cough effectiveness (i.e. effective/ineffective clearance of aspiration from the airway) is a promising area of further investigation.

Figure 16: The expulsive, intermediate and voiced components of coughing. From “Sound: a non-invasive measure of cough intensity”, Lee et al (2017), *BMJ Open Respiratory Research*, 4(1), p. 2. CC BY-NC-ND.



CHAPTER 9: Objective Measurement of Cough Strength for Clearance of Penetration and Aspiration on Video- fluoroscopy

9.1 Study Aims and Hypothesis

The aim of this study was to objectively measure the cough strength that is required to expel penetrated and aspirated material from the laryngeal vestibule in patients with dysphagia during video-fluoroscopic swallowing studies (VFSS). Acoustic intensity was chosen as a measure of cough strength, as it represents a non-invasive, inexpensive, clinically applicable means of objectively measuring coughing. It was hypothesized that the decibel level of coughing that is effective at expelling penetrated and aspirated material from the laryngeal vestibule would be significantly higher than the decibel level of coughing that is not effective at expelling penetrated and aspirated material from the laryngeal vestibule. Based on these data, cut-off values of effective/ineffective coughing for clearance of penetration and aspiration would be determined.

9.2 Methods

9.2.1 Study Design

A cross-sectional observational study design was used to answer the research question. Data were collected across two acute hospital settings. VFSS was conducted by hospital clinicians, as per clinical protocol.

9.2.2 Participants

The study was approved by the New Zealand Health and Disability Ethics Committee (HDEC). Locality authorisation of the District Health Board was granted prior to the commencement of data collection and all participants gave informed consent. Patients who were referred for VFSS during May to November 2016 were invited to participate in the study. Data were included from patients who demonstrated airway invasion (i.e. aspiration and/or penetration) and a subsequent cough response on VFSS, defined as a forced expulsive manoeuvre, associated with a characteristic sound (Morice et al., 2007). From a total of eighty-eight patients recruited, twenty-two patients (25%) demonstrated airway invasion and coughing during their VFSS. Data from four of the twenty-two patients were excluded, as coughing was not captured radiographically (i.e. VFSS was not recording for coughing or patient positioning precluded a clear view of the upper airway during coughing). A subsequent five of the twenty-two studies were excluded due to VFSS equipment failure during the study, which meant the studies were not available for later analysis. A total of 13 patients (mean age: 72 years, range: 29-95, 9 males) were included in the final analysis. The nature of dysphagia varied across participants; neurological (n = 5), head and neck cancer (n= 4), unknown aetiology (n = 4).

9.2.3 Instrumentation and Instrumentation Reliability

Coughing in response to penetration and aspiration was recorded with a lapel microphone (RODE smartLav+), connected to an iPad Mini (iOS 10.3.2) installed with an audio recording application (RODE Rec). The reliability of the audio recording instrumentation was confirmed prior to data collection. A calibrated sound source (Bruel & Kjaer, model: 4230, 94 dB – 1000 Hz) - calibrated with a Bruel & Kjaer precision sound level meter (type: 2203) - was used to evaluate the reliability of the instrumentation at a consistent placement of the microphone (i.e. 3 cm from the calibrated sound source). The calibrated sound source was placed on a table and

using a ruler, a mark was placed 3 cm from the sound source. The microphone was taped over the mark for each recording to ensure consistency of placement. A total of twelve recordings were made. Each time, the microphone was removed and re-placed at the 3 cm mark before recordings were made. This was to mimic potential variation that may arise in the acoustic signal from placement of the microphone across participants in the study. After each recording was made, it was exported to a laptop computer and imported into Audacity®, a free acoustic analysis software, that was also used for the acoustic analyses of the main study. The start and end of the acoustic signal was manually selected from the waveform. Two sound parameters - the root mean square (RMS) and peak decibel level – were chosen for analysis. The choice of these two sound parameters was motivated by clinical feasibility. Both parameters could be automatically calculated from a selected acoustic waveform using Audacity® (wave-stats program). The RMS of the cough signal was also used by Lee and colleagues (2017) and was found to strongly correlate with cough flow, esophageal pressure and subjective judgements of cough strength. A < 10% coefficient of variation of the two chosen sound parameters was deemed acceptable, as described by Lee et al. (2017). The coefficient of variation (% CV) for the RMS dBFS was 1.9 % and for the peak dBFS was 4.5%. These data suggest the reliability of the two sound parameters, at a consistent microphone placement, is acceptable (Lee et al., 2017).

It was later hypothesized that, despite placing the microphone at a consistent anatomical location across participants, there may be variation in the mouth-to-mic distance across participants. In order to estimate this variation, the mouth-to-mic distance across a cohort of 24 healthy males and females was evaluated. The mic-to-mouth distance was defined as the distance in cm between anterior to the tragus of the ear (where the microphone was positioned), and the corner of the mouth. The mean (SD) mouth-to-mic distance for males was 11.9 (0.35)

cm, and for females 11.0 (0.54) cm. The variance in mouth-to-mic distance across males and females was 0.42 cm, suggesting the position of the microphone was similar across participants.

9.2.4 Procedures

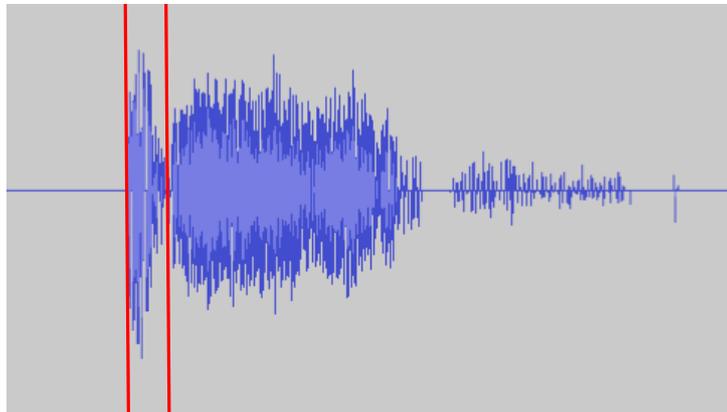
The microphone was placed anterior to the tragus of participants' ears, facing towards the mouth, and secured with medical tape. The placement of the microphone at the tragus of the ear was for hygiene reasons, and ensured it was out of the trajectory of coughing. VFSS were conducted by hospital clinicians, as per clinical protocol, in the radiology suite. Participants were administered a standard protocol of volumes and consistencies (3 x sip of water, 3 x sip of thickened juice, 3 x teaspoons apple puree, 3 x bites of peaches/banana, 3 x bites of white bread, 3 x bites of hard cracker, continuous drinking from a cup), mixed with barium contrast agent (Liquid Polibar, E-Z paste, E-Z-EM Canada Inc.), which were modified as required for patient safety by their treating clinician. Images were recorded in the anteroposterior and lateral planes. The researcher observed for spontaneous coughing in response to airway invasion of food or fluid during the VFSS study. This was defined as a forced expulsive manoeuvre against a closed glottis that was associated with a characteristic cough sound (Morice et al., 2007). Throat clearing, forced exhalations, or coughing elicited in response to clinicians' command were not included within this definition. The bolus type that evoked coughing was recorded on the data collection form. If the patient did not cough during the VFSS, the audio recording was deleted. If the patient coughed in response to penetration or aspiration during the VFSS, the audio recording was saved and exported to a laptop computer. A copy of the coughing event on video-fluoroscopy, was saved by the researcher for later analysis offline.

9.3 Data Extraction

9.3.1 Acoustic Data Extraction

Audio recordings were downloaded to a laptop computer and imported into Audacity®. Coughing sounds that corresponded to the airway invasion and coughing on VFSS were extracted from the acoustic waveform and saved as a separate file. All coughs were visible on the audio waveform. There was no perceived ambient noise during the cough events on the recordings. For all participants, only the expulsive phase of the first cough to aspiration or penetration was analysed only. Subsequent coughing events were not captured radiographically for all participants, as the fluoroscopy was stopped after the first or second cough, as deemed appropriate by the radiographers running the studies. Analysis of the first expulsive event ensured consistency across participants who had different cough frequencies within the cough epoch. The start and end of the expulsive phase of the first coughs was manually selected from the waveform. They were identified by large excursions on the acoustic waveform, and were confirmed perceptually, by listening to the audio recording. The start of the cough was defined as the point at which the acoustic signal increased in amplitude from baseline, and was associated with the onset of the characteristic cough sound, identified perceptually, by listening to the audio recording. The end of the cough was defined as the point at which the acoustic signal returned to baseline, or, in cases where the acoustic signal did not return to baseline, the point at which the next cough or an inhalation could be heard on the audio recording (see Figure 17). Based on the outcome of the instrumentation reliability evaluation (described above), RMS was chosen as the acoustic parameter of cough strength, as it had a lower coefficient of variation. RMS was evaluated in decibel level relative to full scale (dBFS), which represents the unit of measurement of acoustic intensity in electronic equipment, where zero is the maximum acoustic intensity level.

Figure 17: An example of an expulsive phase of coughing (i.e. between the red lines), followed by the intermediate and voiced components (Lee et al, 2017), extracted from the Audacity software.



9.3.1.1 VFSS Data Extraction

Instances of airway invasion and reflexive coughing were evaluated by two trained speech-language therapists using the judgement criteria below (Table 27). A third speech-language therapist was consulted for any videos for which consensus was not reached.

Table 27: Criteria for judgement of effective versus ineffective clearance of airway invasion on VFSS.

Aspiration	Effective Clearance	Material enters the airway, passes below the level of the vocal folds and is ejected from the laryngeal vestibule by coughing.
	Ineffective Clearance	Material enters the airway, passes below the level of the vocal folds and is not ejected from the laryngeal vestibule by coughing.
Penetration	Effective Clearance	Material enters the airway above the level of the vocal folds and is ejected from the laryngeal vestibule by coughing.
	Ineffective Clearance	Material enters the airway, above the level of the vocal folds and is not ejected from the laryngeal vestibule by coughing.

9.4 Data Analysis

Group means and standard deviations (SD) of the acoustic intensity (dBFS) of coughing for each subject group were calculated. Statistical comparisons were not made for this study due to the limited number of participants and unexpected findings, described below.

9.5 Results

From the thirteen patients included in the study, ten instances of coughing to aspiration, and seven instances of coughing to penetration were analysed (three patients were included in both groups).

9.5.1 Reflexive Coughing to Aspiration

All coughing events to aspiration were in response to thin liquids. No coughs were effective at expelling aspirate from the laryngeal vestibule. The mean acoustic intensity of spontaneous reflexive coughing to aspiration was -46.9 (SD = 5.4) (95% CI, -50.33, -43.53) dBFS.

9.5.2 Reflexive Coughing to Penetration

Coughing to penetration was in response to a range of bolus textures (water n = 3, mildly thick juice n = 3, banana n = 1). Four coughs (57%) were effective (1 water, 2 mildly thick juice, 1 banana) and three (43%) were ineffective (2 water, 1 mildly thick juice) in clearing penetration from the laryngeal vestibule. The mean acoustic intensity of effective coughing to penetration was -44.0 (SD = 7.3), (95% CI = -51.14, -36.86) dBFS. The mean acoustic intensity of ineffective coughing to penetration was -42.9 (SD = 2.0) (95% CI, -45.21, -40.59) dBFS. The mean difference in acoustic intensity between effective and ineffective coughing to penetration was 1.1 dBFS.

9.6 Discussion

The intent of this study was to determine the cough strength, as measured by acoustic intensity, that is required to expel penetration and aspiration from the laryngeal vestibule in patients with dysphagia. However, conclusions could not be reached, due to a number of unanticipated methodological limitations and unexpected findings. No patient demonstrated clearance of aspiration on the first cough event, and VFSS recording were limited to the first cough, precluding evaluation of the effectiveness of subsequent coughing events. However, there are a number of observations from the current data that warrant discussion. An important, yet unanticipated finding from this study was the ineffectiveness of coughing for expelling aspiration from the laryngeal vestibule. In all ten cases of coughing to aspiration, the bolus remained at a sub-glottic level, despite participants eliciting a spontaneous cough in response. In contrast, coughing was observed to expel penetration from the laryngeal vestibule. These findings raise tentative questions on whether coughing has the capacity to expel sub-glottic material from the airway, or whether only material sitting in the supra-glottic space are expelled by high velocity airflow during the expulsive phase of the first cough response.

Coughing is widely recognized in the dysphagia literature as the mechanism by which the airways are cleared of secretions and/or foreign materials (Hegland, Troche, et al., 2014; Hutcheson et al., 2018; Laciuga et al., 2016; Miles et al., 2014; Pitts et al., 2008; Troche, Brandimore, Okun, et al., 2014; Watts et al., 2016). As a result, it was hypothesized that some participants would effectively expel aspiration from the laryngeal vestibule in the current study. However, there is a lack of empirical evidence to support the role of coughing in expelling material from the airway in healthy individuals, or patients with dysphagia. While numerous studies demonstrate a relationship between impaired cough strength and presence of penetration and aspiration (Pitts et al., 2008; Pitts et al., 2010; Plowman et al., 2016; Smith

Hammond et al., 2009), no study has investigated the relationship between cough strength and clearance of penetration and aspiration.

In the respiratory physiology literature, it has been demonstrated that mucus in the central airway (i.e. trachea and mainstem bronchi) is effectively expelled by coughing (Camner, Mossberg, Philipson, & Strandberg, 1979; Dickey, 2018; Hasani, Pavia, Agnew, & Clarke, 1994; King, Brock, & Lundell, 1985; Van der Schans, 2007; Zahm et al., 1991). This suggests that coughing can effectively expel sub-glottic material. However, mucus clearance in these studies is initiated by volitional coughing after a full inspiration (Camner et al., 1979; Hasani et al., 1994; Pavia, Agnew, & Clarke, 1987). As a result, these findings cannot be extrapolated to spontaneous coughing to aspiration, due to differences in neurological control and physiological patterns (i.e. presence/absence of a preceding inspiration) between these two cough types. It is also important to note that some of these studies use machine models of stimulated coughing to expel artificial mucus in an artificial airway (King et al., 1985; Zahm et al., 1991). These models reproduce the biomechanics of coughing, but cannot emulate the afferent pathway of coughing, which is essential for cough elicitation in humans (Mazzone, 2016; Polverino et al., 2012; Sant'Ambrogio, 1987). Thus, these findings must be interpreted with caution. Furthermore, the extent to which clearance of mucus mimics clearance of aspiration is unknown. The viscosity of mucus differs to that of aspirated food and fluid. Viscosity is known to be an important factor in the effectiveness of coughing (Rubin, 2014). Pathologic mucus, with higher viscosity and elasticity, is less easily expelled from the airway (Fahy & Dickey, 2010). It has also been suggested that extremely watery mucus is not easily expelled by coughing (Rubin, MacLeod, Sturgess, & King, 1991). As all aspiration and cough events in the current study were in response to thin fluids, it is unknown whether the viscosity of the aspirate may have affected the ability of the cough to expel the material from the airway.

A recent review by Steele and colleagues (2017) acknowledges that PAS scores of four (i.e. material enters the airway, contacts the vocal folds and is ejected from the airway) and six (i.e. material enters the airway, passes below the vocal folds and is ejected from the airway) are rare scores in dysphagia research and clinical practice. This corroborates the findings of the current study and further questions the role of coughing in expelling aspirate from the airway. However, an important consideration is whether the findings of this study, and the reports by Steele and colleagues (2017), are attributed to limitations of the methods used to evaluate clearance of aspiration. There are a number of methodological limitations in the current study that must be taken into consideration in interpreting the results.

The first expulsive maneuver to penetration and aspiration was evaluated. This is typically the LER (Widdicombe et al., 2011), although, it is important to note that no physiologic measures were made to confirm this. As outlined in Chapter 2, the LER is a specific “anti-aspiration” mechanism (Widdicombe et al., 2011 p. 312), elicited in response to vocal fold stimulation (Korpas & Jakus, 2000). The absence of an initial inspiration is advantageous for preventing material entering further into the airway (Widdicombe et al., 2011). However, the LER arises from lower lung volumes, as it is not preceded by an inhalation (Korpas & Jakus, 2000). Thus, its capacity to expel sub-glottic material may be less than that of a cough preceded by a deep inspiration. Furthermore, limiting evaluation of cough effectiveness to the first cough may not provide an accurate representation of the effectiveness of coughing within a cough epoch, which is likely to involve both LERs and subsequent coughing preceded by inspiration (Fontana & Widdicombe, 2007; Widdicombe et al., 2011). As outlined in the methods section, methodological limitations precluded analysis of subsequent coughing events. The fluoroscopy was stopped after the second cough to aspiration in eight out of ten VFSS studies. However,

multiple coughs to aspiration were heard on the audio recordings. As this was an observational study, the aspects of the VFSS procedure were not controlled. Thus, a request could not be made that the fluoroscopic recordings continue until the end of the cough epoch.

However, this observation raises a number of important issues. Firstly, do clinicians routinely observe for effective/ineffective *clearance* of penetration and aspiration during VFSS? Clearance of penetration and aspiration are not reported in previously published research. The most recently published standardized tool for VFSS evaluation – MBSImp (Martin-Harris et al., 2008) – also does not include cough, or cough effectiveness within the criteria for quantification of swallowing impairment or swallowing safety. It is also important to note that there is lack of standardization of VFSS procedures across clinics and laboratories (Martin-Harris & Jones, 2008), and an absence of guidelines on how cough effectiveness should be evaluated. It is unclear how long fluoroscopic recordings should be continued after airway invasion to maximize the chance of accurately evaluating cough effectiveness, and whether judgements of cough effectiveness should be made after the first cough, or a cough epoch. This may explain why PAS scores of four and six are rare in dysphagia research and clinical practice (Steele & Grace-Martin, 2017), and also offers insight into the limitations of the current data in making definitive conclusions regarding the role of coughing in expelling aspiration.

The role of cough frequency in clearing aspiration from the laryngeal vestibule warrants discussion. In reviewing the audio files of coughing to aspiration, it became apparent that cough frequency may play an important role in clearing aspirate from the airway. This is supported by findings in the respiratory physiology literature, in which rapid successive coughing was found to induce a significant increase in mucus clearance, when compared to a single cough in a simulated cough model (Zahm et al., 1991). This finding contradicts a study in healthy adults

(n = 20), that demonstrated a reduction in the mechanical effectiveness (as measured by the peak expiratory flow rate and cough expired volume) of each subsequent capsaicin induced cough (Hegland, Troche, & Davenport, 2013). However, there was no evaluation of the functional effectiveness of coughing in this study. Thus, whether this would translate to more or less effective clearance or aspiration, or endogenous material is not known. Further studies are necessary to evaluate the role of cough frequency in clearing aspiration on VFSS. It is possible that repeated coughing to aspiration may reflect awareness that a bolus has entered the airway and volitional attempts to clear the bolus. In this sense, repeated coughing to aspiration may reflect better sensory perception. However, further research is required to substantiate these hypotheses.

The results of the current study demonstrate that coughing has the ability to expel penetration from the supraglottic airway, but this was only observed in a small number of participants (i.e. four out of seven participants). The reason for relatively few examples of coughing to penetration may be explained by the focus of this study on coughing, rather than a broader range of airway clearance behaviours. During data collection, it was observed that in many cases of penetration, the bolus was effectively cleared with swallowing, throat clearing, or expectorating. These findings are in line with the theoretical model by Troche and colleagues (2014) that describes a continuum of airway protective mechanisms. In the current study, it was decided to evaluate forced expulsive manoeuvres that were associated with a characteristic cough sound (Morice et al., 2007). This enabled the acoustic intensity of the same airway clearance mechanism to be evaluated across individuals. However, it is acknowledged that this precluded analysis of many attempts at airway clearance from the study. The mean difference between coughing that effectively versus ineffectively expelled penetration was 1.1 dBFS. There was a large standard deviation in the decibel level of effective coughing to penetration

and overlapping confidence intervals between the two groups, suggesting no true difference in these estimates. Therefore, acoustic intensity may not be sensitive in discriminating between effective and ineffective clearance of penetration on VFSS. However, due to the limited sample size and consequent lack of statistical analyses of the data, these results must be interpreted with caution.

