Citric acid inhalation cough challenge

Establishing normative data

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ABSTRACT

One of the most elusive challenges in the diagnosis and treatment of dysphagia is the reliable identification of silent aspiration (aspiration in the absence of cough). The citric acid inhalation cough challenge offers potential for aiding in identification of silent aspiration; however clinical application of this technique is currently problematic due to an absence of normative data. Therefore, this study aimed to establish a normative data set for the Citric-Acid Inhalation Cough Challenge, as administered with facemask method. 80 healthy subjects will participate in this study, constituting 2 age groups: above and below 60 years, with equal gender representation. On 3 separate trials, they will be asked to passively inhale, via a facemask, nebulised citric acid of concentrations ranging from 08M to 2.6M with placebo interspersed. 'Natural cough thresholds' (NCT) and 'Suppressed Cough Thresholds' (SCT) will be reached when subjects cough on at least 2 out of 3 trials. The majority (92.5%) of participants reached Natural Cough Threshold by 0.8M, with 68% demonstrating Suppressed Cough Threshold also at this concentration. There were no significant differences found between males and females ($p<0.05$) for either NCT ($p=0.9885$) or SCT ($p=0.44$). Whilst no difference was found between youngers and elders for NCT ($p=0.7254$), there was a significant difference for SCT ($p=0.018$), with youngers better able to suppress cough. Over 90% of healthy people were found to elicit cough at 0.8M, inferring that this level would be an adequate guide for use by clinicians testing for presence/absence of cough.

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CHAPTER 1

Introduction

The term dysphagia has its origin in the Greek words ‘dys’ meaning ‘difficulty’ and ‘phagia’ meaning ‘to eat’ and refers to disorders of swallowing and deglutition. The inadequacies of the human swallowing mechanism have been known for over a century, with Charles Darwin (1859) noting “the strange fact that every particle of food or drink which we swallow has to pass over the orifice of the trachea, with some risk of falling into the lungs.” (p.191). It is now thought that the human vocal tract has evolved this lowered positioning of the larynx in order to allow for the dual function of both speech and swallowing (Lieberman, 1993; Arsuaga, Martinez & Anton, 2005; Christiansen & Kirby, 2003). A uniquely human feature, when compared with all other terrestrial mammals (most of which can swallow and breathe simultaneously), this has significantly heightened the risk of choking whenever eating or drinking (Lieberman, 1993; Knight, Studdert-Kennedy & Hurford, 2000). In addition to this already precarious state of ‘normal swallowing’, dysphagia can also arise as a result of many different aetiologies including neurological and structural disorders. As such, humans rely on a host of mechanisms to protect the airways from aspiration, arguably one of the most important being the cough reflex (Pantaleo, Bongiani & Mutolo, 2002; Hutchings, Morris, Eccles & Jawad, 1993).

However, the cough reflex itself can be damaged or even absent after neurological illness such as stroke (Addington, Stephens, Widdicombe & Rekab, 2005). This effectively leaves many dysphagic patients without a critical mechanism of airway protection. These patients are at increased risk of developing aspiration pneumonia (Ramsay, Smithard & Kalra, 2006; Addington, Stephens & Gilliland, 1999; Nakajoh et al., 2000), which in turn can lead to increased morbidity and mortality (Ramsay, Smithard & Kalra, 2006). Currently, we have no reliable way of identifying the integrity of the cough reflex. The citric acid inhalation cough challenge holds much potential towards helping to achieve this goal.

CHAPTER 2

Literature Review

2.1 Cough

2.1.1 What is cough?

Fontana (2008) defines cough as "an airway defensive reflex consisting of an inspiratory phase followed by a forced expiratory effort initially against a closed glottis, followed by active glottal opening and rapid expiratory flow" (p. S3). When the tracheobronchial receptors are stimulated, or cough occurs voluntarily, cough is initiated with an inspiratory phase, accompanied by contraction of the diaphragmatic and laryngeal abductor muscles (Fontana & Lavorini, 2006). However, if the stimulation arises at the level of the true vocal folds and upper tracheal areas, there is no preliminary inspiration, and this is known as the 'expiratory reflex' (as distinct from the cough reflex) (Fontana, 2008). This reflex is characterised by the glottis closing off, as a result of contraction of laryngeal adductor muscles, accompanied by contraction of the expiratory muscles. The 'power' of the cough in expelling any foreign objects/material comes from the sudden opening of the glottis, whilst expiratory muscles continue to contract strongly (Fontana & Lavorini, 2006). In reality, it is usually a combination of both true cough (i.e. laryngeal cough preceded by inspiration) and expiratory reflex which act to protect the lower airways (Fontana, 2008). The effectiveness of these two reflexes as a defense mechanism is increased further by the supporting reflex responses of bronchoconstriction and increased airway secretion (Karlsson, St Ambrogio & Widdicombe, 1988).

2.1.2 Clinical significance of cough

In the healthy population, the laryngeal cough reflex protects the supraglottic larynx from aspiration of food or fluids (Addington et al., 2005). Nakajoh et al. (2000) demonstrated an inverse relationship between strength of cough reflex in post stroke patients and the incidence of patients developing pneumonia.