It is also important to acknowledge that the acoustic intensity of coughing to penetration is in response to different bolus types (i.e. water, thickened fluids and solids). Similar to the way in which the composition and quantity of secretions in the airway can influence the acoustic signal (Smith Hammond et al., 2001), it is possible that the texture (e.g. thin fluid, thick fluid, puree, solid) and quantity of food or fluid in the airway may influence the acoustic signal. Due to the limited number of participants, it was not possible to analyse coughing in response to penetration according to specific bolus types, providing a notable limitation in interpreting the acoustic intensity of coughing to penetration.

In conclusion, the findings from this study have highlighted a number of gaps in the literature and uncovered further questions regarding the role of coughing in clearing aspiration. In light of the results and acknowledged methodological limitations, the next logical question that arises is: does coughing expel aspiration from the laryngeal vestibule in patients with dysphagia? In addressing this question, it is important that VFSS protocols are tailored to ensure the role of coughing can be adequately evaluated. This is vital information that is likely to have important implications on assessment and management of patients with dystussia and dysphagia in the future.

CHAPTER 10: Acoustic Intensity of Citric Acid Induced Coughing in Healthy Individuals and Patients with Dysphagia Following Stroke

10.1 Study Aims and Hypotheses

Objective measurement of cough strength during citric acid CRT remains challenging in clinical practice and is often based on unreliable subjective evaluation (Laciuga et al., 2016; Miles & Huckabee, 2013; Miles et al., 2014). This study aimed to determine whether acoustic intensity (i.e. decibel levels) was sensitive to differences in citric acid induced coughing between stroke patients with dysphagia and healthy individuals. Acoustic intensity was chosen as a measure of cough strength as it represents a non-invasive, inexpensive, clinically applicable means of measuring coughing. Healthy individuals were assumed to have adequate citric acid induced cough strength, and thus, were used as a reference population against which patients with dysphagia were compared. It was hypothesized that the decibel level of citric acid induced coughing would be significantly lower in patients with dysphagia compared to healthy controls. As a secondary objective, this study aimed to determine whether there was a difference in the number of coughs (i.e. cough frequency) to citric acid CRT in healthy individuals and patients with dysphagia. This objective arose from the findings of Chapter 9, where cough frequency could not be captured on VFSS.

10.2 Methods

10.2.1 Participants

Twelve patients with dysphagia following stroke, and sixteen aged-matched healthy controls were recruited. All participants were 65 years or older (mean age: 79 years, range: 67-94 years). Stroke patients with dysphagia were recruited from a sub-acute rehabilitation ward. Patients were eligible to participate in the study if they were on the clinical dysphagia caseload, and on a modified diet, as documented in the clinical notes. Patients were excluded if they had an absent cough to citric acid CRT on admission into the stroke ward (documented in the clinical notes), were unable to sign informed consent, drowsy, medically unstable, tracheostomized, requiring oxygen support or where participation would be contraindicated (e.g. patients with spinal injuries or increased intracranial pressure), according to clinical CRT guidelines. Healthy controls were excluded from the study if they had a history of any neurogenic disorders, a clinically significant respiratory disease (e.g. asthma, COPD, chronic bronchitis, emphysema), gastro-esophageal reflux, were taking ACE inhibitor or codeine-based drugs, were smokers, or had a recent (< 2 weeks) acute upper respiratory tract infection (URTI). This study was approved by the New Zealand Health and Disability Ethics Committee (HDEC). Locality authorisation of the District Health Board was granted prior to the commencement of data collection and all participants gave informed consent.

10.2.2 Equipment & Preparation

A RODE smartLav+ microphone was connected to an iPad Mini (iOS 10.3.2) with the RODE Rec audio recording application. This was the same instrumentation that was used in the previous study (Chapter 9). Given the clinical focus of this study, a validated clinical method of citric acid CRT (Miles, Moore, et al., 2013) was used to induce coughing in healthy individuals and patients with dysphagia. Citric acid (0.6 mol/L) diluted with 0.9% saline was

delivered via face-mask with a disposable nebulizer (Hudson Micro Mist Nebulizer, Standard Connector & Adult Mask, Hudson, RCI, NC, USA) (Miles, Moore, et al., 2013). A PulmoMate® Compressor Nebulizer (model 4650I) (DeVilbiss Healthcare LLC, Pennsylvania, US), with a restricted flow output of 6.6 L/min, was connected to the nebulizer.

10.2.3 Protocol

All participants were seated upright for citric acid CRT and cough acoustic measurements. The microphone was placed anterior to the tragus, facing towards the mouth, and secured with medical tape. The placement of the microphone at the tragus was for hygiene reasons, and ensured it was out of the trajectory of expelled secretions during coughing. The compressor was placed as far away from participants as possible to prevent interference in the sound recordings. Participants initially performed 15 s of tidal-breathing of 0.9% saline solution via the facemask, to acclimate them to the sound and sensation of the nebuliser, as recommended in the European Respiratory Society (ERS) Guidelines (Morice et al., 2007). Participants subsequently completed up to 15 s of tidal-breathing during nebulization of 0.6 mol/L citric acid, until a C₂ cough response (defined as 2 consecutive coughs within 15 s) was produced (Morice et al., 2007). Participants were instructed to “breathe in and out through your mouth and try not to cough”. This aimed to prevent participants from producing a volitional cough in response to a sub-threshold tussigenic stimuli. Citric acid was presented up to three times to each participant, with at least 30 seconds between trials to prevent tachyphylaxis (Morice et al., 2007). The test was stopped when participants achieved a C₂ response on two of three trials (referred to herein as CRT₁ and CRT₂). If no cough response was elicited on two of three trials data from the patient was excluded. Audio recordings of the C₂ cough sounds were saved and exported to a laptop computer for later analysis.

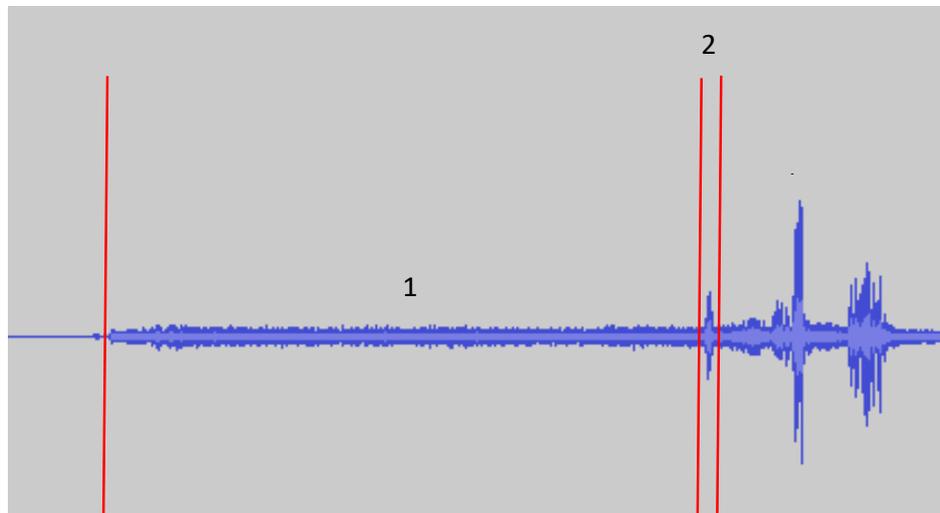
10.3 Data Extraction

10.3.1 Acoustic Data Extraction

Audio recordings were downloaded to a laptop computer and imported into an acoustic analysis software, Audacity®. All coughs were above the level of any background noise and were visible on the acoustic waveform. For all participants, the acoustic intensity of the first cough of each CRT trial was analysed. This ensured consistency across participants who had different cough frequencies within the cough epoch. The start and end of each cough was manually selected from the waveform. They were identified by large excursions on the acoustic waveform, and were confirmed perceptually, by listening to the audio recording. The start of the cough was defined as the point at which the acoustic signal increased in amplitude from baseline. Baseline, in this case, was the noise artefact from the nebulizer (see Figure 18). The end of the cough was defined as the point at which the acoustic signal returned to baseline (see Figure 18).

The peak dBFS of the selected acoustic signal was extracted using the ‘wave-stats’ plugin on Audacity®. It was originally intended to analyse the root mean square (RMS). However, as RMS is a value of the average, or continuous power of the acoustic signal, it was determined that the noise from the nebuliser may cause variability in the RMS signal due to varying room acoustics and position of the nebuliser across individuals. In this case, the peak dBFS was chosen as the acoustic parameter of citric acid induced coughing, as it was above the noise of the nebulizer for all participants. dBFS represents the unit of measurement of acoustic intensity in electronic equipment, where zero is the maximum acoustic intensity level. The number of coughs occurring up to 30 seconds following elicitation of the first C₂ response was counted for all participants for each CRT trial.

Figure 18: Example of a cough to citric acid CRT on the Audacity software (1 = the noise artefact from the nebulizer, 2 = the first cough to citric acid CRT). The subsequent bursts on the waveform represent additional cough events.



10.4 Data Analysis

IMM SPSS Statistics, Version 23 (IMP Corporation, Armonk, New York, USA) was used to analyse the data. An a priori alpha level of 0.05 was used. A between-subject three-way ANOVA was used to analyse the effects of gender (male/female), group (healthy/dysphagia) and trial (i.e. CRT₁ and CRT₂) on decibel level (dBFS) of citric acid induced coughing. Gender is known to influence cough sensitivity (Dicpinigaitis & Rauf, 1998). Thus, it was included as an independent variables to evaluate if any effects were related to gender. Previous studies have demonstrated a trial effect with acoustic intensity of citric acid CRT induced coughing (Mills et al., 2017). Thus CRT trial was included as an independent variable. to evaluate if any effects were related to CRT trial. An independent samples t-test was used to compare the number of coughs (cough frequency) within 30 seconds following citric acid inhalation between healthy individuals and patients with dysphagia across both cough trials.

10.5 Results

All participants completed the citric acid cough test without adverse events. One patient with dysphagia (female) completed one CRT and declined further participation. Thus, 12 cough recordings are included for CRT₁, and 11 cough recordings for CRT₂ in the dysphagia cohort. A summary of the data is provided in Table 27.

Table 28: Summary of the acoustic intensity of citric acid induced coughing across trials and groups.

Group	Trial	Mean peak dBFS	SD	n
Healthy	CRT ₁	-24.26	5.0	16
	CRT ₂	-23.51	5.1	16
	Total	-23.90	5.0	32
Dysphagia	CRT ₁	-28.18	3.00	12
	CRT ₂	-26.65	6.26	11*
	Total	-27.45	4.79	23

*One female patient with dysphagia did not complete a second CRT.

10.5.1 Acoustic Intensity of Coughing

Results showed no significant interaction effect between gender, group and trial on decibel level of citric acid induced coughing, ($F(1, 47) = 0.138, p = 0.712$). There was no evidence of a main effect of gender ($F(1, 47) = 0.012, p = 0.913$), or trial ($F(1, 47) = 0.613, p = 0.438$). However, there was a main effect of group ($F(1, 47) = 5.647, p = .022$), with healthy individuals having higher decibel levels of citric acid induced coughing than the patients with dysphagia. Pairwise comparisons between the two groups revealed a mean difference of 3.53

dBFS (95% CI, 0.54 – 6.5, $p = 0.022$). Estimated marginal means of the effects of group on decibel level of citric acid induced coughing are showed in Table 28.

Table 29: Estimated marginal means for the effects of group on decibel level of citric acid induced coughing.

Group	Mean dBFS	SE	95% Confidence Intervals	
			Lower Limit	Upper Limit
Healthy	-23.88	0.909	-25.71	-22.10
Dysphagia	-27.41	1.17	-29.77	-25.05

SE = standard error. 0 dbFS = maximum acoustic intensity.

10.5.2 Cough Frequency

There was no difference in the cough frequency between healthy individuals and patients with dysphagia ($t(53) = 2.21, p = 0.68$). The mean (SD) number of coughs for healthy individuals was 6.7 (2.62) and was 5.1 (2.63) for patients with dysphagia.

10.6 Discussion

The main finding from this study is that acoustic intensity, as measured by peak dBFS, is sensitive to differences in citric acid induced coughing between stroke patients with dysphagia and healthy individuals. Decibel level (dBFS) of citric acid induced coughing was 15% lower in stroke patients, compared to healthy controls. Current clinical protocols for citric acid CRT rely on subjective judgement of the cough response (i.e. perceptually strong or weak) (Miles, Moore, et al., 2013; Miles, Zeng, et al., 2013; Perry et al., 2019). However, research indicates that clinicians lack confidence and reliability in making such judgements (Laciuga et al., 2016; Miles & Huckabee, 2013; Miles et al., 2014). A key intent of the current research was to contribute to the development of a clinically applicable, objective measure of the strength of citric acid induced coughing. The use of acoustic intensity was favoured over measures of cough airflow or respiratory muscle activity, as it can be assessed without the need for complex and expensive instrumentation (Mills et al., 2017; Umayahara et al., 2018a). As a result, it is more easily translated into clinical practice. However, the *meaning* of ‘cough strength’ in the current study warrants discussion. Healthy individuals were assumed to have adequate citric acid induced cough strength, and thus, were used as a reference population against which patients with dysphagia were compared. However, if a strong cough is defined as a cough which effectively expels secretions and/or foreign material from the airway (as discussed in Chapter 8), the validity of acoustic intensity of citric acid induced coughing in measuring ‘cough strength’ is unknown. In designing these studies, it was hypothesized that Chapter 9 would provide insight into the validity of acoustic intensity as a measure of cough strength. However, such conclusions were not possible. As a result, conclusions of the current study are limited to stating that healthy individuals have a *louder* cough to CRT than patients with dysphagia. Whether the cough is *stronger* in healthy individuals, in terms of clearance of aspiration, remains to be directly tested.

The source of the cough sound is an important consideration in making sense of these findings. As outlined in Chapter 8, the cough sound is recognized as a function of airflow turbulence in the respiratory tract, and is influenced by the magnitude of the expiratory airflow (Sarkar et al., 2015; Von & Isshiki, 1965). A strong linear correlation between airflow and acoustic intensity of voluntary coughing has been demonstrated (Lee et al., 2017). Given that airflow turbulence is the process by which the airway is cleared of secretions and foreign materials (Button & Button, 2013; Clarke, 1989), tentative conclusions may be made regarding potential differences in airway clearance mechanisms between the two groups. However, other studies have reported poor correlation between acoustic intensity and airflow during citric acid induced coughing (Mills et al., 2017). Thus, in the absence of aerodynamic measures of coughing in the current study, caution must be made in drawing such conclusions.

Another important consideration is the *nature* of lower acoustic intensity of citric acid induced coughing. It is possible that patients with dysphagia had blunted sensory input, and thus, could more effectively diminish or suppress the intensity of their cough output as they were instructed to ‘try not to cough’. In contrast, for healthy individuals, the capacity for suppression or diminishing the intensity of cough output may be less, resulting in a higher acoustic intensity. In this respect, reduced cough output may be secondary to reduced sensory input. Differences in the sensory perception of the citric acid aerosol was not evaluated between the two groups. It is possible that asking participants to rate their urge-to-cough (UTC) may have provided more insight into the perceived intensity and higher order processing of the tussigenic stimulus between the two groups.

Although a statistically significant difference was identified between the two groups, there were large standard deviations in the decibel levels of coughing in healthy individual and patients with dysphagia and almost overlapping confidence intervals. This questions whether the findings would have clinical significance in reliably discriminating strong versus weak coughing. Alternatively, it is possible that some of the patients in the dysphagia group had adequate cough strength. The patients included in this study had a diagnosis of dysphagia based on a clinical swallowing evaluation, rather than instrumental evaluation. It is not known whether they had an effective or ineffective cough for expelling aspirate from the airway. Inclusion of patients who were observed to ineffectively expel aspiration from the airway on VFSS may have resulted in a larger difference between the two groups. Furthermore, longitudinal data on the development of aspiration pneumonia in these patients were not collected, but may have added substantial value to the findings.

The methodological limitations of the citric acid CRT used in the current study must be acknowledged. Methods of citric acid CRT that optimize test-retest reproducibility (used in Chapters 6 and 7) were not considered clinically applicable for bedside evaluation of cough strength. As a result, the nebulizer output and the dose of aerosol delivered across participants and tests (i.e. CRT₁, CRT₂) may have differed, due to the use of a fixed-time (15 s) inhalation method, and the absence of a dosimeter. Differences in inhaled volumes of citric acid between stroke patients and healthy individuals may explain the difference in the acoustic intensity of the cough response. This probability is based on the knowledge that a reduction in lung volumes and chest wall movements are common after stroke (Billinger, Coughenour, Mackay-Lyons, & Ivey, 2012). By not controlling for inhaled volume of citric acid across participants, it is possible that the observed differences in the acoustic intensity of coughing between healthy

individuals and stroke patients may be secondary to differences in inhaled volumes of citric acid across groups.

In summary, the implications of these findings suggest that more research is necessary in order to draw definitive conclusions on the validity of differences in acoustic intensity of citric acid induced coughing between healthy individuals and stroke patients with dysphagia. Evaluation of acoustic intensity of coughing is clinically applicable. All measures were made at patients' bedside without difficulty. Future studies should determine whether acoustic intensity differs between patients who develop aspiration pneumonia, versus those that do not. Development of a cut-off value of acoustic intensity of coughing for risk of aspiration pneumonia would be clinically valuable.

SECTION V: CONCLUSIONS

CHAPTER 11: Conclusions

This research program was inspired by the relationship between dystussia and dysphagia. The studies offer a substantial contribution to the literature by enhancing our understanding of assessment and modulation of the sensorimotor cough response, which can be applied to improving methods of assessment and rehabilitation of dystussia in patients with dysphagia. The findings from this thesis develop knowledge across three specific domains: (1) methods of citric acid cough reflex testing (CRT), (2) modulation of the sensorimotor cough response through sensory stimulation, and (3) the use acoustic intensity as a measure of cough strength for clearance of penetration and aspiration.

Lack of standardization and inadequate data on test-retest variability of the citric acid cough thresholds necessitated further investigation into methods of citric acid CRT. Chapter 5 represents the first study to systematically evaluate methods of citric acid CRT used across disciplines. The findings revealed substandard reporting of methods of citric acid CRT, and lack of progress in standardization since publication of the ERS guidelines over a decade ago. For the dysphagia researcher and clinician, these findings offer insight into the factors – such as the type of nebulizer used, the nebulizer output, methods of citric acid preparation - that may influence the diagnostic precision of the test in identifying patients at risk of silent aspiration. This is critical, as risk of negative outcomes, such as aspiration pneumonia and death, are higher in patients with silent aspiration. Standardization of citric acid CRT would enhance cough assessment in the dysphagia literature, affording better communication among researchers, and provision of cohesive practice recommendations to clinicians. It is anticipated that publication of these findings may contribute towards the development of standards of methods of citric acid CRT for research and clinical practice in the dysphagia literature, and

highlight the potential implications of changes in methodology on the outcome of the test. In the meantime, it is crucial that methods of citric acid CRT are adequately reported in published manuscripts to allow for full replication and interpretation of study outcomes.

It is acknowledged the methods of citric acid CRT for research and clinical practice may differ. For research, where CRT may be used as an outcome measure to monitor changes in cough sensitivity, methods of CRT may require instrumentation and protocols that optimize dose to dose reproducibility across trails, such as the use of a dosimeter, a breath activated nebulizer and testing the reproducibility of the nebulizer output. For clinical use, complex instrumentation and protocols may render the test infeasible. An alternative method, where a single concentration of a tussigenic aerosol is free-flowing from a facemask for a fixed time (e.g. 15 seconds, or 1-minute) may be more applicable as a screening tool for identifying patients who may be at risk of silent aspiration (Miles, Moore, et al., 2013; Wakasugi et al., 2008). However, caution may be warranted in using this method to monitor changes in cough sensitivity within or across individuals over time, as differences in the volume of citric acid inhaled may influence the outcome of the test.

Further methodological studies on citric acid CRT are justified to extend understanding of the influence of instrumentation (e.g. nebulizer type, nebulizer flow rate) and CRT protocols (e.g. number of inhalations, solvent used in citric acid solutions), on citric acid cough thresholds and test-retest reliability. These data may provide empirically-based rationale for the use of one method over another. In view of the neurophysiology associated with differing cough types, different methods of citric acid CRT may be advantageous to evaluate the integrity of different cough pathways (e.g. the viscerosensory versus somatosensory cough pathways). Furthermore, the use of different tussive agents (e.g. capsaicin), or different methods of

eliciting cough (e.g. laryngeal air puff stimulation via endoscopy) in a comprehensive cough test protocol may have merit for assessing distinct neural cough pathways. This would enable clinicians or researchers to gain insight into the precise pathophysiology of dystussia in patients with dysphagia, and the effects of different cough therapies.

The current research has provided valuable insight into test-retest variability of the citric acid CRT. The observed habituation to citric acid CRT over the three assessment sessions may represent a confounding variable that has implications on the use of the test as a viable outcome measure. These findings have relevance for the use of the test in the dysphagia literature, but also across disciplines, where citric acid CRT is used to evaluate the effects of anti-tussive medications and cough therapies in respiratory diseases. Quantification of this habituation effect was essential to interpret the effects of a sensory stimulation protocol that was subsequently evaluated. The habituation effect, combined with the observation that no change in citric acid cough thresholds were observed for the stimulation groups may, in fact, represent a sensitization effect of distilled water inhalations on cough sensitivity.

Quantification of test-retest variability of citric acid CRT in a dysphagic population has never been evaluated, but warrants investigation. It is not known whether patients would demonstrate the same habituation effect as healthy individuals, or whether a downregulated sensory system would show a lack of habituation on subsequent testing. This information is crucial for the use of the citric acid CRT as an outcome measure to evaluate the effects of cough therapies in patient populations. Furthermore, this information would be clinically valuable to current CRT protocols, where repeating the test may be used to determine recovery of the sensorimotor cough response and guide clinical decision making regarding oral intake (Perry et al., 2019).

This research provides preliminary evidence that cough sensitivity can be safely modulated in response to distilled water inhalations in healthy individuals. It would be premature to come to any conclusions regarding the effects of the sensory stimulation protocol in an impaired sensory system. However, these findings provide the foundation for future studies to evaluate the safety and efficacy of the sensory stimulation protocol in patients with laryngeal sensory deficits, and subsequent silent aspiration. The ultimate question is whether the sensory stimulation protocol could mitigate the risk of aspiration pneumonia, by enhancing the sensorimotor cough response to aspiration. It is also possible that enhancing laryngeal sensitivity may improve airway protective mechanisms, such as timely glottic closure in response to a misdirected bolus, thus preventing aspiration. The sensory stimulation protocol is non-invasive, relatively inexpensive and portable, thus could be used in the clinical setting, hospital setting or in patients' homes. Furthermore, it does not require activate participation on the patient's behalf, allowing inclusion of patients with cognitive impairment, if deemed ethically appropriate. Future research into the safety and efficacy of the sensory stimulation protocol for patients with dysphagia should be prioritized, to determine whether it would represent a viable treatment option for patients with absent or blunted cough sensitivity and subsequent silent aspiration.

Interestingly, this research indicated the potential for cough sensitivity to be *decreased* following 0.9% saline inhalations. Suppressed cough thresholds increased by almost double the test-retest variability that is expected when repeating the test on two alternate days. This may represent an area of further investigation for patients with chronic cough, where the goal is to reduce cough sensitivity. Collaborations between chronic cough and dysphagia cough research may provide insights into how cough is impaired in different diseases that result in contrasting clinical presentations (i.e. hyper- versus hypo- cough sensitivity). Whether similar

neural networks are involved in both diseases is unclear. A recent study speculates that impairment of the descending neural networks of coughing, that are responsible for inhibiting or facilitating the sensorimotor cough response, may give rise to up-regulated (e.g. chronic cough) or down-regulated coughing (i.e. silent aspiration) (McGovern et al., 2017). Evaluation of the neural networks activated by tussigenic aerosols in patients with chronic cough versus silent aspiration under imaging, may provide further insights into the neural control of hyper- versus hypo- cough sensitivity. It was suggested that the descending neural networks of coughing may provide a therapeutic target for normalising the sensorimotor cough response for both disorders (McGovern et al., 2017). However, methods of activating and modulating this pathway remain unknown. The underlying neural networks of the saline and distilled water inhalation protocols used in the current research are not known, but in light of their differential effects on cough sensitivity, warrant further investigation.

The findings of this research were not able to support or refute the use of acoustic intensity as a measure of cough strength. There are many improvements to this study design that are necessary before definitive conclusions can be made. If VFSS methods are adapted to evaluate the effectiveness of coughing in clearing aspirate from the airway for longer periods after aspiration events, and the current findings are upheld, this raises questions on the role of coughing as an airway clearance mechanism. It is interesting that no study has empirically investigated the role of coughing in clearing the airway of aspirate material. Despite this, it remains a commonly held belief that coughing should be prompted, if not reflexively initiated following episodes of aspiration in patients with dysphagia. Video-fluoroscopy provides an ideal opportunity to evaluate the sensorimotor cough response to aspiration. Further research should evaluate whether coughing can expel aspirate from the airway, and what type of cough or airway clearance behaviours are triggered in response to aspiration. The current thesis

focused on the sensorimotor cough response, but it is possible that less overt airway clearance mechanisms, such as throat clearing or forced exhalations, are more effective. This information may also guide interpretation of cough and airway clearance behaviours in response to citric acid CRT.

11.1 Final Remarks

Research in the field of assessment and management of dysphagia and dystussia is still in its infancy. This research program has enhanced understanding of assessment and modulation of the sensorimotor cough response and provides important groundwork for future studies. Moving forward, it would be advantageous to incorporate an integrated model of cough evaluation in patients with dysphagia in the clinical setting, that may include assessment of motor, sensory and cognitive (i.e. UTC) components of the sensorimotor cough response. The current research offers important first steps towards achieving this goal, in terms of quantifying the test-retest variability of citric acid cough thresholds and UTC ratings in healthy individuals, and evaluating novel methods of cough strength testing in patient populations.

Alongside enhancing our methods of assessment, it is important to continue to develop novel approaches to rehabilitate dystussia in patients with dysphagia. The distilled water sensory stimulation protocol was shown to be safe and potentially sensitize the cough response in a cohort of healthy adults. This research provides the first steps in developing a potential treatment for sensory cough impairments. However, further work is required to evaluate the safety and efficacy of the sensory stimulation protocol in patients with laryngeal sensory deficits. This may present different challenges, as patients would be characterized as having relatively high, or completely absent, baseline cough thresholds and may not perceive the distilled water inhalations. Furthermore, whether the method of citric acid CRT described in

this thesis would be sensitive to detecting changes in citric acid cough thresholds in patient populations is unknown. It is possible that smaller increments of citric acid at higher concentrations, where patients with laryngeal sensory deficits are more likely to cough, may be required (e.g. 0.8, 1.0, 1.2, 1.4, 1.6 mol/L etc)

Future work should focus on (1) evaluating test-retest reliability of citric acid cough thresholds and UTC ratings in patient populations, with the addition of smaller increments of higher concentrations citric acid (2) evaluating the safety and efficacy of the proposed distilled water sensory stimulation protocol in patients with laryngeal sensory deficits and (3) determining the role of coughing, and other airway clearance behaviours, in expelling aspirate from the airway. Continued research in this field is essential to reduce the negative consequences associated with the comorbidity of dysphagia and dystussia that is commonly seen in patients following neurological injury.

Appendices

Appendix 1: Information Leaflets and Consent Forms



Participant Information Sheet

You are invited to participate in a research project on Cough Strength Testing

What is the project about?

A strong cough is important to protect our lungs if food/drink goes down the wrong way. Results from the study will give us more information on how we can measure cough strength to identify patients with a strong or weak cough.

Why should I participate in the study?

- Whether or not you take part is **your choice**
- If you do not want to take part, you don't have to give a reason. It will not affect your care in any way.
- If you want to take part now, but change your mind later, you can pull out of the study at any time.

What will I need to do?

- You will need to sign a consent form. We can help you with this.
- We will need to know some information about you.
- You will be asked to wear a small microphone on your ear during a cough reflex test.
- The Cough Reflex test involves wearing a face mask that is connected to a nebuliser. The air omitted form the face mask contains citric acid (the acid in oranges and lemons). This air may/may not make you cough.
- The test will be repeated three times.
- No additional time is required after this.
- If you cough during your x-ray swallow study, the researcher will keep a copy of the audio file and the x-ray of your swallow study for further analysis.

- If you do not cough, your audio recording will be deleted and your information will not be included in the final analysis.

What happens after this?

- We will keep your information at the Rose Centre for Stroke Recovery and Research, St Georges Medical Centre.
- Your name will be removed from all paperwork and you will be assigned a code number.
- All information will be kept safely on a password protected computer.
- The data will be stored for 10 years; after that it will be deleted.
- The results of the study will be included in the researcher's MSc thesis and may be submitted for publication in a peer reviewed journal. If you would like a copy of the study when it's complete, please indicate this on the consent form.

Are there any risks?

- There are no risks in taking part in the study. Your participation will not affect your care in any way.
- You will have the opportunity to ask questions and to find out more information from the researcher.

What if I decide I do not want to be involved in the study?

- You can withdraw from the study at any time by contacting the primary investigator.
- If you do not wish to contact the primary investigator, you can contact your speech and language therapist who can inform the primary investigator on your behalf.

You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you would like to participate in the study, please sign the consent form that accompanies this information leaflet, and bring it to your x-ray swallow study.

What if I have more Questions?

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Email: emma.wallace@pg.canterbury.ac.nz / Phone: 027-456-21-69

Supervisor: **Prof Maggie-Lee Huckabee.**

The University of Canterbury Rose Centre for Stroke Recovery and Research.

Email: maggie-lee.huckabee@canterbury.ac.nz / Phone: +64 3364 2014

Maori Health Support: <http://www.health.govt.nz/our-work/populations/maori-health>.



Consent Form

- I have been given a full explanation of this project and have had the opportunity to ask questions.
- I understand what is required of me if I agree to take part in the research.
- I understand that participation is voluntary and I may withdraw at any time without penalty. Withdrawal of participation will also include the withdrawal of any information I have provided, if this is still possible.
- I understand that any information or opinions I provide will be kept confidential to the researcher and supervisors, and that any published or reported results will not identify the participants.
- I understand that a thesis is a public document and will be available through the UC Library.
- I understand that all data collected for the study will be kept in locked and secure facilities and/or in password-protected electronic form and will be destroyed after ten years.
- I understand that I can contact the researcher Emma Wallace, (emma.wallace@pg.canterbury.ac.nz) or her supervisor Maggie-Lee Huckabee (maggie-lee.huckabee@canterbury.ac.nz) for further information.

Optional: I would like to receive a summary of the findings. If so, please provide email address:

By signing below, I agree with the statements above, and to participate in this research project.

Print name of participant: _____

Signature of participant: _____

Date: _____

Participant Information Sheet

You are invited to participate in a research project on Cough Strength Testing

What is the project about?

A strong cough is important to protect our lungs if food/drink goes down the wrong way. Results from this study will give us more information on how we can measure cough strength, to help us identify patients in hospital with a weak cough.

Why should I participate in the study?

- Whether or not you take part is **your choice**.
- If you do not want to take part, you don't have to give a reason. It will not affect your care in any way.
- If you want to take part now, but change your mind later, you can pull out of the study at any time.

What will I need to do?

- You will need to sign a consent form. We can help you with this.
- We will need to know some information about you e.g. past medical history.
- You will be asked to wear a small microphone next to your ear during a cough reflex test.
- The Cough Reflex test involves wearing a face mask. Air comes out of the face mask and it may/may not make you cough.
- The test will be repeated three times. It will take in total no more than 30 minutes.
- No additional time is required after this.

What Happens Next?

- We will keep your information at the Rose Centre for Stroke Recovery and Research, St Georges Medical Centre.
- Your name will be removed from all paperwork and you will be assigned a code number.
- All information will be kept safely on a password protected computer.
- The data will be stored for 10 years; after that it will be deleted.
- The results of the study will be included in the researcher's MSc thesis and may be submitted for publication in a peer reviewed journal. If you would like a copy of the study when it's complete, please indicate this on the consent form.

Are there any risks?

- There are no risks in taking part in the study.
- You will have the opportunity to ask questions and to find out more information from the researcher.
- You can withdraw from the study at any time by advising the primary investigator.

You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

What if I have more Questions?

Principal Investigator: **Emma Wallace**

Email: emma.wallace@pg.canterbury.ac.nz

Phone: 027-456-21-69

Supervisor: **Prof Maggie-Lee Huckabee.**

The University of Canterbury Rose Centre for Stroke Recovery and Research.

Email: maggie-lee.huckabee@canterbury.ac.nz

Phone: +64 3364 2014

Maori Health Support: If you require Māori cultural support talk to your whānau in the first instance. Alternatively, you may contact the administrator for He Kamaka Waiora (Māori Health Team) by telephoning 09 486 8324 ext 2324, or contact catherine.grant@cdhb.health.nz



Consent Form

Project Title: Cough Strength Testing in Acute Dysphagia Management

- I have been given a full explanation of this project and have had the opportunity to ask questions.
- I understand what is required of me if I agree to take part in the research.
- I understand that participation is voluntary and I may withdraw at any time without penalty. Withdrawal of participation will also include the withdrawal of any information I have provided, if this is still possible.
- I understand that any information or opinions I provide will be kept confidential to the researcher and supervisors, and that any published or reported results will not identify the participants.
- I understand that a thesis is a public document and will be available through the UC Library.
- I understand that all data collected for the study will be kept in locked and secure facilities and/or in password-protected electronic form and will be destroyed after ten years.
- I understand that I can contact the researcher Emma Wallace, (emma.wallace@pg.canterbury.ac.nz) or her supervisor Maggie-Lee Huckabee (maggie-lee.huckabee@canterbury.ac.nz) for further information.

Optional: I would like to receive a summary of the findings. If so, please provide email address:

By signing below, I agree with the statements above, and to participate in this research project.

Print name of participant: _____

Signature of participant: _____

Date: _____



Participant Information Sheet

Reliability of Citric Acid Cough Reflex Testing in Healthy Participants

What is this project about?

The cough reflex test (CRT) is currently used on all stroke patients admitted to hospital in the Canterbury District Health Board (CDHB) in New Zealand. This is a useful test that tells us about patients' cough sensitivity and whether they can protect their airway if food or liquid goes down the wrong way. The CRT can also tell us how sensitive a healthy person's cough reflex is, depending on what concentration of citric acid they cough to. This is called the reflexive cough threshold. This study examines whether a person's cough threshold changes over 5 days, and if so, by how much, when we control for the volume and inspiratory flow rate of citric acid inhalations in healthy participants.

What will I need to do?

- Participants will include healthy males and females, over the age of 18 with no known history of any respiratory diseases. Any participant with a history of neurogenic disorders, gastro-esophageal reflux, individuals taking ACE inhibitor drugs or codeine based drugs, smokers, or participants with an upper respiratory tract infection in the previous 2 week will be excluded from participating in the study.
- Data collection will take place across 3 locations: The Rose Centre for Stroke Recovery and Research, The University of Canterbury and at local nursing and residential homes, to facilitate participation of both young and elderly individuals.
- To ensure confidentiality, your name will be removed from all paperwork and you will be assigned a code number.
- Participation will involve **1 hour, 3 days per week (Monday, Wednesday and Friday) within one week.**
- You will be required to brush your teeth immediately prior to testing. A new unused toothbrush will be provided for you.

- Each day you will be required to inhale increasing concentrations of citric acid through a facemask. The volume and flow rate of each citric acid inhalation will be controlled.
- You will have 30 s short breaks in-between inhalations.
- The mists may or may/not make you cough.

What happens after this?

- All information collected from you will be kept on a password protected computer.
- The data will be stored for 10 years, after which it will be deleted.
- The results of the study will be included in the researcher's PhD thesis and may be submitted for publication in a peer reviewed journal. No information that could identify you will be included in these publications
- If you would like a copy of the results of the study, please indicate this on the consent form.

Are there any risks?

- There are no risks associated with single sessions of citric acid cough reflex testing.
- If you feel uncomfortable, we can immediately stop the study.

What if I decide I do not want to be involved in the study?

- If you want to take part now, but change your mind later, **you can withdraw from study at any time, without any consequences.**
- You can do this by contacting the primary investigator, Emma Wallace (e-mail: emma.wallace@pg.canterbury.ac.nz or phone: 027-456-21-69).

What if I have more questions?

For more information, please contact the principal investigator, or her supervisor:

Principal Investigator:

Emma Wallace, PhD Candidate

The University of Canterbury Rose Centre
for Stroke Recovery and Research.

Email: emma.wallace@pg.canterbury.ac.nz

Phone: +64 33642307/ 027-456-21-69

Supervisor:

Dr. Phoebe Macrae

The University of Canterbury Rose Centre
for Stroke Recovery and Research.

Email: phoebe.macrae@canterbury.ac.nz

Phone: +64 33642032

Undergraduate Student:

Alicia Ang, BSLP Year 4

The University of Canterbury

Email: ata72@uclive.ac.nz

Phone: 021-076-6975



Consent Form

“Methodological Study: Reliability of Citric Acid Cough Reflex Testing in Healthy Participants”

- I have been given a full explanation of this project and have had the opportunity to ask questions.
- I understand what is required of me if I agree to take part in the research.
- I understand that participation is voluntary and I may withdraw at any time without penalty. Withdrawal of participation will also include the withdrawal of any information I have provided, if this is still possible.
- I understand that any information or opinions I provide will be kept confidential to the researcher and supervisors, and that any published or reported results will not identify the participants.
- I understand that a thesis is a public document and will be available through the UC Library.
- I understand that all data collected for the study will be kept in locked and secure facilities and/or in password-protected electronic form and will be destroyed after ten years.
- I understand that I can contact the researcher Emma Wallace, (emma.wallace@pg.canterbury.ac.nz) or her supervisor Dr. Phoebe Macrae (phoebe.macrae@canterbury.ac.nz) for further information.

Optional: I would like to receive a summary of the findings. If so, please provide email address:

By signing below, I agree with the statements above, and to participate in this research project.

Print name of participant: _____

Signature of participant: _____

Date: _____



Participant Information Sheet

You are invited to take part in research on cough rehabilitation

“Modulation of the Cough Reflex using Ultrasonically Nebulized Distilled

Water (UNDW) in Healthy Participants”

What is the purpose of this study?