2.1.3 Disordered cough reflex

Following stroke or other neurological illness, cough can be weakened or not present at all (Addington et al., 1999), resulting in the phenomena of what is termed 'silent aspiration'. Silent aspiration can be defined as 'the occurrence of aspiration before, during or after swallowing in the absence of cough' (Smith-Hammond, 2008, p. 157S). Addington et al. (2005) coined a term "brainstem shock" which they defined as a "global neurological condition involving a transient or permanent impairment of one or more of the following vital functions: the reticular activating system, respiratory drive or the LCR (laryngeal cough reflex)" (p. 7). Widdicombe, Eccles & Fontana (2006) suggest that this mechanism is physiologically important in that the act of coughing increases cerebrospinal fluid pressure, which may exacerbate the brain injury.

Whilst some degree of silent aspiration is normal within the healthy population, in terms of prognosis in dysphagic patients, it can be associated with increased morbidity and mortality (Ramsay et al., 2006). One of the most elusive challenges in the diagnosis and treatment of dysphagia is the reliable identification of silent aspiration. Bedside swallowing assessments and pulse oximetry have both been shown to have poor sensitivity in identifying silent aspiration when compared with videofluoroscopy (Ramsay, et al., 2006). However, even videofluoroscopy has its limitations, and if variations are present in swallowing function, it is possible that this 'snap-shot' in time may miss them. Thus, there is a strong clinical need for development of more reliable ways of identifying patients at risk of silent aspiration.

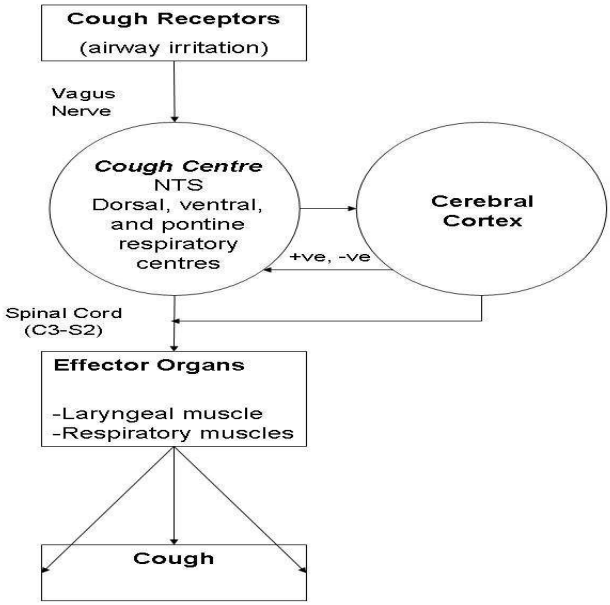
2.1.4 Neurophysiology of cough

The cough reflex arc follows the same basic pattern across all species, including humans (Canning, 2008). Cough receptors in the larynx or tracheobronchial tree are activated by mechanical or chemical stimulation. The sensory afferent nerves then discharge an action potential which is conveyed to the brainstem for processing (Canning, 2008). If the specificity and intensity

of the afferent nerve signal is sufficient, the cough reflex will be triggered (Chang, 1999), and an action potential is carried via the efferent nerves to the effector organs, which consist of the larynx and muscles of respiration (see Figure 1).

Whilst the general reflex arc is well known, there has been a significant amount of work conducted into ascertaining the specific neural mechanisms of the cough (Coleridge & Coleridge, 1994; Shannon et al., 2004; Pantaleo et al., 2002; Miller & Yates, 1993; Widdicombe 1998). The main afferent receptors for cough are dispersed throughout the larynx and tracheobronchial tree; these areas constitute the main stimulatory areas for the cough reflex (Fontana & Lavorini, 2006). Sensory information from afferent receptors in the larynx is carried by the superior laryngeal nerve (of the vagus), whilst receptors distributed lower down throughout the tracheobronchial tree and pleural areas are subserved by the pulmonary branch of the vagus (Widdicombe, 1998). However, there are also non-respiratory areas such as the proximal gastrointestinal tract, external auditory meatus and tympanic membrane (also innervated by vagal sensory terminals) which are able to initiate cough when stimulated (Fontana & Lavorini, 2006). Whilst historically, it was thought that the glossopharyngeal and trigeminal nerves also served cough receptors, it is now known that the sites for eliciting cough are exclusively confined to areas innervated by the vagus nerve (Chang, 1999).

Figure 1**.**
Cough model showing reflexive and voluntary pathways. Reflexive: Cough receptors are triggered by airway irritation. An action potential is conveyed via vagus nerve to cough centres in the pons and medulla, which integrate the sensory information and send another action potential down an efferent pathway (consisting of spinal cord segments C3-S2) to the effector organs, resulting in a cough (Shannon et al., 2004). Pathways for voluntary cough are not known, but thought to involve feedback from the cerebral cortex.