The purpose of this study is to learn whether it is possible to safely alter the cough reflex in healthy participants using ultrasonically nebulized distilled water.

Research has shown that many stimuli (e.g. cigarette smoke, menthol, oral stimulation, capsaicin, citric acid and distilled water) can alter the cough reflex. Distilled water has been chosen for this study because it's non-toxic and has a neutral pH, making it safer than other stimuli.

The results of this study will provide information on how we can alter cough reflex sensitivity in patients with an absent cough reflex, who are unable to protect their airway in the event of aspiration (when food or liquid go down the wrong way).

The study is being conducted by the Rose Centre for Stroke Recovery and Research in conjunction with the Respiratory Physiology Lab at Christchurch Hospital.

What will my participation in the study involve?

Participants will include healthy males and females, over the age of 18 with no known history of asthma or any respiratory diseases. Any participant with a history of neurogenic disorders, gastro-esophageal reflux, individuals taking ACE inhibitor drugs or codeine-based drugs,

smokers, or participants with an upper respiratory tract infection in the previous 2 week will be excluded from participating in the study for their safety.

A brief medical history will be taken on the first day, to determine your eligibility to participate in the study. Your name will be removed from any health information collected during the study (e.g. spirometry results, medical history questionnaire).

Participation will involve 5 consecutive days, 1 session per day (morning or afternoon), with a maximum of 2 hours per session at the Respiratory Physiology Lab in Christchurch Hospital.

Each session will involve breathing a mist from a nebulizer in 4-minute cycles for up to an hour. You will have short breaks in-between nebulizer inhalations. You will be assigned to one of three groups with different types of mists and stimulations. The mists may or may/not make you cough, depending on what group you're assigned to.

One day 1, 3 and 5, you will breathe a mist of citric acid at increasing levels until you cough. This is an outcome measure that tells us how sensitive your cough is. We will also take spirometry measurements each day to ensure that your airway is not under stress (bronchoconstriction) from the inhalations.

What are the possible benefits and risks of this study?

Distilled water has been shown to cause bronchoconstriction in asthmatics thus, if you have a history of asthma, even as a child, it is important to make the researcher aware of this because you are not safe to participate in the study. There are no known risks of bronchoconstriction for those who have no history of asthma, however, you may/may not experience a change in breathing pattern or an increased respiratory rate during the inhalations. If you feel uncomfortable at any stage, we can immediately stop the study.

A trained researcher will be present for all procedures and medical doctor will be available at all times.

Spirometry will be performed on any participant who shows signs of bronchoconstriction. Data collection will be stopped and treatment with a bronchodilator will be provided to reverse signs of bronchoconstriction if indicated.

All participants will be provided with the contact details of Dr. Michael Epton, a medical doctor and respiratory physician who is part of the research team, should they experience any respiratory distress after treatment sessions.

Who pays for the study?

Participant will not incur any costs

Participants will be reimbursed \$100 for their time and costs (transport) in participating in the study.

What if something goes wrong?

There are no known side effects of this treatment protocol and no known risk of bronchoconstriction for those with no history of asthma. However, in the unlikely event that you are injured in this study, you would be eligible **to apply** for compensation from ACC, just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

What are my rights?

Participation in the study is voluntary and you are free to decline to participate, or to withdraw from the research at any practicable time, without experiencing any disadvantage.

You will have the right to access information about you collected as part of the study.

You will be informed of any new information about adverse or beneficial effects related to the study that becomes available during the study that may have an impact on their health

Participants' names will be removed from any data collected as part of the study. All participants will be assigned a code number, which will appear on all documents.

What happens after the study, or if I change my mind?

Any data collected as part of the study will be kept in a password protected computer or locked cabinet in a secure room at the Rose Centre for Stroke Recovery and Research for 10 years,

after which it will be destroyed. The results will be included in the researcher's PhD thesis, which will be submitted in February 2019 and may be submitted for publication in a peer review journal.

Who can I contact for more information or if I have any concerns?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Emma Wallace, PhD Candidate

027-456-21-69

emma.wallace@pg.canterbury.ac.nz

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050

Fax: 0800 2 SUPPORT (0800 2787 7678)

Email: advocacy@hdc.org.nz

For Maori health support please contact :

Hector Matthews, Executive Director, Maori and Pacific Health, CDHB

03-364-41-69

hector.matthews@cdhb.health.nz

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone: 0800 4 ETHICS

Email: hdecs@moh.govt.nz



Consent Form

“Modulation of the Cough Reflex using Ultrasonically Nebulized Distilled Water (UNDW) in Healthy Participants”

Please tick to indicate you consent to the following

I have read, or have had read to me in my first language, and I understand the Participant Information Sheet.

I have been given sufficient time to consider whether or not to participate in this study.

I have had the opportunity to use a legal representative, whanau/ family support or a friend to help me ask questions and understand the study.

I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.

I consent to the research staff collecting and processing my information, including information about my health.

If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed. Yes No

I consent to my GP or current provider being informed about my participation in the study and of any significant abnormal results obtained during the study AND I consent to providing the contact details of my GP to the researcher. Yes No

I agree to an approved auditor appointed by the New Zealand Health and Disability Ethic Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study.

I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.

I understand the compensation provisions in case of injury during the study.

I know who to contact if I have any questions about the study in general.

I understand my responsibilities as a study participant.

I wish to receive a summary of the results from the study.

Yes

No

Declaration by participant:

I hereby consent to take part in this study.

Participant's name:

Signature:

Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature:

Date:

Contact Details for GP:

Name: _____

Address: _____

Phone Number: _____

Appendix 2: Pre-Participation Questionnaire



Pre-Participation Questionnaire

Please answer all questions to assess your eligibility to participate in the study.

- | | | |
|--|-------|----|
| ▪ Are you under the age of 18 years? | YES / | NO |
| ▪ Have you ever had a stroke or brain injury? | YES / | NO |
| ▪ Are you on medication for gastro-esophageal reflux? | YES / | NO |
| ▪ Do you have any respiratory diseases? | YES / | NO |
| ▪ Are you taking ACE inhibitor drugs for blood pressure? | YES / | NO |
| ▪ Are you taking codeine? | YES / | NO |
| ▪ Are you a smoker? | YES / | NO |
| ▪ Have you had a chest infection in the past 2 weeks? | YES / | NO |

If you answered YES to any of the questions above, you are not suitable to participate in the study.

Appendix 3: Search Strategy for Systematic Review of Methods of Citric Acid CRT

- 1 coughing/ or chronic cough/ or experimental coughing/ or irritative coughing/
- 2 cough*.tw.
- 3 1 or 2
- 4 citric acid/
- 5 citric [acid.tw.](#)
- 6 4 or 5
- 7 3 and 6

Appendix 4: Citric Acid Concentrations for Systematic Review of Methods of Citric Acid CRT

Table 30: Citric acid concentrations - single dose method

Citric Acid Concentration (mol/L)	Citric Acid Concentration (as reported in the study)	Studies (first author, year)
0.04 mol/L	0.68% citric acid	Ogihara, 1991, Morice, 1987, Morice, 1992
0.05 mol/L	1% (w/v)	Guillen-Sola, 2015, Lee, 2014, Wakasugi, 2014, Wakasugi, 2008, Sato, 2012
0.09 mol/L	18 g/L	Kenia, 2008
0.2 mol/L	0.2 M	Stone, 1992
0.3 mol/L	5% (w/v)	Packman, 1991, Winning, 1986, Packman, 1977, Winning, 1988, Winning, 1985, Grattan, 1995
0.5 mol/L	10% (w/v)	Enright, 1980, Griffin, 1982, Moghadamnia, 2003, Abdul- Manap, 1999, D'Souza, 1988, Van Meerhaeghe, 1986, Hull, 2002, Hull, 2002, Lavietes, 1998, Rostami-Hodjegan, 2001
	100 g/L	Cross, 1994

0.6 mol/L	0.6 mol/L	Holmes, 2016
0.8 mol/L	15% (w/v)	Noel, 1962
1 mol/L	20% (w/v)	Lavorini, 2007, Lee, 2013
1.3 mol/L	25 % (w/v)	Bickerman, 1965, Calesnick, 1967
1.6 mol/L	30% w/v	Nishino, 2008
0.000033 mol per 0.125 ml *reported as the dose delivered	33 μ mol	Morice, 1994
Threshold concentration (C5)	Threshold concentration (C5)	Smith, 2012
Threshold concentration	Threshold concentration	Bickerman, 1960
The concentration that participants produced 3 - 6 coughs.	The concentration that participants produced 3 - 6 coughs.	Winther, 1970

Table 31: Citric acid concentrations - dose-response method

Citric Acid Concentration (mol/L)	Citric Acid Concentration (as reported in the study)	Increments	Studies (first author, year)
0.00001 – 0.0033 mol/L	2.5 - 640 mg/L	doubling	Barros, 1991, Barros, 1990
0.0001 - 1.33 mol/L	0.03 - 256 g/L	NR	Boulet, 1994
0.0001 – 0.5 mol/L	10 ⁻⁴ - 0.5 M	NR	Di Franco, 2001
0.001 – 1 mol/L	1mM – 1,000 mM	log incremental	Wright, 2010 (Mefar) Pecova, 2007, Ramsay, 2008
		incremental	Kastelik, 2002
0.001 - 0.8 mol/L	0.3125 -160 g/L	doubling	Thompson, 2009
0.001 - 1 mol/L	1 mM to 1 M	half log, quarter log and linear	Leow, 2012
0.001 - 1.9 mol/L	0.03 - 36% w/v	doubling	Nakajoh, 2000
0.001 - 0.9 mol/L	0.03 - 18% w/v	doubling	Katsumata, 1991

0.002 – 0.8 mol/L	0.3125 – 160 g/L	doubling	Mason, 1999, Barry, 1997
0.003 - 1.9 mol/L	0.7 - 360 g/L	doubling	Gui, 2010, Gui, 2014, Yamanda, 2008, Kanezaki, 2010, Sato, 2016, Ebihara, 2011, Watando, 2004, Ebihara, 2003, Gui, 2012
0.003 - 0.5 mol/L	0.7 - 90 g/L	doubling	Kashiwazaki, 2013
0.003 - 1.7 mol/L	0.625- 320 g/L	doubling	Schmidt, 1997
0.005 – 2.7 mol/	1 – 512 g/L	doubling	Auffarth, 1991, Ziora, 2005, Auffarth, 1991
0.0078 – 1 mol/L	7.8 – 1,000 mM	doubling	Vilardell, 2017 Wright, 2010 (KoKo)
0.007 - 1.7 mol/L	0.125 - 32 %	x 4 concentrations	Midgren, 1992
0.05 - 2.6 mol/L	1 - 50% w/v	random 1, 2, 5, 10, 20, 50%	Rees, 1983
0.01 - 1 mol/L	0.25 - 20% w/v	random	Empey, 1976
0.01 – 1 mol/L	10 – 1,000 mM	10, 30, 100, 300 mM, and 1 mol/L	Laude, 1993
		Log incremental	Wong, 1999, Gorden, 1997, Morice, 1992 (b), Wong, 1999

0.01 – 3.3 mol/L	2.5 – 640 mg/ml ⁻¹	doubling	Guglieminotti, 2005, Gayat, 2007, Guglieminotti, 2007
0.03 - 1.7 mol/L	0.5 - 32% w/v	doubling	Pounsford, 1985
0.03 – 1 mol/L	30 – 1,000 mM	30, 100, 300, 1,000mM	Janssens, 2014, Janssens, 2015
0.03 – 1 mol/L	0.03 – 1 mol/L	ascending	Smith, 2006, Smith, 2006
		doubling	Decalmer, 2007
0.03 – 4 mol/L	0.03 – 4 mol/L	ascending	West, 2012
		doubling	Smith, 2010, Young, 2009, Marsden, 2008, Kelsall, 2009
0.03 - 3.3 mol/L	0.5 - 63% of crystalline citric monohydrate	0.5, 1, 2, 4, 6, 8, 12, 16, 24, 32, 38 63% of crystalline citric monohydrate	Taylor, 1988
0.03 - 1 mol/L	0.5% -20%	0.5%, 1%, 2.5%	Empey, 1979
0.03 - 3.5 mol/L	5.2 - 675 g/L	multiples of 1.5	Riordan, 1994
0.05 - 3.3 mol/L	10 - 640 g/L	doubling	Kondo, 1998
0.05 - 5.2 mol/L	1 - 100% w/v	random order of 1%, 2%, 5%, 10%, 20%,	Vianna, 1988

		50%, and 100% (w/v)	
0.05 - 1.6 mol/L	1 - 30%	0.5%, 1%, 2.5%	Karttunen, 1987
0.0625 - 2 mol/L	62.5 - 2000 mM	doubling	Lin, 1999
0.07 - 1.3 mol/L	1.25%- 25%	1.25%, 2.5%, 5%, 7.5%, 10%, 15%, 20%, 25%	Bickerman, 1957 (first study)
0.07 - 4.2 mol/L	1.25 - 80% w/v	doubling	Belcher, 1986
0.1 - 1.2 mol/L	0.1 - 1.2 mol/L	0.1 mol/L	Perry, 2017 Monroe, 2014
0.1 - 2.6 mol/L	2 - 50 g/100ml	Stepwise (0, 2, 4, 10, 20, 35, 50g/100ml)	Stockwell, 1993
0.1 - 0.5 mol/L	2.5 - 10% w/v	doubling	Bickerman, 1954
0.1 - 0.8 mol/L	2 - 15% w/v	2% (w/v), 5%, 10%, 15%	Berkowitz, 1973
0.125-0.2 mol/L	0.125 - 0.2 M/L	doubling	Dilworth, 1990
0.2 - 1 mol/L	0.3% -20%	doubled every 3 mins	Cox, 1984
0.2 - 1.7 mol/L	4 - 32% w/v	doubling	Rietveld, 2000

0.3 - 1 mol/L	5 - 20% w/v	doubling	Costello, 1985
0.3 - 1.3 mol/L	5 - 25% w/v	5% increments	Winther, 1970 (CRT training period)
		NR	Sevelius, 1965
0.4 - 0.8 mol/L	0.4 - 0.8 mol/L	0.2 mol	Kallesen, 2016, Miles, 2013, Kelly, 2016
		0.4 mol	Mills, 2017
0.5 - 5.2 mol/L	10 - 1000 g/L	(10, 20, 50, 100, 500 mg/ml and 1 g/ml,	Behera, 1995
0.5 mol/L - no maximum dose stated	10%	5% and 1%	Karttunen, 1988
0.5 mol/L - no maximum dose stated	10%	6% then 2%	Karttunen, 1988
0.5 - 1 mol/L	10 - 20% w/v	NR	Simonsson, 1967
0.5 - 2 mol/L	10 - 40% w/v	NR	Bossi, 1988
0.8 - 1.2 mol/L	0.8 - 1.2 M/L	Two concentrations only	Miles, 2013
0.8 - 2.6 mol/L	0.8 - 2.6 M/L	0.2 mol/L	Monroe, 2014
1 mol/L and 0.5 mol/L	200 g/l and 100g/l	NA	Thomson, 1979

1mol/L - 1.95mM	1mol/L - 1.95mM	serial dilutions	Faruqi, 2011
six serial dilutions from a 1 mol/L stock solution of citric acid	six serial dilutions	serial dilutions	Mincheva, 2014
Starting dose not specified. Max dose of 0.1 mol/L	Starting dose not specified. Max dose of 20%	NR	Uzubalis, 1989

References

- Addington, W. R., Stephens, R. E., Gilliland, K., & Rodriguez, M. (1999). Assessing the laryngeal cough reflex and the risk of developing pneumonia after stroke. *ARCHIVES OF PHYSICAL MEDICINE AND REHABILITATION*, 80(2), 150-154.
- Addington, W. R., Stephens, R. E., Phelipa, M. M., Widdicombe, J., & Ockey, R. R. (2008). Intra-abdominal pressures during voluntary and reflex cough. *Cough (London, England)*, 4(1), 2-2.
- Allegra, L., & Bianco, S. (1980). Non-specific broncho-reactivity obtained with an ultrasonic aerosol of distilled water. *Eur J Respir Dis Suppl*, 106, 41-49.
- Amaris, M., Dua, K. S., Naini, S. R., Samuel, E., & Shaker, R. (2012). Characterization of the upper esophageal sphincter response during cough. *Chest*, 142(5), 1229-1236.
- Ambalavanar, R., Tanaka, Y., Selbie, W. S., & Ludlow, C. L. (2004). Neuronal activation in the medulla oblongata during selective elicitation of the laryngeal adductor response. *J Neurophysiol*, 92(5), 2920-2932.
- Anderson, J. W., Sant'Ambrogio, F. B., Mathew, O. P., & Sant'Ambrogio, G. (1990). Water-responsive laryngeal receptors in the dog are not specialized endings. *Respir Physiol*, 79(1), 33-43.
- Anderson, S. D., Schoeffel, R. E., & Finney, M. (1983). Evaluation of ultrasonically nebulised solutions for provocation testing in patients with asthma. *Thorax*, 38(4), 284-291.
- Ando, A., Smallwood, D., McMahon, M., Irving, L., Mazzone, S. B., & Farrell, M. J. (2016). Neural correlates of cough hypersensitivity in humans: evidence for central sensitisation and dysfunctional inhibitory control. *Thorax*, 71(4), 323-329.

- Antunes, M. B., & Cohen, N. A. (2007). Mucociliary clearance--a critical upper airway host defense mechanism and methods of assessment. *Curr Opin Allergy Clin Immunol*, 7(1), 5-10.
- Athukorala, R. P., Jones, R. D., Sella, O., & Huckabee, M. L. (2014). Skill training for swallowing rehabilitation in patients with Parkinson's disease. *Arch Phys Med Rehabil*, 95(7), 1374-1382.
- Auffarth, B., de Monchy, J. G., van der Mark, T. W., Postma, D. S., & Koeter, G. H. (1991a). Citric acid cough threshold and airway responsiveness in asthmatic patients and smokers with chronic airflow obstruction. *Thorax*, 46(9), 638-642.
- Barber, C. M., Curran, A. D., Bradshaw, L. M., Morice, A. H., Rawbone, R., & Fishwick, D. (2005). Reproducibility and validity of a Yan-style portable citric acid cough challenge. *Pulmonary Pharmacology & Therapeutics*, 18(3), 177-180.
- Barros, M. J., Zammattio, S. J., & Rees, P. J. (1990). Importance of inspiratory flow rate in the cough response to citric acid inhalation in normal subjects. *Clinical science (London, England : 1979)*, 78(5), 521-525.
- Barros, M. J., Zammattio, S. L., & Rees, P. J. (1991). Effect of changes in inspiratory flow rate on cough responses to inhaled capsaicin. *Clinical science (London, England : 1979)*, 81(4), 539-542.
- Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1-48.
- Bates, D. V., Fish, B. R., Hatch, T. F., Mercer, T. T., & Morrow, P. E. (1966). Deposition and retention models for internal dosimetry of the human respiratory tract. Task group on lung dynamics. *Health Phys*, 12(2), 173-207.
- Belcher, N., & Rees, P. J. (1986). Effects of pholcodine and salbutamol on citric acid induced cough in normal subjects. *Thorax*, 41(1), 74-75.

- Beste, C., & Dinse, H. R. (2013). Learning without training. *Current biology : CB*, 23(11), R489-R499.
- Bianchi, A. L., & Gestreau, C. (2009). The brainstem respiratory network: an overview of a half century of research. *Respir Physiol Neurobiol*, 168(1-2), 4-12.
- Bianchi, C., Baiardi, P., Khirani, S., & Cantarella, G. (2012). Cough peak flow as a predictor of pulmonary morbidity in patients with dysphagia. *American Journal of Physical Medicine and Rehabilitation*, 91(9), 783-788.
- Bickerman, H. A., & Barach, A. L. (1954). The experimental production of cough in human subjects induced by citric acid aerosols; preliminary studies on the evaluation of antitussive agents. *The American journal of the medical sciences*, 228(2), 156-163.
- Bickerman, H. A., German, E., Cohen, B. M., & Itkin, S. E. (1957). The cough response of healthy human subjects stimulated by citric acid aerosol. II. Evaluation of antitussive agents. *The American journal of the medical sciences*, 234(2), 191-206.
- Billinger, S. A., Coughenour, E., Mackay-Lyons, M. J., & Ivey, F. M. (2012). Reduced cardiorespiratory fitness after stroke: biological consequences and exercise-induced adaptations. *Stroke research and treatment*, 2012, 959120-959111.
- Boe, J., Dennis, J., O'driscoll, B., Bauer, T., Carone, M., Dautzenberg, B., . . . Lannefors, L. (2001). European Respiratory Society Guidelines on the use of nebulizers. *European Respiratory Journal*, 18(1), 228-242.
- Boggs, D. F., & Bartlett, J. D. (1982). Chemical specificity of a laryngeal apneic reflex in puppies. *J Appl Physiol Respir Environ Exerc Physiol*, 53(2), 455.
- Bolser, D. C. (1991). Fictive cough in the cat. *J Appl Physiol (1985)*, 71(6), 2325-2331.
- Bolser, D. C., Gestreau, C., Morris, K. F., Davenport, P. W., & Pitts, T. E. (2013). Central neural circuits for coordination of swallowing, breathing, and coughing: predictions

- from computational modeling and simulation. *Otolaryngol Clin North Am*, 46(6), 957-964.
- Bolser, D. C., Pitts, T. E., Davenport, P. W., & Morris, K. F. (2015). Role of the dorsal medulla in the neurogenesis of airway protection. *Pulmonary Pharmacology & Therapeutics*, 35, 105-110.
- Bonham, A. C., Sekizawa, S. i., & Joad, J. P. (2004). Plasticity of central mechanisms for cough. *Pulmonary Pharmacology & Therapeutics*, 17(6), 453-470.
- Boulet, L. P., Milot, J., Boutet, M., St. Georges, F., & Laviolette, M. (1994). Airway inflammation in nonasthmatic subjects with chronic cough. *American Journal of Respiratory and Critical Care Medicine*, 149(2 I), 482-489.
- Boushey, H. A., Richardson, P. S., Widdicombe, J., & Wise, J. C. (1974). The response of laryngeal afferent fibres to mechanical and chemical stimuli. *J Physiol*, 240(1), 153-175.
- Brandimore, A. E., Hegland, K. W., Okun, M. S., Davenport, P. W., & Troche, M. S. (2017). Voluntary upregulation of reflex cough is possible in healthy older adults and Parkinson's disease. *J Appl Physiol (1985)*, 123(1), 19-26.
- Britton, D., Benditt, J. O., Merati, A. L., Miller, R. M., Stepp, C. E., Boitano, L., . . . Yorkston, K. M. (2014). Associations between laryngeal and cough dysfunction in motor neuron disease with bulbar involvement. *Dysphagia*, 29(6), 637-646.
- Brodsky, M. B., McFarland, D. H., Dozier, T. S., Blair, J., Ayers, C., Michel, Y., . . . Martin-Harris, B. (2010). Respiratory-swallow phase patterns and their relationship to swallowing impairment in patients treated for oropharyngeal cancer. *Head Neck*, 32(4), 481-489.

- Brouwer, A. F. J., Roorda, R. J., & Brand, P. L. P. (2007). Comparison between peak expiratory flow and FEV1 measurements on a home spirometer and on a pneumotachograph in children with asthma. *Pediatric Pulmonology*, *42*(9), 813-818.
- Button, B. M., & Button, B. (2013). Structure and function of the mucus clearance system of the lung. *Cold Spring Harbor perspectives in medicine*, *3*(8), a009720.
- Calesnick, B., & Christensen, J. A. (1967). Latency of cough response as a measure of antitussive agents. *Clinical Pharmacology and Therapeutics*, *8*(3), 374-380.
- Camner, P., Mossberg, B., Philipson, K., & Strandberg, K. (1979). Elimination of test particles from the human tracheobronchial tract by voluntary coughing. *Scand J Respir Dis*, *60*(2), 56-62.
- Canning, B. J., Chang, A. B., Bolser, D. C., Smith, J. A., Mazzone, S. B., & McGarvey, L. (2014). Anatomy and neurophysiology of cough: CHEST Guideline and Expert Panel report. *Chest*, *146*(6), 1633-1648.
- Canning, B. J., Mazzone, S. B., Meeker, S. N., Mori, N., Reynolds, S. M., & Udem, B. J. (2004). Identification of the tracheal and laryngeal afferent neurons mediating cough in anaesthetized guinea-pigs. *Journal of Physiology*, *557*(2), 543-558.
- Chadha, T. S., Birch, S., Allegra, L., & Sackner, M. A. (1984). Effects of ultrasonically nebulized distilled water on respiratory resistance and breathing pattern in normals and asthmatics. *Bull Eur Physiopathol Respir*, *20*(3), 257-262.
- Cheney, F. W., Jr., & Butler, J. (1968). The effects of ultrasonically-produced aerosols on airway resistance in man. *Anesthesiology*, *29*(6), 1099-1106.
- Cheng, Y. S. (2014). Mechanisms of pharmaceutical aerosol deposition in the respiratory tract. *AAPS PharmSciTech*, *15*(3), 630-640.
- Clarke, S. W. (1989). Rationale of airway clearance. *Eur Respir J Suppl*, *7*, 599s-603s.

- Clayton, N. A., Carnaby, G. D., Peters, M. J., & Ing, A. J. (2014). Impaired laryngopharyngeal sensitivity in patients with COPD: The association with swallow function. *International Journal of Speech-Language Pathology*, *16*(6), 615-623.
- Coates, A. L., Wanger, J., Cockcroft, D. W., Culver, B. H., Carlsen, K.-H., Diamant, Z., . . . Sterk, P. J. (2017). ERS technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *European Respiratory Journal*, *49*(5), 1601526.
- Cohen, J., Postma, D. S., Douma, W. R., Vonk, J. M., De Boer, A. H., & ten Hacken, N. H. T. (2011). Particle size matters: diagnostics and treatment of small airways involvement in asthma. *European Respiratory Journal*, *37*(3), 532-540.
- Costello, J. F., Dunlop, L. S., & Gardiner, P. J. (1985). Characteristics of prostaglandin induced cough in man. *British Journal of Clinical Pharmacology*, *20*(4), 355-359.
- Cox, I. D., Wallis, P. J. W., & Apps, M. C. P. (1984). An electromyographic method of objectively assessing cough intensity and use of the method to assess effects of codeine on the dose-response curve to citric acid. *British Journal of Clinical Pharmacology*, *18*(3), 377-382.
- Cunningham, J. J., Halum, S. L., Butler, S. G., & Postma, G. N. (2007). Intraobserver and interobserver reliability in laryngopharyngeal sensory discrimination thresholds: a pilot study. *Ann Otol Rhinol Laryngol*, *116*(8), 582-588.
- D'Souza, G. A., Jindal, S. K., Malik, S. K., Kumar, B., & Kaur, S. (1988). Airway response to aerosol inhalations in leprosy. *The Indian journal of medical research*, *87*, 190-193.
- Daintith, J. (2008). *A Dictionary of Chemistry*.
- Davenport, P. W. (2008). Urge-to-cough: what can it teach us about cough? *Lung*, *186 Suppl 1*, S107-111.

- Dawes, C. (1987). Physiological Factors Affecting Salivary Flow Rate, Oral Sugar Clearance, and the Sensation of Dry Mouth in Man. *Journal of Dental Research*, 66(1_suppl), 648-653.
- de Bruin, P. F., de Bruin, V. M., Lees, A. J., & Pride, N. B. (1993). Effects of treatment on airway dynamics and respiratory muscle strength in Parkinson's disease. *Am Rev Respir Dis*, 148(6 Pt 1), 1576-1580.
- Decalmer, S. C., Webster, D., Kelsall, A. A., McGuinness, K., Woodcock, A. A., & Smith, J. A. (2007). Chronic cough: how do cough reflex sensitivity and subjective assessments correlate with objective cough counts during ambulatory monitoring? *Thorax*, 62(4), 329-334.
- Di Franco, A., Dente, F. L., Giannini, D., Vagaggini, B., Conti, I., Macchioni, P., . . . Paggiaro, P. L. (2001). Effects of inhaled corticosteroids on cough threshold in patients with bronchial asthma. *Pulmonary Pharmacology & Therapeutics*, 14(1), 35-40.
- Dickey, B. F. (2018). What it takes for a cough to expel mucus from the airway. *Proceedings of the National Academy of Sciences of the United States of America*, 115(49), 12340.
- Dickinson, R. S., Morjaria, J. B., Wright, C. E., & Morice, A. H. (2014). Is opiate action in cough due to sedation? *Therapeutic advances in chronic disease*, 5(5), 200-205.
- Dicpinigaitis, P. V. (2003). Short- and long-term reproducibility of capsaicin cough challenge testing. *Pulmonary Pharmacology & Therapeutics*, 16(1), 61-65.
- Dicpinigaitis, P. V. (2007). Experimentally induced cough. *Pulmonary Pharmacology & Therapeutics*, 20(4), 319-324.
- Dicpinigaitis, P. V., & Rauf, K. (1998). The Influence of Gender on Cough Reflex Sensitivity. *Chest*, 113(5), 1319-1321.
- Dicpinigaitis, P. V., Rhoton, W. A., Bhat, R., & Negassa, A. (2012). Investigation of the urge-to-cough sensation in healthy volunteers. *Respirology*, 17(2), 337-341.

- Dicpinigaitis, P. V., Sitkauskiene, B., Stravinskaite, K., Appel, D. W., Negassa, A., & Sakalauskas, R. (2006). Effect of smoking cessation on cough reflex sensitivity. *European Respiratory Journal*, 28(4), 786-790.
- Dilworth, J. P., Pounsford, J. C., & White, R. J. (1990). Cough threshold after upper abdominal surgery. *Thorax*, 45(3), 207-209.
- Dinse, H. R., & Tegenthoff, M. (2015). Evoking plasticity through sensory stimulation: Implications for learning and rehabilitation. *e-Neuroforum*, 6(1), 11-20.
- Doeltgen, S. H., Dalrymple-Alford, J., Ridding, M. C., & Huckabee, M. L. (2010). Differential effects of neuromuscular electrical stimulation parameters on submental motor-evoked potentials. *Neurorehabilitation & Neural Repair*, 24(6), 519-527.
- Doty, R. W. (1951). Influence of stimulus pattern on reflex deglutition. *Am J Physiol*, 166(1), 142-158.
- Doty, R. W., & Bosma, J. F. (1956). An electromyographic analysis of reflex deglutition. *J Neurophysiol*, 19(1), 44-60.
- Dua, K. S., Ren, J., Bardan, E., Xie, P., & Shaker, R. (1997). Coordination of deglutitive glottal function and pharyngeal bolus transit during normal eating. *Gastroenterology*, 112, 73-83.
- Ebihara, S., Sekiya, H., Miyagi, M., Ebihara, T., & Okazaki, T. (2016). Dysphagia, dystussia, and aspiration pneumonia in elderly people. *J Thorac Dis*, 8(3), 632-639.
- Eccles, R. (2009). Central mechanisms IV: conscious control of cough and the placebo effect. *Handb Exp Pharmacol*(187), 241-262.
- Edstrom, L. (1970). Selective changes in the sizes of red and white muscle fibres in upper motor lesions and Parkinsonism. *J Neurol Sci*, 11(6), 537-550.
- Ekberg, O. (1982). Closure of the laryngeal vestibule during deglutition. *Acta Otolaryngol*, 93(1-2), 123-129.

- Empey, D. W., Laitinen, L. A., Jacobs, L., Gold, W. M., & Nadel, J. A. (1976). Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Am Rev Respir Dis*, *113*(2), 131-139.
- Eschenbacher, W. L., Boushey, H. A., & Sheppard, D. (1984). Alteration in osmolarity of inhaled aerosols cause bronchoconstriction and cough, but absence of a permeant anion causes cough alone. *Am Rev Respir Dis*, *129*(2), 211-215.
- Fahy, J. V., & Dickey, B. F. (2010). Airway mucus function and dysfunction. *N Engl J Med*, *363*(23), 2233-2247.
- Faruqi, S., Molyneux, I. D., Fathi, H., Wright, C., Thompson, R., & Morice, A. H. (2011). Chronic cough and esomeprazole: a double-blind placebo-controlled parallel study. *Respirology (Carlton, Vic.)*, *16*(7), 1150-1156.
- Feng, C., Wang, H., Lu, N., Chen, T., He, H., Lu, Y., & Tu, X. M. (2014). Log-transformation and its implications for data analysis. *Shanghai archives of psychiatry*, *26*(2), 105-109.
- Finucane, T. E., & Bynum, J. P. W. (1996). Use of tube feeding to prevent aspiration pneumonia. *The Lancet*, *348*(9039), 1421-1424.
- Folinsbee, L. J. (1989). Human health effects of exposure to airborne acid. *Environmental Health Perspectives*, *79*, 195-199.
- Fontana, G. A. (2008). Before We Get Started: What Is a Cough? *Lung*, *186*(S1), 3-6.
- Fontana, G. A., Pantaleo, T., Lavorini, F., Benvenuti, F., & Gangemi, S. (1998). Defective motor control of coughing in Parkinson's disease. *American Journal of Respiratory & Critical Care Medicine*, *158*(2), 458-464.
- Fontana, G. A., Pantaleo, T., Lavorini, F., Boddi, V., & Panuccio, P. (1997). A noninvasive electromyographic study on threshold and intensity of cough in humans. *European Respiratory Journal*, *10*(5), 983-989.

- Fontana, G. A., Pantaleo, T., Lavorini, F., Maluccio, N. M., Mutolo, D., & Pistolesi, M. (1999). Repeatability of cough-related variables during fog challenges at threshold and suprathreshold stimulus intensity in humans. *The European respiratory journal*, *13*(6), 1447.
- Fontana, G. A., & Widdicombe, J. (2007). What is cough and what should be measured? *Pulmonary Pharmacology & Therapeutics*, *20*(4), 307-312.
- Forbes, S., Latimer, J., Sreenivasan, P. K., & McBain, A. J. (2016). Simultaneous Assessment of Acidogenesis-Mitigation and Specific Bacterial Growth-Inhibition by Dentifrices. *PLoS ONE*, *11*(2), e0149390.
- Fraser, C., Power, M., Hamdy, S., Rothwell, J., Hobday, D., Hollander, I., . . . Thompson, D. (2002). Driving plasticity in human adult motor cortex is associated with improved motor function after brain injury. *Neuron*, *34*(5), 831-840.
- Fujiwara, K., Kawamoto, K., Shimizu, Y., Fukuhara, T., Koyama, S., Kataoka, H., . . . Takeuchi, H. (2017). A novel reflex cough testing device. *BMC Pulm Med*, *17*(1), 19.
- Garcia, J. M., & Chambers, E. (2010). Managing dysphagia through diet modifications. *The American journal of nursing*, *110*(11), 26.
- Garon, B. R., Sierzant, T., & Ormiston, C. (2009). Silent aspiration: results of 2,000 video fluoroscopic evaluations. *J Neurosci Nurs*, *41*(4), 178-185.
- Gauld, L. M. (2009). Airway clearance in neuromuscular weakness. *Developmental medicine and child neurology*, *51*(5), 350.
- Gayat, E., Lecarpentier, E., Retout, S., Bedairia, E., Batallan, A., Bonay, M., . . . Guglielminotti, J. (2007). Cough reflex sensitivity after elective Caesarean section under spinal anaesthesia and after vaginal delivery. *British journal of anaesthesia*, *99*(5), 694-698.

- Gestreau, C., Grelot, L., & Bianchi, A. L. (2000). Activity of respiratory laryngeal motoneurons during fictive coughing and swallowing. *Exp Brain Res*, *130*(1), 27-34.
- Gestreau, C., Milano, S., Bianchi, A. L., & Grelot, L. (1996). Activity of dorsal respiratory group inspiratory neurons during laryngeal-induced fictive coughing and swallowing in decerebrate cats. *Exp Brain Res*, *108*(2), 247-256.
- Giraldo-Cadavid, L. F., Burguete, J., Rueda, F., Galvis, A. M., Castaneda, N., Agudelo-Otalora, L. M., . . . Fernandez, S. (2017). Reliability of a laryngo-pharyngeal esthesiometer and a method for measuring laryngo-pharyngeal mechano-sensitivity in a prospectively recruited cohort of patients. *Eur Arch Otorhinolaryngol*, *274*(7), 2861-2870.
- Godde, B., Spengler, F., & Dinse, H. R. (1996). Associative pairing of tactile stimulation induces somatosensory cortical reorganization in rats and humans. *Neuroreport*, *8*(1), 281-285.
- Godde, B., Stauffenberg, B., Spengler, F., & Dinse, H. R. (2000). Tactile coactivation-induced changes in spatial discrimination performance. *J Neurosci*, *20*(4), 1597-1604.
- Gomes, G. F., Pisani, J. C., Macedo, E. D., & Campos, A. C. (2003). The nasogastric feeding tube as a risk factor for aspiration and aspiration pneumonia. *Curr Opin Clin Nutr Metab Care*, *6*(3), 327-333.
- Grattan, T., Marshall, A., Higgins, K., & Morice, A. (1995). The effect of inhaled and oral dextromethorphan on citric acid induced cough in man. *British Journal of Clinical Pharmacology*, *39*(3), 261-263.
- Guglielminotti, J., Rackelboom, T., Tesniere, A., Panhard, X., Mentre, F., Bonay, M., . . . Desmonts, J. M. (2005). Assessment of the cough reflex after propofol anaesthesia for colonoscopy. *British journal of anaesthesia*, *95*(3), 406-409.

- Guglielminotti, J., Tesniere, A., Rackelboom, T., Mantz, J., Vecchierini, M.-F., Bonay, M., . . . Desmots, J.-M. (2007). Cough reflex sensitivity is decreased in female obese patients with obstructive sleep apnea. *Respiratory Physiology & Neurobiology*, *158*(1), 83-87.
- Gui, P., Ebihara, S., Ebihara, T., Kanezaki, M., Kashiwazaki, N., Ito, K., & Kohzuki, M. (2012). Urge-to-cough and dyspnea conceal perception of pain in healthy adults. *Respiratory Physiology & Neurobiology*, *181*(2), 214-219.
- Gui, P., Ebihara, S., Kanezaki, M., Suda, C., Nikkuni, E., Ebihara, T., . . . Kohzuki, M. (2010). Gender differences in perceptions of urge to cough and dyspnea induced by citric acid in healthy never smokers. *Chest*, *138*(5), 1166-1172.
- Haji, A., Kimura, S., & Ohi, Y. (2013). A model of the central regulatory system for cough reflex. *Biol Pharm Bull*, *36*(4), 501-508.
- Haji, A., Ohi, Y., & Kimura, S. (2012). Cough-related neurons in the nucleus tractus solitarius of decerebrate cats. *Neuroscience*, *218*, 100-109.
- Hannawi, Y., Hannawi, B., Rao, C. P., Suarez, J. I., & Bershady, E. M. (2013). Stroke-associated pneumonia: major advances and obstacles. *Cerebrovasc Dis*, *35*(5), 430-443.
- Hans, R., Thomas, S., Garla, B., Dagli, R. J., & Hans, M. K. (2016). Effect of Various Sugary Beverages on Salivary pH, Flow Rate, and Oral Clearance Rate amongst Adults. *Scientifica (Cairo)*, *2016*, 5027283.
- Hansson, L., Wollmer, P., Dahlback, M., & Karlsson, J. A. (1992). Regional sensitivity of human airways to capsaicin-induced cough. *Am Rev Respir Dis*, *145*(5), 1191-1195.
- Hasani, A., Pavia, D., Agnew, J. E., & Clarke, S. W. (1994). Regional lung clearance during cough and forced expiration technique (FET): effects of flow and viscoelasticity. *Thorax*, *49*(6), 557-561.
- Hegland, K. W., Bolser, D. C., & Davenport, P. W. (2012). Volitional control of reflex cough. *Journal of Applied Physiology*, *113*(1), 39-46.

- Hegland, K. W., Okun, M. S., & Troche, M. S. (2014). Sequential Voluntary Cough and Aspiration or Aspiration Risk in Parkinson's Disease. *Lung, 192*(4), 601-608.
- Hegland, K. W., Pitts, T., Bolser, D. C., & Davenport, P. W. (2011). Urge to cough with voluntary suppression following mechanical pharyngeal stimulation. *Bratislavske Lekarske Listy, 112*(3), 109-114.
- Hegland, K. W., Troche, M. S., Brandimore, A., Okun, M. S., & Davenport, P. W. (2016). Comparison of Two Methods for Inducing Reflex Cough in Patients With Parkinson's Disease, With and Without Dysphagia. *Dysphagia, 31*(1), 66-73.
- Hegland, K. W., Troche, M. S., Brandimore, A. E., Davenport, P. W., & Okun, M. S. (2014). Comparison of voluntary and reflex cough effectiveness in Parkinson's disease. *Parkinsonism Relat Disord, 20*(11), 1226-1230.
- Hegland, K. W., Troche, M. S., & Davenport, P. W. (2013). Cough expired volume and airflow rates during sequential induced cough. *Frontiers in physiology, 4*, 167.
- Heyder, J. (2004). Deposition of Inhaled Particles in the Human Respiratory Tract and Consequences for Regional Targeting in Respiratory Drug Delivery. *Proceedings of the American Thoracic Society, 1*(4), 315-320.
- Higgins, J., & Green, S. (2011). Cochrane Handbook for Systematic Reviews of Interventions (Version 5.1.0) *The Cochrane Collaboration*.
- Hiss, S. G., & Postma, G. N. (2003). Fiberoptic Endoscopic Evaluation of Swallowing. *Laryngoscope, 113*, 1386-1393.
- Hiss, S. G., Strauss, M., Treole, K., Stuart, A., & Boutilier, S. (2004). Effects of age, gender, bolus volume, bolus viscosity, and gustation on swallowing apnea onset relative to lingual bolus propulsion onset in normal adults. *J Speech Lang Hear Res, 47*(3), 572-583.

- Holas, M. A., Holas, M. A., DePippo, K. L., Depippo, K. L., Reding, M. J., & Reding, M. J. (1994). Aspiration and relative risk of medical complications following stroke. *Archives of Neurology*, *51*(10), 1051-1053.
- Hollie, M. C., Malone, R. A., Skufca, R. M., & Nelson, H. S. (1991). Extreme Variability in Aerosol Output of the DeVilbiss 646 Jet Nebulizer*. *Chest*, *100*(5), 1339-1344.
- Holma, B. (1989). Effects of inhaled acids on airway mucus and its consequences for health. *Environ Health Perspect*, *79*, 109-113.
- Holmes, S. (2016). A service evaluation of cough reflex testing to guide dysphagia management in the postsurgical adult head and neck patient population. *Curr Opin Otolaryngol Head Neck Surg*, *24*(3), 191-196.
- Horner, J., & Massey, E. W. (1988). Silent aspiration following stroke. *Neurology*, *38*(2), 317-319.
- Huckabee, M.-L., & Lamvik-Gozdzikowska, K. (2018). Reconsidering Rehabilitation for Neurogenic Dysphagia: Strengthening Skill in Swallowing. *Current Physical Medicine and Rehabilitation Reports*, *6*(3), 186-191.
- Hull, D., Wright, C., Cronin, J., Dobroszi, D., Hoke, S., Noronha, A., . . . Morice, A. (2002). Optimised delivery of an antitussive improves effectiveness. *European Respiratory Review*, *12*(85), 270-274.
- Humbert, I. A., Sunday, K. L., Karagiorgos, E., Vose, A. K., Gould, F., Greene, L., . . . Rivet, A. (2018). Swallowing Kinematic Differences Across Frozen, Mixed, and Ultrathin Liquid Boluses in Healthy Adults: Age, Sex, and Normal Variability. *J Speech Lang Hear Res*, *61*(7), 1544-1559.
- Hutcheson, K. A., Barrow, M. P., Warneke, C. L., Wang, Y., Eapen, G., Lai, S. Y., . . . Lewin, J. S. (2018). Cough strength and expiratory force in aspirating and nonaspirating

- postradiation head and neck cancer survivors: Expiratory Function and Aspiration in HNC. *The Laryngoscope*, 128(7), 1615-1621.
- Hutchings, H. A., Eccles, R., Smith, A. P., & Jawad, M. S. (1993b). Voluntary cough suppression as an indication of symptom severity in upper respiratory tract infections. *The European respiratory journal*, 6(10), 1449.
- Hutchings, H. A., Morris, S., Eccles, R., & Jawad, M. S. (1993a). Voluntary suppression of cough induced by inhalation of capsaicin in healthy volunteers. *Respiratory Medicine*, 87(5), 379-382.
- Inamoto, Y., Fujii, N., Saitoh, E., Baba, M., Okada, S., Katada, K., . . . Palmer, J. B. (2011). Evaluation of swallowing using 320-detector-row multislice CT. Part II: kinematic analysis of laryngeal closure during normal swallowing. *Dysphagia*, 26(3), 209-217.
- Jafari, S., Prince, R. A., Kim, D. Y., & Paydarfar, D. (2003). Sensory regulation of swallowing and airway protection: a role for the internal superior laryngeal nerve in humans. *J Physiol*, 550(Pt 1), 287-304.
- James, A., Kapur, K., & Hawthorne, A. B. (1998). Long-term outcome of percutaneous endoscopic gastrostomy feeding in patients with dysphagic stroke. *Age Ageing*, 27(6), 671-676.
- Janssens, T., Brepoels, S., Dupont, L., & Van den Bergh, O. (2015). The impact of harmfulness information on citric acid induced cough and urge-to-cough. *Pulmonary Pharmacology & Therapeutics*, 31, 9-14.
- Janssens, T., Silva, M., Davenport, P. W., Van Diest, I., Dupont, L. J., & Van den Bergh, O. (2014). Attentional modulation of reflex cough. *Chest*, 146(1), 135-141.
- Jean, A. (2001). Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiol Rev*, 81(2), 929-969.

- Jean, A., & Car, A. (1979). Inputs to the swallowing medullary neurons from the peripheral afferent fibers and the swallowing cortical area. *Brain Res*, 178(2-3), 567-572.
- Johansson, A. K., Lingström, P., Imfeld, T., Birkhed, D., Sahlgrenska, a., Göteborgs, u., . . . Institute of Odontology, D. o. C. (2004). Influence of drinking method on tooth-surface pH in relation to dental erosion. *European Journal of Oral Sciences*, 112(6), 484-489.
- Joos, G. F., & Connor, B. (2003). Indirect airway challenges. *European Respiratory Journal*, 21(6), 1050.
- Jordan, D. (2001). Central nervous pathways and control of the airways. *Respir Physiol*, 125(1-2), 67-81.
- Karlsson, J. A. (1996). The role of capsaicin-sensitive C-fibre afferent nerves in the cough reflex. *Pulmonary Pharmacology*, 9(5-6), 315-321.
- Karttunen, P. (1988b). Assessment of the antitussive effect of vadocaine hydrochloride using citric acid-induced cough in healthy volunteers. *Arzneimittel-Forschung*, 38(4A), 639-641.
- Kastelik, J. A., Thompson, R. H., Aziz, I., Ojoo, J. C., Redington, A. E., & Morice, A. H. (2002). Sex-related differences in cough reflex sensitivity in patients with chronic cough. *American Journal of Respiratory and Critical Care Medicine*, 166(7), 961-964.
- Kattenstroth, J. C., Kalisch, T., Peters, S., Tegenthoff, M., & Dinse, H. R. (2012). Long-term sensory stimulation therapy improves hand function and restores cortical responsiveness in patients with chronic cerebral lesions. Three single case studies. *Front Hum Neurosci*, 6, 244.
- Kattenstroth, J. C., Kalisch, T., Sczesny-Kaiser, M., Greulich, W., Tegenthoff, M., & Dinse, H. R. (2018). Daily repetitive sensory stimulation of the paretic hand for the treatment of sensorimotor deficits in patients with subacute stroke: RESET, a randomized, sham-controlled trial. *BMC neurology*, 18(1), 2-2.

- Kavalcikova-Bogdanova, N., Buday, T., Plevkova, J., & Song, W. J. (2016). Chronic Cough as a Female Gender Issue. *Advances in Experimental Medicine and Biology*, 905, 69.
- Kawasaki, A., Fukuda, H., Shiotani, A., & Kanzaki, J. (2001). Study of movements of individual structures of the larynx during swallowing. *Auris Nasus Larynx*, 28(1), 75-84.
- Kelsall, A., Decalmer, S., McGuinness, K., Woodcock, A., & Smith, J. A. (2009). Sex differences and predictors of objective cough frequency in chronic cough. *Thorax*, 64(5), 393-398.
- Kenia, P., Houghton, T., & Beardsmore, C. (2008). Does inhaling menthol affect nasal patency or cough? *Pediatric Pulmonology*, 43(6), 532-537.
- Kidd, D., Lawson, J., Nesbitt, R., & MacMahon, J. (1995). The natural history and clinical consequences of aspiration in acute stroke. *Qjm*, 88(6), 409-413.
- Kimura, Y., Takahashi, M., Wada, F., & Hachisuka, K. (2013). Differences in the peak cough flow among stroke patients with and without dysphagia. *Journal of UOEH*, 35(1), 9-16.
- King, M., Brock, G., & Lundell, C. (1985). *Clearance of mucus by simulated cough* (Vol. 58).
- Kleim, J. A., & Jones, T. A. (2008). Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage. *J Speech Lang Hear Res*, 51(1), S225-239.
- Kollarik, M., Ru, F., & Udem, B. J. (2007). Acid-sensitive vagal sensory pathways and cough. *Pulmonary Pharmacology & Therapeutics*, 20(4), 402-411.
- Kollarik, M., & Udem, B. J. (2002). Mechanisms of acid-induced activation of airway afferent nerve fibres in guinea-pig. *J Physiol*, 543(Pt 2), 591-600.
- Korpas, J., & Jakus, J. (2000). The expiration reflex from the vocal folds. *Acta physiologica Hungarica*, 87(3), 201.

- Korpas, J., Misik, A., & Kalocsayova, G. (1975). The expiration reflex in man. *Physiol Bohemoslov*, 24(3), 249-252.
- Kulnik, S. T., Birring, S. S., Hodsoll, J., Moxham, J., Rafferty, G. F., & Kalra, L. (2016). Higher cough flow is associated with lower risk of pneumonia in acute stroke. *Thorax*, 71(5), 474-475.
- Kulnik, S. T., MacBean, V., Birring, S. S., Moxham, J., Rafferty, G. F., & Kalra, L. (2015). Accuracy of portable devices in measuring peak cough flow. *PHYSIOLOGICAL MEASUREMENT*, 36(2), 243-257.
- Laciuga, H., Brandimore, A. E., Troche, M. S., & Hegland, K. W. (2016). Analysis of Clinicians' Perceptual Cough Evaluation. *Dysphagia*.
- Landers, M., Barker, G., Wallentine, S., McWhorter, J. W., & Peel, C. (2003). A comparison of tidal volume, breathing frequency, and minute ventilation between two sitting postures in healthy adults. *Physiother Theory Pract*, 19(2), 109-119.
- Langmore, S. E., Terpenning, M. S., Schork, A., Chen, Y., Murray, J. T., Lopatin, D., & Loesche, W. J. (1998). Predictors of aspiration pneumonia: how important is dysphagia? *Dysphagia*, 13(2), 69-81.
- Laude, E. A., Higgins, K. S., & Morice, A. H. (1993). A comparative study of the effects of citric acid, capsaicin and resiniferatoxin on the cough challenge in guinea-pig and man. *Pulmonary Pharmacology*, 6(3), 171-175.
- Lavietes, M. H., Smeltzer, S. C., Cook, S. D., Modak, R. M., & Smaldone, G. C. (1998). Airway dynamics, oesophageal pressure and cough. *The European respiratory journal*, 11(1), 156-161.
- Lavorini, F., Fontana, G. A., Pantaleo, T., Camiciottoli, G., Castellani, W., Maluccio, N. M., & Pistolesi, M. (2001). Fog-induced respiratory responses are attenuated by nedocromil

- sodium in humans. *American Journal of Respiratory & Critical Care Medicine*, 163(5), 1117-1120.
- Lavorini, F., Fontana, G. A., Pantaleo, T., Geri, P., Piumelli, R., Pistolesi, M., & Widdicombe, J. (2007). Fog-induced Cough with Impaired Respiratory Sensation in Congenital Central Hypoventilation Syndrome. *American Journal of Respiratory and Critical Care Medicine*, 176(8), 825-832.
- Lee, J. Y., Kim, D. K., Seo, K. M., & Kang, S. H. (2014). Usefulness of the simplified cough test in evaluating cough reflex sensitivity as a screening test for silent aspiration. *Ann Rehabil Med*, 38(4), 476-484.
- Lee, K., & Birring, S. S. (2012). Cough. *Medicine*, 40(4), 173-176.
- Lee, K. K., Matos, S., Ward, K., Rafferty, G. F., Moxham, J., Evans, D. H., & Birring, S. S. (2017). Sound: a non-invasive measure of cough intensity. *BMJ Open Respiratory Research*, 4(1), e000178.
- Lee, M. G., Macglashan, D. W., Jr., & Undem, B. J. (2005). Role of chloride channels in bradykinin-induced guinea pig airway vagal C-fibre activation. *J Physiol*, 566(Pt 1), 205-212.
- Lee, S. C., Kang, S.-W., Kim, M. T., Kim, Y. K., Chang, W. H., & Im, S. H. (2013). Correlation between voluntary cough and laryngeal cough reflex flows in patients with traumatic brain injury. *ARCHIVES OF PHYSICAL MEDICINE AND REHABILITATION*, 94(8), 1580-1583.
- Lekoubou, A., & Ovbiagele, B. (2016). Prevalance and influence of chronic obstructive pumony disease on stroke outcomes in hospitalized stroke patients. *eNeurologicalSci*, 6, 21-24.

- Leonard, R. J. P., White, C. M. A., McKenzie, S. M. S., & Belafsky, P. C. M. D. P. (2014). Effects of Bolus Rheology on Aspiration in Patients with Dysphagia. *Journal of the Academy of Nutrition and Dietetics*, *114*(4), 590-594.
- Leow, L. P., Beckert, L., Anderson, T., & Huckabee, M.-L. (2012). Changes in chemosensitivity and mechanosensitivity in aging and Parkinson's disease. *Dysphagia*, *27*(1), 106.
- Lin, K. H., Lai, Y. L., Wu, H. D., Wang, T. Q., & Wang, Y. H. (1999). Cough threshold in people with spinal cord injuries. *Phys Ther*, *79*(11), 1026-1031.
- Logemann, J. A., Kahrilas, P. J., & Cheng, J. (1992). Closure mechanism of laryngeal vestibule during swallowing. *Am J Physiol*, *21*(262), G338-G344.
- Loke, C., Lee, J., Sander, S., Mei, L., & Farella, M. (2016). Factors affecting intra-oral pH - a review. *J Oral Rehabil*, *43*(10), 778-785.
- Lowry, R. H., Wood, A. M., & Higenbottam, T. W. (1988). Effects of pH and osmolarity on aerosol-induced cough in normal volunteers. *Clinical science (London, England : 1979)*, *74*(4), 373.
- Magni, C., Chellini, E., Lavorini, F., Fontana, G. A., & Widdicombe, J. (2011). Voluntary and reflex cough: Similarities and differences. *Pulmonary Pharmacology & Therapeutics*, *24*(3), 308-311.
- Mamun, K., & Lim, J. (2005). Role of nasogastric tube in preventing aspiration pneumonia in patients with dysphagia. *Singapore Med J*, *46*(11), 627-631.
- Mandal, N. G. (2006). Respirometers including spirometer, pneumotachograph and peak flow meter. *Anaesthesia & Intensive Care Medicine*, *7*(1), 1-5.
- Mann, G. (2002). *MASA, the Mann Assessment of Swallowing Ability*: Singular Thomson Learning.

- Martin, B. J., Logemann, J. A., Shaker, R., & Dodds, W. J. (1994). Coordination between respiration and swallowing: respiratory phase relationships and temporal integration. *Journal of Applied Physiology*, *76*(2), 714-723.
- Martin-Harris, B. (2006). Coordination of respiration and swallowing. *GI Motility online*.
- Martin-Harris, B. (2008). Clinical implications of respiratory-swallowing interactions. *Curr Opin Otolaryngol Head Neck Surg*, *16*(3), 194-199.
- Martin-Harris, B., Brodsky, M. B., Michel, Y., Castell, D. O., Schleicher, M., Sandidge, J., . . . Blair, J. (2008). MBS Measurement Tool for Swallow Impairment—MBSImp: Establishing a Standard. *Dysphagia*, *23*(4), 392-405.
- Martin-Harris, B., Brodsky, M. B., Michel, Y., Ford, C. L., Walters, B., & Heffner, J. (2005). Breathing and swallowing dynamics across the adult lifespan. *Arch Otolaryngol Head Neck Surg*, *131*, 762-770.
- Martin-Harris, B., & Jones, B. (2008). The videofluorographic swallowing study. *Physical medicine and rehabilitation clinics of North America*, *19*(4), 769-viii.
- Matsuo, K., Hiiemae, K. M., Gonzalez-Fernandez, M., & Palmer, J. B. (2008). Respiration during feeding on solid food: alterations in breathing during mastication, pharyngeal bolus aggregation, and swallowing. *J Appl Physiol* (1985), *104*(3), 674-681.
- Matsuo, K., & Palmer, J. B. (2009). Coordination of Mastication, Swallowing and Breathing. *Jpn Dent Sci Rev*, *45*(1), 31-40.
- Mazzone, S. B. (2016). A Cough Is a Cough, Is It Not? Neurophenotypes Define Patients with Chronic Cough. *American Journal of Respiratory and Critical Care Medicine*, *193*(12), 1324-1326.
- Mazzone, S. B., Cole, L. J., Ando, A., Egan, G. F., & Farrell, M. J. (2011). Investigation of the Neural Control of Cough and Cough Suppression in Humans Using Functional Brain Imaging. *The Journal of Neuroscience*, *31*(8), 2948.

- Mazzone, S. B., & McGovern, A. E. (2006). Na⁺-K⁺-2Cl⁻ cotransporters and Cl⁻ channels regulate citric acid cough in guinea pigs. *J Appl Physiol (1985)*, *101*(2), 635-643.
- Mazzone, S. B., McLennan, L., McGovern, A. E., Egan, G. F., & Farrell, M. J. (2007). Representation of capsaicin-evoked urge-to-cough in the human brain using functional magnetic resonance imaging. *American Journal of Respiratory & Critical Care Medicine*, *176*(4), 327-332.
- Mazzone, S. B., Mori, N., & Canning, B. J. (2005). Synergistic interactions between airway afferent nerve subtypes regulating the cough reflex in guinea-pigs. *J Physiol*, *569*(2), 559-573.
- Mazzone, S. B., & Udem, B. J. (2016). Vagal Afferent Innervation of the Airways in Health and Disease. *Physiol Rev*, *96*(3), 975-1024.
- McCullough, G. H., & Martino, R. (2013). Clinical Evaluation of Patients with Dysphagia: Importance of History Taking and Physical Exam. In R. Shaker, C. Easterling, P. C. Belafsky, & G. N. Postma (Eds.), *Manual of Diagnostic and Therapeutic Techniques for Disorders of Deglutition* (pp. 11-30). New York, NY: Springer New York.
- McCullough, G. H., Rosenbek, J. C., Wertz, R. T., McCoy, S., Mann, G., & McCullough, K. (2005). Utility of clinical swallowing examination measures for detecting aspiration post-stroke. *J Speech Lang Hear Res*, *48*(6), 1280-1293.
- McGarvey, L. P. A., Heaney, L. G., Lawson, J. T., Johnston, B. T., Scally, C. M., Ennis, M., . . . MacMahon, J. (1998). Evaluation and outcome of patients with chronic non-productive cough using a comprehensive diagnostic protocol. *53*(9), 738-743.
- McGovern, A. E., Ajayi, I. E., Farrell, M. J., & Mazzone, S. B. (2017). A neuroanatomical framework for the central modulation of respiratory sensory processing and cough by the periaqueductal grey. *Journal of Thoracic Disease*, *9*(10), 4098-4107.

- McGovern, A. E., Davis-Poynter, N., Yang, S. K., Simmons, D. G., Farrell, M. J., & Mazzone, S. B. (2015). Evidence for multiple sensory circuits in the brain arising from the respiratory system: an anterograde viral tract tracing study in rodents. *Brain Struct Funct*, 220(6), 3683-3699.
- McGovern, A. E., Driessen, A. K., Simmons, D. G., Powell, J., Davis-Poynter, N., Farrell, M. J., & Mazzone, S. B. (2015). Distinct Brainstem and Forebrain Circuits Receiving Tracheal Sensory Neuron Inputs Revealed Using a Novel Conditional Anterograde Transsynaptic Viral Tracing System. *The Journal of Neuroscience*, 35(18), 7041.
- Mei, N., Condamin, M., & Boyer, A. (1980). The composition of the vagus nerve of the cat. *Cell Tissue Res*, 209(3), 423-431.
- Menetrey, D., & Basbaum, A. I. (1987). Spinal and trigeminal projections to the nucleus of the solitary tract: a possible substrate for somatovisceral and viscerovisceral reflex activation. *J Comp Neurol*, 255(3), 439-450.
- Midgren, B., Hansson, L., Karlsson, J. A., Simonsson, B. G., & Persson, C. G. A. (1992). Capsaicin-induced cough in humans. *American Review of Respiratory Disease*, 146(2), 347-351.
- Miles, A., & Huckabee, M.-L. (2013). Intra- and inter-rater reliability for judgement of cough following citric acid inhalation. *International Journal of Speech-Language Pathology*, 15(2), 209-215.
- Miles, A., McFarlane, M., & Huckabee, M. L. (2014). Inter-rater reliability for judgment of cough following citric acid inhalation after training. *Speech, Language and Hearing*, 17(4), 204-209.
- Miles, A., Moore, S., McFarlane, M., Lee, F., Allen, J., & Huckabee, M. L. (2013). Comparison of cough reflex test against instrumental assessment of aspiration. *Physiology & Behavior*, 118, 25-31.

- Miles, A., Zeng, I. S., McLauchlan, H., & Huckabee, M. L. (2013). Cough reflex testing in Dysphagia following stroke: a randomized controlled trial. *Journal of Clinical Medicine Research*, 5(3), 222-233.
- Miller, A. J. (1972). Characteristics of the swallowing reflex induced by peripheral nerve and brain stem stimulation. *Exp Neurol*, 34(2), 210-222.
- Miller, M. R., Dickinson, S. A., & Hitchings, D. J. (1992). The accuracy of portable peak flow meters. *Thorax*, 47(11), 904-909.
- Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., . . . Wanger, J. (2005). Standardisation of spirometry. 26(2), 319-338.
- Mills, C., Jones, R., & Huckabee, M. L. (2017). Measuring voluntary and reflexive cough strength in healthy individuals. *Respiratory Medicine*, 132, 95-101.
- Mincheva, R. K., Kralimarkova, T. Z., Rasheva, M., Dimitrov, Z., Nedeva, D., Staevska, M., . . . Popov, T. A. (2014). A real - life observational pilot study to evaluate the effects of two-week treatment with montelukast in patients with chronic cough. *Cough (London, England)*, 10(1), 2.
- Monroe, M. D., Manco, K., Bennett, R., & Huckabee, M.-L. (2014). Citric acid cough reflex test: Establishing normative data. *Speech, Language and Hearing*, 17(4), 216-224.
- Moore, V. C. (2012). Spirometry: step by step. *Breathe*, 8(3), 232.
- Morice, A. H., Brown, M. J., Lowry, R., & Higenbottam, T. (1987). Angiotensin-converting enzyme and the cough reflex. *Lancet*, 2(8568), 1116-1118.
- Morice, A. H., Fontana, G. A., Belvisi, M. G., Birring, S. S., Chung, K. F., Dicpinigaitis, P. V., . . . Widdicombe, J. (2007). ERS guidelines on the assessment of cough. *European Respiratory Journal*, 29(6), 1256-1276.
- Morice, A. H., Higgins, K. S., & Yeo, W. W. (1992). Adaptation of cough reflex with different types of stimulation. *European Respiratory Journal*, 5(7), 841-847.

- Morice, A. H., Kastelik, J. A., & Thompson, R. (2001). Cough challenge in the assessment of cough reflex. *British Journal of Clinical Pharmacology*, 52(4), 365-375.
- Morice, A. H., Marshall, A. E., Higgins, K. S., & Grattan, T. J. (1994). Effect of inhaled menthol on citric acid induced cough in normal subjects. *Thorax*, 49(10), 1024-1026.
- Mu, L., & Sanders, I. (2000). Sensory nerve supply of the human oro- and laryngopharynx: a preliminary study. *Anat Rec*, 258(4), 406-420.
- Murdoch, J. R., & Lloyd, C. M. (2010). Chronic inflammation and asthma. *Mutation research*, 690(1-2), 24-39.
- Mutolo, D. (2017). Brainstem mechanisms underlying the cough reflex and its regulation. *Respir Physiol Neurobiol*, 243, 60-76.
- Nakajoh, K., Nakagawa, T., Sekizawa, K., Matsui, T., Arai, H., & Sasaki, H. (2000). Relation between incidence of pneumonia and protective reflexes in post-stroke patients with oral or tube feeding. *Journal of Internal Medicine*, 247(1), 39-42.
- Nakashima, T., Maeda, K., Tahira, K., Taniguchi, K., Mori, K., Kiyomiya, H., & Akagi, J. (2018). Silent aspiration predicts mortality in older adults with aspiration pneumonia admitted to acute hospitals: Silent aspiration in pneumonia. *Geriatrics & Gerontology International*, 18(6), 828-832.
- Nakazawa, H., Sekizawa, K., Ujiie, Y., Sasaki, H., & Takishima, T. (1993). Risk of Aspiration Pneumonia in the Elderly. *Chest*, 103(5), 1636-1637.
- Nejla, S., Fujimura, M., & Kamio, Y. (2000). Comparison between tidal breathing and dosimeter methods in assessing cough receptor sensitivity to capsaicin. *Respirology*, 5(4), 337-342.
- Nishino, T., Isono, S., Shinozuka, N., & Ishikawa, T. (2008). Coughing induced by airway irritation modulates the sensation of air hunger. *J Physiol*, 586(2), 649-658.

- Nishino, T., Tagaito, Y., & Isono, S. (1996). Cough and Other Reflexes on Irritation of Airway Mucosa in Man. *Pulmonary Pharmacology*, 9(5-6), 285-292.
- Noel, P. R. (1962). The evaluation of a new cough suppressant, "Detigon," in general practice. *British Journal of Diseases of the Chest*, 56(2), 70-77.
- O'Callaghan, C., & Barry, P. W. (1997). The science of nebulised drug delivery. *Thorax*, 52 Suppl 2(Supplement 2), S31-S44.
- O'Keefe, S. T. (2018). Use of modified diets to prevent aspiration in oropharyngeal dysphagia: is current practice justified? *BMC geriatrics*, 18(1), 167-167.
- OECD, O. f. E. C.-o. a. D. (2001). Citric Acid, SIDS Initial Assessment Profile. *United Nations Environment Documents Repository*.
- Ogihara, T., Mikami, H., Katahira, K., & Otsuka, A. (1991). Comparative study of the effects of three angiotensin converting enzyme inhibitors on the cough reflex. *American Journal of Hypertension*, 4(1), 46S-51S.
- Ohmae, Y., Logemann, J. A., Kaiser, P., Hanson, D. G., & Kahrilas, P. J. (1995). Timing of glottic closure during normal swallow. *Head Neck Surg*, 17, 394-402.
- Palmer, J. B., Hiiemae, K. M., Matsuo, K., & Haishima, H. (2007). Volitional control of food transport and bolus formation during feeding. *Physiology & Behavior*, 91(1), 66-70.
- Pavia, D., Agnew, J. E., & Clarke, S. W. (1987). Cough and mucociliary clearance. *Bull Eur Physiopathol Respir*, 23 Suppl 10, 41s-45s.
- Pearson, W. G., Jr., Hindson, D. F., Langmore, S. E., & Zumwalt, A. C. (2013). Evaluating swallowing muscles essential for hyolaryngeal elevation by using muscle functional magnetic resonance imaging. *Int J Radiat Oncol Biol Phys*, 85(3), 735-740.
- Pearson, W. G., Jr., Langmore, S. E., Yu, L. B., & Zumwalt, A. C. (2012). Structural analysis of muscles elevating the hyolaryngeal complex. *Dysphagia*, 27(4), 445-451.

- Pecova, R., Javorkova, N., Kudlicka, J., & Tatar, M. (2007). Tussigenic agents in the measurement of cough reflex sensitivity. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society*, 58 Suppl 5(Pt 2), 531-538.
- Perry, S., & Huckabee, M. L. (2017). Does diurnal variation in cough reflex testing exist in healthy young adults? *Respir Physiol Neurobiol*, 239, 70-74.
- Perry, S. E., Miles, A., Fink, J. N., & Huckabee, M. L. (2019). The Dysphagia in Stroke Protocol Reduces Aspiration Pneumonia in Patients with Dysphagia Following Acute Stroke: a Clinical Audit. *Transl Stroke Res*, 10(1), 36-43.
- Pikus, L., Levine, M. S., Yang, Y. X., Rubesin, S. E., Katzka, D. A., Laufer, I., & Gefter, W. B. (2003). Videofluoroscopic studies of swallowing dysfunction and the relative risk of pneumonia. *AJR Am J Roentgenol*, 180(6), 1613-1616.
- Pitts, T. (2014). Airway Protective Mechanisms. *Lung*, 192(1), 27-31.
- Pitts, T., Bolser, D., Rosenbek, J., Troche, M., Okun, M. S., & Sapienza, C. (2009). Impact of expiratory muscle strength training on voluntary cough and swallow function in Parkinson disease. *Chest*, 135(5), 1301-1308.
- Pitts, T., Bolser, D., Rosenbek, J., Troche, M., & Sapienza, C. (2008). Voluntary cough production and swallow dysfunction in Parkinson's disease. *Dysphagia*, 23(3), 297-301.
- Pitts, T., Rose, M. J., Mortensen, A. N., Poliacek, I., Sapienza, C. M., Lindsey, B. G., . . . Bolser, D. C. (2013). Coordination of cough and swallow: a meta-behavioral response to aspiration. *Respir Physiol Neurobiol*, 189(3), 543-551.
- Pitts, T., Troche, M. S., Mann, G., Rosenbek, J., Okun, M. S., & Sapienza, C. (2010). USING voluntary cough to detect penetration and aspiration during oropharyngeal swallowing in patients with parkinson disease. *Chest*, 138(6), 1426-1431.

- Pleger, B., Dinse, H. R., Ragert, P., Schwenkreis, P., Malin, J. P., & Tegenthoff, M. (2001). Shifts in cortical representations predict human discrimination improvement. *Proceedings of the National Academy of Sciences*, *98*(21), 12255-12260.
- Pleger, B., Foerster, A. F., Ragert, P., Dinse, H. R., Schwenkreis, P., Malin, J. P., . . . Tegenthoff, M. (2003). Functional imaging of perceptual learning in human primary and secondary somatosensory cortex. *Neuron*, *40*(3), 643-653.
- Plowman, E. K., Watts, S. A., Robison, R., Tabor, L., Dion, C., Gaziano, J., . . . Gooch, C. (2016). Voluntary Cough Airflow Differentiates Safe Versus Unsafe Swallowing in Amyotrophic Lateral Sclerosis. *Dysphagia*, *31*(3), 383-390.
- Polverino, M., Poverino, F., Fasolino, M., Andò, F., Alfieri, A., & De Blasio, F. (2012). Anatomy and neuro-pathophysiology of the cough reflex arc. *Multidisciplinary Respiratory Medicine*, *7*(1), 5-5.
- Power, M., Fraser, C., Hobson, A., Rothwell, J. C., Mistry, S., Nicholson, D. A., . . . Hamdy, S. (2004). Changes in pharyngeal corticobulbar excitability and swallowing behavior after oral stimulation. *Am J Physiol Gastrointest Liver Physiol*, *286*(1), G45-50.
- Prinz, J. F., & Lucas, P. W. (1997). An optimization model for mastication and swallowing in mammals. *Proc Biol Sci*, *264*(1389), 1715-1721.
- Quanjer, P. H., Stanojevic, S., Cole, T. J., Baur, X., Hall, G. L., Culver, B. H., . . . Stocks, J. (2012). Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *European Respiratory Journal*, *40*(6), 1324-1343.
- Ra, J. Y., Hyun, J. K., Ko, K. R., & Lee, S. J. (2014). Chin tuck for prevention of aspiration: effectiveness and appropriate posture. *Dysphagia*, *29*(5), 603-609.
- Rai, Z. L., Fowles, H. E., Wright, C., Howard, J., & Morice, A. H. (2018). The effect of pH on citric acid cough challenge: A randomised control trial in chronic cough and healthy volunteers. *Respir Physiol Neurobiol*, *257*, 51-54.

- Ramsay, J., Wright, C., Thompson, R., Hull, D., & Morice, A. H. (2008). Assessment of antitussive efficacy of dextromethorphan in smoking related cough: objective vs. subjective measures. *British Journal of Clinical Pharmacology*, *65*(5), 737-741.
- Ramsey, D. J., Smithard, D. G., & Kalra, L. (2003). Early assessments of dysphagia and aspiration risk in acute stroke patients. *Stroke*, *34*(5), 1252-1257.
- Regan, J., Walshe, M., & Tobin, W. O. (2010). Immediate effects of thermal-tactile stimulation on timing of swallow in idiopathic parkinson's disease. *Dysphagia*, *25*(3), 207-215.
- Ricciardolo, F. L. (2001). Mechanisms of citric acid-induced bronchoconstriction. *Am J Med*, *111 Suppl 8A*, 18S-24S.
- Ricciardolo, F. L., Rado, V., Fabbri, L. M., Sterk, P. J., Di Maria, G. U., & Geppetti, P. (1999). Bronchoconstriction induced by citric acid inhalation in guinea pigs: role of tachykinins, bradykinin, and nitric oxide. *American Journal of Respiratory and Critical Care Medicine*, *159*(2), 557.
- Ricco, M. M., Kummer, W., Biglari, B., Myers, A. C., & Udem, B. J. (1996). Interganglionic segregation of distinct vagal afferent fibre phenotypes in guinea-pig airways. *J Physiol*, *496 (Pt 2)*, 521-530.
- Rietveld, S., Beest, I. V., & Everaerd, W. (2000). Psychological confounds in medical research: the example of excessive cough in asthma. *Behaviour Research and Therapy*, *38*(8), 791-800.
- Robbins, J., Butler, S. G., Daniels, S. K., Diez Gross, R., Langmore, S., Lazarus, C. L., . . . Rosenbek, J. (2008). Swallowing and dysphagia rehabilitation: translating principles of neural plasticity into clinically oriented evidence. *J Speech Lang Hear Res*, *51*(1), S276-300.

- Rossi, B., Siciliano, G., Carboncini, M. C., Manca, M. L., Massetani, R., Viacava, P., & Muratorio, A. (1996). Muscle modifications in Parkinson's disease: myoelectric manifestations. *Electroencephalogr Clin Neurophysiol*, *101*(3), 211-218.
- Rostami-Hodjegan, A., Abdul-Manap, R., Wright, C. E., Tucker, G. T., & Morice, A. H. (2001). The placebo response to citric acid-induced cough: pharmacodynamics and gender differences. *Pulmonary Pharmacology & Therapeutics*, *14*(4), 315-319.
- Rubin, B. K. (2014). Secretion properties, clearance, and therapy in airway disease. *Translational respiratory medicine*, *2*, 6-6.
- Rubin, B. K., MacLeod, P. M., Sturgess, J., & King, M. (1991). Recurrent respiratory infections in a child with fucosidosis: is the mucus too thin for effective transport? *Pediatric Pulmonology*, *10*(4), 304-309.
- Ryan, G., Dolovich, M. B., Obminski, G., Cockcroft, D. W., Juniper, E., Hargreave, F. E., & Newhouse, M. T. (1981). Standardization of inhalation provocation tests: influence of nebulizer output, particle size, and method of inhalation. *J Allergy Clin Immunol*, *67*(2), 156-161.
- Saconato, M., Chiari, B. M., Lederman, H. M., & Gonçalves, M. I. R. (2015). Effectiveness of Chin-tuck Maneuver to Facilitate Swallowing in Neurologic Dysphagia. *International Archives of Otorhinolaryngology*, *20*(1), 013-017.
- Saetta, M., Di Stefano, A., Turato, G., De Caro, R., Bordignon, D., Holgate, S. T., & Fabbri, L. M. (1995). Fatal asthma attack during an inhalation challenge with ultrasonically nebulized distilled water. *J Allergy Clin Immunol*, *95*(6), 1285-1287.
- Salam, A., Tilluckdharry, L., Amoateng-Adjepong, Y., & Manthous, C. A. (2004). Neurologic status, cough, secretions and extubation outcomes. *Intensive Care Medicine*, *30*(7), 1334-1339.

- Sanders, I., Wu, B. L., Mu, L., Li, Y., & Biller, H. F. (1993). The innervation of the human larynx. *Arch Otolaryngol Head Neck Surg*, *119*(9), 934-939.
- Sant'Ambrogio, G. (1987). Afferent pathways for the cough reflex. *Bull Eur Physiopathol Respir*, *23 Suppl 10*, 19s-23s.
- Sarkar, M., Madabhavi, I., Niranjana, N., & Dogra, M. (2015). Auscultation of the respiratory system. *Annals of thoracic medicine*, *10*(3), 158-168.
- Sato, M., Tohara, H., Iida, T., Wada, S., Inoue, M., & Ueda, K. (2012). Simplified cough test for screening silent aspiration. *ARCHIVES OF PHYSICAL MEDICINE AND REHABILITATION*, *93*(11), 1982-1986.
- Schmidt, D., Jörres, R. A., & Magnussen, H. (1997). Citric acid-induced cough thresholds in normal subjects, patients with bronchial asthma, and smokers. *European journal of medical research*, *2*(9), 384-388.
- Schmidt, J., Holas, M., Halvorson, K., & Reding, M. (1994). Videofluoroscopic evidence of aspiration predicts pneumonia and death but not dehydration following stroke. *Dysphagia*, *9*(1), 7-11.
- Schoeffel, R. E., Anderson, S. D., & Altounyan, R. E. (1981). Bronchial hyperreactivity in response to inhalation of ultrasonically nebulised solutions of distilled water and saline. *Br Med J (Clin Res Ed)*, *283*(6302), 1285-1287.
- Sekizawa, K., Ujiie, Y., Itabashi, S., Sasaki, H., & Takishima, T. (1990). Lack of cough reflex in aspiration pneumonia. *Lancet*, *335*(8699), 1228-1229.
- Sevelius, H., Lester, P. D., & Colmore, J. P. (1965). Objective evaluation of antitussive agents. *Clinical Pharmacology and Therapeutics*, *6*(2), 146-151.
- Shaker, R., Dodds, W. J., Dantas, R. O., Hogan, W. J., & Arndorfer, R. C. (1990). Coordination of deglutitive glottic closure with oropharyngeal swallowing. *Gastroenterology*, *98*(6), 1478-1484.

- Shaker, R., Dua, K. S., Ren, J., Xie, P., Funahashi, A., & Schapira, R. M. (2002). Vocal cord closure pressure during volitional swallow and other voluntary tasks. *Dysphagia*, *17*(1), 13-18.
- Sheppard, D., Rizk, N. W., Boushey, H. A., & Bethel, R. A. (1983). Mechanism of cough and bronchoconstriction induced by distilled water aerosol. *Am Rev Respir Dis*, *127*(6), 691.
- Shi, Y., Liu, H., Wang, Y., Cai, M., & Xu, W. (2018). *Theory and Application of Audio-Based Assessment of Cough %J Journal of Sensors* (Vol. 2018).
- Silverman, E. P., Carnaby, G., Singletary, F., Hoffman-Ruddy, B., Yeager, J., & Sapienza, C. (2016). Measurement of Voluntary Cough Production and Airway Protection in Parkinson Disease. *Arch Phys Med Rehabil*, *97*(3), 413-420.
- Silverman, E. P., Carnaby-Mann, G., Pitts, T., Davenport, P., Okun, M. S., & Sapienza, C. (2014). Concordance and discriminatory power of cough measurement devices for individuals with Parkinson disease. *Chest*, *145*(5), 1089-1096.
- Simonsson, B. G., Jacobs, F. M., & Nadel, J. A. (1967). Role of autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airway disease. *The Journal of clinical investigation*, *46*(11), 1812-1818.
- Smina, M., Salam, A., Khamiees, M., Gada, P., Amoateng-Adjepong, Y., & Manthous, C. A. (2003). Cough peak flows and extubation outcomes. *Chest*, *124*(1), 262-268.
- Smith, E. C., Denyer, J., & Kendrick, A. H. (1995). Comparison of twenty three nebulizer/compressor combinations for domiciliary use. *The European respiratory journal*, *8*(7), 1214.
- Smith Hammond, C. (2008). Cough and aspiration of food and liquids due to oral pharyngeal Dysphagia. *Lung*, *186 Suppl 1*, S35-40.

- Smith Hammond, C. A., & Goldstein, L. B. (2006). Cough and aspiration of food and liquids due to oral-pharyngeal dysphagia: ACCP evidence-based clinical practice guidelines. *Chest*, *129*(1 Suppl), 154S.
- Smith Hammond, C. A., Goldstein, L. B., Horner, R. D., Ying, J., Gray, L., Gonzalez-Rothi, L., & Bolser, D. C. (2009). Predicting aspiration in patients with ischemic stroke: comparison of clinical signs and aerodynamic measures of voluntary cough. *Chest*, *135*(3), 769-777.
- Smith Hammond, C. A., Goldstein, L. B., Zajac, D. J., Gray, L., Davenport, P. W., & Bolser, D. C. (2001). Assessment of aspiration risk in stroke patients with quantification of voluntary cough. *Neurology*, *56*(4), 502-506.
- Smith, J. (2007). Ambulatory methods for recording cough. *Pulmonary Pharmacology & Therapeutics*, *20*(4), 313-318.
- Smith, J., Owen, E., Earis, J., & Woodcock, A. (2006). Effect of codeine on objective measurement of cough in chronic obstructive pulmonary disease. *The Journal of Allergy and Clinical Immunology*, *117*(4), 831-835.
- Smith, J. A., Aliverti, A., Quaranta, M., McGuinness, K., Kelsall, A., Earis, J., & Calverley, P. M. (2012). Chest wall dynamics during voluntary and induced cough in healthy volunteers. *J Physiol*, *590*(3), 563-574.
- Smith, J. A., McGarvey, L. P. A., Badri, H., Satia, I., Warren, F., Siederer, S., . . . Marks-Konczalik, J. (2017). Effects of a novel sodium channel blocker, GSK2339345, in patients with refractory chronic cough. *International journal of clinical pharmacology and therapeutics*, *55*(9), 712-719.
- Smithard, D. G., O'Neill, P. A., Park, C., England, R., Renwick, D. S., Wyatt, R., . . . Martin, D. F. (1998). Can bedside assessment reliably exclude aspiration following acute stroke? *Age Ageing*, *27*(2), 99-106.

- Snyder, J. S., Schwiedrzik, C. M., Vitela, A. D., & Melloni, L. (2015). How previous experience shapes perception in different sensory modalities. *Front Hum Neurosci*, *9*, 594.
- Sohn, D., Park, G. Y., Koo, H., Jang, Y., Han, Y., & Im, S. (2018). Determining Peak Cough Flow Cutoff Values to Predict Aspiration Pneumonia Among Patients With Dysphagia Using the Citric Acid Reflexive Cough Test. *Arch Phys Med Rehabil*, *99*(12), 2532-2539.e2531.
- Spektor, D. M., Yen, B. M., & Lippmann, M. (1989). Effect of concentration and cumulative exposure of inhaled sulfuric acid on tracheobronchial particle clearance in healthy humans. *Environmental Health Perspectives*, *79*, 167-172.
- Splaingard, M. L., Hutchins, B., Sulton, L. D., & Chaudhuri, G. (1988). Aspiration in rehabilitation patients: videofluoroscopy vs bedside clinical assessment. *Arch Phys Med Rehabil*, *69*(8), 637-640.
- Steele, C. M., & Grace-Martin, K. (2017). Reflections on Clinical and Statistical Use of the Penetration-Aspiration Scale. *Dysphagia*, *32*(5), 601-616.
- Steele, C. M., & Miller, A. J. (2010). Sensory input pathways and mechanisms in swallowing: a review. *Dysphagia*, *25*(4), 323-333.
- Stephens, R. E., Addington, W. R., Miller, S. P., & Anderson, J. W. (2003). Videofluoroscopy of the diaphragm during voluntary and reflex cough in humans. *Am J Phys Med Rehabil*, *82*(5), 384.
- Stephens, R. E., Addington, W. R., & Widdicombe, J. (2003). Effect of acute unilateral middle cerebral artery infarcts on voluntary cough and the laryngeal cough reflex. *AMERICAN JOURNAL OF PHYSICAL MEDICINE & REHABILITATION*, *82*(5), 379-383.

- Stockwell, M., Lang, S., Yip, R., Zintel, T., White, C., & Gallagher, C. G. (1993). Lack of importance of the superior laryngeal nerves in citric acid cough in humans. *J Appl Physiol* (1985), 75(2), 613-617.
- Storey, A. T. (1968). A functional analysis of sensory units innervating epiglottis and larynx. *Exp Neurol*, 20(3), 366-383.
- Subburaj, S., Parvez, L., & Rajagopalan, T. G. (1996). Methods of Recording and Analysing Cough Sounds. *Pulmonary Pharmacology*, 9(5), 269-279.
- Suiter, D. M., & Leder, S. B. (2008). Clinical utility of the 3-ounce water swallow test. *Dysphagia*, 23(3), 244-250.
- Sullivan, C. E., Murphy, E., Kozar, L. F., & Phillipson, E. A. (1978). Waking and ventilatory responses to laryngeal stimulation in sleeping dogs. *J Appl Physiol Respir Environ Exerc Physiol*, 45(5), 681-689.
- Tabor-Gray, L. C., Gallestagui, A., Vasilopoulos, T., & Plowman, E. K. (2019). Characteristics of impaired voluntary cough function in individuals with amyotrophic lateral sclerosis. *Amyotrophic lateral sclerosis & frontotemporal degeneration*, 1-6.
- Takara, G. N., Ruas, G., Pessoa, B. V., Jamami, L. K., Di Lorenzo, V. A., & Jamami, M. (2010). Comparison of five portable peak flow meters. *Clinics (Sao Paulo)*, 65(5), 469-474.
- Tanaka, Y., Yoshida, Y., & Hirano, M. (1995). Expression of Fos-protein activated by tactile stimulation on the laryngeal vestibulum in the cat's lower brain stem. *J Laryngol Otol*, 109(1), 39-44.
- Tandon, R., Smaldone, G. C., & McPeck, M. (1997). Measuring Nebulizer Output: Aerosol Production vs Gravimetric Analysis. *Chest*, 111(5), 1361-1365.
- Taylor, D. R., Reid, W. D., Pare, P. D., & Fleetham, J. A. (1988). Cigarette smoke inhalation patterns and bronchial reactivity. *Thorax*, 43(1), 65-70.

- Teismann, I. K., Steinsträter, O., Warnecke, T., Suntrup, S., Ringelstein, E. B., Pantev, C., & Dziewas, R. (2009). Tactile thermal oral stimulation increases the cortical representation of swallowing. *BMC Neurosci*, *10*(1), 71-71.
- Terzi, N., Orlikowski, D., Aegerter, P., Lejaille, M., Ruquet, M., Zalcman, G., . . . Lofaso, F. (2007). Breathing-swallowing interaction in neuromuscular patients: a physiological evaluation. *American Journal of Respiratory & Critical Care Medicine*, *175*(3), 269-276.
- Thomson, N. C. (1979). The effect of different pharmacological agents on respiratory reflexes in normal and asthmatic subjects. *Clinical Science*, *56*(3), 235-241.
- Thorpe, C. W., Toop, L. J., & Dawson, K. P. (1992). Towards a quantitative description of asthmatic cough sounds. *European Respiratory Journal*, *5*(6), 685.
- Troche, M. S., Brandimore, A. E., Godoy, J., & Hegland, K. W. (2014). A framework for understanding shared substrates of airway protection. *Journal of Applied Oral Science*, *22*(4), 251-260.
- Troche, M. S., Brandimore, A. E., Okun, M. S., Davenport, P. W., & Hegland, K. W. (2014). Decreased cough sensitivity and aspiration in Parkinson disease. *Chest*, *146*(5), 1294.
- Troche, M. S., Okun, M. S., Rosenbek, J. C., Musson, N., Fernandez, H. H., Rodriguez, R., . . . Sapienza, C. M. (2010). Aspiration and swallowing in Parkinson disease and rehabilitation with EMST: a randomized trial. *Neurology*, *75*(21), 1912-1919.
- Troche, M. S., Schumann, B., Brandimore, A. E., Okun, M. S., & Hegland, K. W. (2016). Reflex Cough and Disease Duration as Predictors of Swallowing Dysfunction in Parkinson's Disease. *Dysphagia*, *31*(6), 757-764.
- Tsujimura, T., Udemgba, C., Inoue, M., & Canning, B. J. (2013). Laryngeal and tracheal afferent nerve stimulation evokes swallowing in anaesthetized guinea pigs. *J Physiol*, *591*(18), 4667-4679.

- Umayahara, Y., Soh, Z., Sekikawa, K., Kawae, T., Otsuka, A., & Tsuji, T. (2018a). A Mobile Cough Strength Evaluation Device Using Cough Sounds. *Sensors (Basel)*, 18(11).
- Umayahara, Y., Soh, Z., Sekikawa, K., Kawae, T., Otsuka, A., & Tsuji, T. (2018b). Estimation of Cough Peak Flow Using Cough Sounds. *Sensors (Basel)*, 18(7).
- Urbaniak, G. C., & Plous, S. (2013). Research Randomizer (Version 4.0). [*Computer Software*].
- Utell, M. J. (1985). Effects of inhaled acid aerosols on lung mechanics: an analysis of human exposure studies. *Environ Health Perspect*, 63, 39-44.
- Uzubalis, R. I., Frewin, D. B., Bushell, M. K., & McEvoy, R. D. (1989). The effect of angiotensin converting enzyme inhibitors (ACE-I) and selective β 1-antagonists on bronchial reactivity and the cough reflex in man. *European Journal of Clinical Pharmacology*, 37(5), 467-470.
- Van der Schans, C. P. (2007). Bronchial mucus transport. *Respiratory Care*, 52(9), 1150-1156; discussion 1156-1158.
- Van Meerhaeghe, A., Bracamonte, M., Willeput, R., & Sergysels, R. (1986). Airway anaesthesia and breathing pattern during exercise in normal subjects and in eucapnic patients with chronic airflow obstruction. *Bulletin europeen de physiopathologie respiratoire*, 22(4), 381-385.
- Vilardell, N., Rofes, L., & Clave, P. (2015). Cough reflex testing in post-stroke patients with oropharyngeal dysphagia. *Dysphagia*, 30(2), 231.
- Vilardell, N., Rofes, L., Nascimento, W. V., Muriana, D., Palomeras, E., & Clave, P. (2017). Cough reflex attenuation and swallowing dysfunction in sub-acute post-stroke patients: prevalence, risk factors, and clinical outcome. *Neurogastroenterology and Motility*, 29(1), e12910.

- Von, L., & Isshiki, N. (1965). An Analysis of Cough at the Level of the Larynx. *Arch Otolaryngol*, 81, 616-625.
- Vose, A., & Humbert, I. (2018). "Hidden in Plain Sight": A Descriptive Review of Laryngeal Vestibule Closure. *Dysphagia*.
- Vovk, A., Bolser, D. C., Hey, J. A., Danzig, M., Vickroy, T., Berry, R., . . . Davenport, P. W. (2007). Capsaicin exposure elicits complex airway defensive motor patterns in normal humans in a concentration-dependent manner. *Pulmonary Pharmacology & Therapeutics*, 20(4), 423-432.
- Wakasugi, Y., Tohara, H., Hattori, F., Motohashi, Y., Nakane, A., Goto, S., . . . Uematsu, H. (2008). Screening Test for Silent Aspiration at the Bedside. *Dysphagia*, 23(4), 364-370.
- Wakasugi, Y., Tohara, H., Nakane, A., Murata, S., Mikushi, S., Susa, C., . . . Uematsu, H. (2014). Usefulness of a handheld nebulizer in cough test to screen for silent aspiration. *Odontology*, 102(1), 76-80.
- Wallace, E., Ang, A., Guiu-Hernandez, E., & Macrae, P. (in prep). Quantifying test retest variability of citric acid cough reflex testing. *Manuscript in preparation*.
- Ward, K., Seymour, J., Steier, J., Jolley, C. J., Polkey, M. I., Kalra, L., & Moxham, J. (2010). Acute ischaemic hemispheric stroke is associated with impairment of reflex in addition to voluntary cough. *European Respiratory Journal*, 36(6), 1383-1390.
- Watando, A., Ebihara, S., Ebihara, T., Okazaki, T., Takahashi, H., Asada, M., & Sasaki, H. (2004). Daily Oral Care and Cough Reflex Sensitivity in Elderly Nursing Home Patients. *Chest*, 126(4), 1066-1070.
- Watts, S. A., Tabor, L., & Plowman, E. K. (2016). To Cough or Not to Cough? Examining the Potential Utility of Cough Testing in the Clinical Evaluation of Swallowing. *Current Physical Medicine and Rehabilitation Reports*, 4(4), 262-276.

- Wen, L.-y., Ni, H., Li, K.-s., Yang, H.-h., Cheng, J., Wang, X., . . . Su, H. (2016). Asthma and Risk of Stroke: A Systematic Review and Meta-analysis. *Journal of Stroke and Cerebrovascular Diseases*, 25(3), 497-503.
- Widdicombe, J., Addington, W., Fontana, G., & Stephens, R. (2011). Voluntary and reflex cough and the expiration reflex; implications for aspiration after stroke. *Pulmonary Pharmacology & Therapeutics*, 24(3), 312-317.
- Widdicombe, J., & Fontana, G. (2006). Cough: What's in a name? *European Respiratory Journal*, 28(1), 10-15.
- Winning, A. J., Hamilton, R. D., & Guz, A. (1988). Ventilation and breathlessness on maximal exercise in patients with interstitial lung disease after local anaesthetic aerosol inhalation. *Clinical science (London, England : 1979)*, 74(3), 275-281.
- Winning, A. J., Hamilton, R. D., & Shea, S. A. (1985). The effect of airway anaesthesia on the control of breathing and the sensation of breathlessness in man. *Clinical Science*, 68(2), 215-225.
- Winning, A. J., Hamilton, R. D., Shea, S. A., & Guz, A. (1986). Respiratory and cardiovascular effects of central and peripheral intravenous injections of capsaicin in man: evidence for pulmonary chemosensitivity. *Clinical science (London, England : 1979)*, 71(5), 519-526.
- Winther, F. O. (1970). Experimentally induced cough in man by citric acid aerosol. An evaluation of a method. *Acta Pharmacologica et Toxicologica*, 28(2), 108-112.
- Wong, C. H., Matai, R., & Morice, A. H. (1999). Cough induced by low pH. *Respiratory Medicine*, 93(1), 58-61.
- Wong, C. H., & Morice, A. H. (1999). Cough threshold in patients with chronic obstructive pulmonary disease. *Thorax*, 54(1), 62-64.

- Woolf, C. R., & Rosenberg, A. (1964). Objective assessment of cough suppressants under clinical conditions using a tape recorded system *Thorax*, *19*, 125-130.
- Wright, C. E., Jackson, J., Thompson, R. L., & Morice, A. H. (2010). Validation of the ERS standard citric acid cough challenge in healthy adult volunteers. *Cough (London, England)*, *6*, 8.
- Wyzga, R. E., & Folinsbee, L. J. (1995). Health effects of acid aerosols. *Water, Air, & Soil Pollution*, *85*(1), 177-188.
- Yamanda, S., Ebihara, S., Ebihara, T., Yamasaki, M., Asamura, T., Asada, M., . . . Arai, H. (2008). Impaired urge-to-cough in elderly patients with aspiration pneumonia. *Cough (London, England)*, *4*(1), 11-11.
- Young, E. C., Brammer, C., Owen, E., Brown, N., Lowe, J., Johnson, C., . . . Smith, J. A. (2009). The effect of mindfulness meditation on cough reflex sensitivity. *Thorax*, *64*(11), 993-998.
- Zahm, J. M., King, M., Duvivier, C., Pierrot, D., Girod, S., & Puchelle, E. (1991). Role of simulated repetitive coughing in mucus clearance. *European Respiratory Journal*, *4*(3), 311.
- Ziora, D., Jarosz, W., Dzielicki, J., Ciekalski, J., Krzywiecki, A., Dworniczak, S., & Kozielski, J. (2005). Citric acid cough threshold in patients with gastroesophageal reflux disease rises after laparoscopic fundoplication. *Chest*, *128*(4), 2458-2464.