Sensory information from laryngeal afferents is carried specifically via the internal branch of the superior laryngeal nerve to the nucleus tractus solitarius (in the medulla) as well as other medullary and pontine regions including the dorsal, ventral and pontine respiratory groups (Fontana & Lavorini, 2006; Mazzone & Geraghty, 2000). Afferent information from the lower airway receptors (carried by the pulmonary branch of the vagus) terminate mainly in the nucleus tractus solitarius (Jordan, 2001). Second order neurons then project extensively to the dorsal, ventral and pontine respiratory nuclei (Fontana & Lavorini, 2006). The specific efferent cough pathways are poorly understood, but are known to include the spinal cord from C3 to S2, the recurrent laryngeal nerve and the spinal nerves (Chang, 1999). The efferent pathways then carry the central motor command from these respiratory groups to the main effectors including the glottis and muscles of respiration (Chang, 1999).

Recently, the importance of the laryngeal afferents in the cough reflex has been questioned. Although bilateral blockage of the superior laryngeal nerve has been found to inhibit cough when elicited by mechanical probing of the laryngeal mucosa, the cough reflex to citric acid inhalation remains intact, suggesting that it is the afferents throughout the tracheobronchial tree which are the major players in cough response to this sort of tussigen (Fontana et al., 1999; Chang, 1999). Additionally, the fact that laryngectomy patients maintain a normal cough threshold suggests that information from the larynx itself is not essential to the cough reflex (Fontana et al., 1999).

2.2 Cough receptors

There are 2 major types of cough receptor found in the respiratory system: mechanoceptors and nociceptors or chemoceptors (also known as C-Fibres) (Mokry & Nosalova, 2007). Within the category of mechanoceptors, there are 2 main groupings: the rapidly adapting receptors (RARs) and the slowly adapting receptors (SARs) (Ho, Gu, Lin, & Lee, 2001). A third type of mechanoceptor, the extrapulmonary mechanoceptor, was identified in the guinea pig by Canning et al. (2004). These were found to be extremely sensitive to touch, but not to other mechanical forms of stimulation including airway smooth muscle contraction. In general the mechanoceptors are all activated by a variety of mechanical stimuli which can include lung inflation and light touch, and usually show no response directly to chemical stimuli (Mazzone, 2005). However, if the action of the chemical stimuli results in a mechanical distortion of the nerve terminal (Widdicombe, 2001), activation will also occur. Another exception to the rule is that substances such as citric acid (i.e. a chemical stimuli) will also activate the mechanoceptors, although the mechanisms responsible for this are not yet understood (Mazzone, 2005). Chemoceptors are found throughout the airways and lungs (Mazzone, 2005), and in contrast are activated by chemical stimuli (including capsaicin, bradykinin and citric acid), but usually not by mechanical (Ho, Gu, Lin, & Lee, 2001).

The afferent receptors responsible for initiating the cough have still not been definitively elucidated (Lee & Undem, 2008), despite it being over 8 decades since the first pioneering research was conducted into this question. Traditionally, it is the rapidly adapting receptors (RARs) which have been thought to be the primary afferent nerve subtype involved in evoking the cough reflex (Widdicombe, 2001; Widdicombe, 1998). However, Mazzone (2005) points out several flaws in this hypothesis. Many substances that produce strong responses from these fibres are not good at evoking the cough response, and in some species, the RARs can be active throughout the respiratory cycle (without presence of cough). Mazzone also highlights the fact that chemoceptors are activated by stimuli that happen to be among the most potent of tussigenic agents (capsaicin, bradykinin and citric acid), and thus may play a role in cough reflex stimulation.

Canning et al. (2004) who identified the extrapulmonary receptor in the guinea-pig, found that whilst the application of capsaicin or other inflammatory mediators fails to exert any effect on these nerves, they are highly sensitive to hypertonic solution as well as rapid decreases in tissue pH (i.e. acidic compounds). When the authors surgically sectioned this nerve in an anaesthetized animal, they were no longer able to elicit cough via acidic (applied topically) or mechanical stimuli. These results suggest that the afferent neurones mediating the cough reflex are distinct from the slowly and rapidly adapting stretch receptors innervating the lungs, and constitute a distinct subtype of mechanofibre, which is also sensitive to changes in pH, whose primary function is control of the cough reflex (Canning et al., 2004). Additionally, the fact that these fibres are absent in species which do not cough, and are the only nerve types in the guinea pig which are capable of eliciting cough regardless of conscious state, make these receptors ideal candidates for the primary afferent nerve fibre involved in the cough response (Mazzone, 2005). As a result, the term “cough receptors” has been re-introduced into the literature for these fibres. Whilst it is not known whether these receptors exist in humans, circumstantial evidence suggests that there are analogous fibres in

man (Shannon, Baekey, Morris & Lindsey, 1998). Cough in response to an acute event such as prandial aspiration is most likely mediated by these 'cough receptors' (Mazzone, 2005).

2.3 Reflexive versus volitional cough

Humans are capable of both volitional and reflex coughing, each of which differs in the organisation of the motor activity involved (Lasserson et al., 2006). Whilst reflexive coughing elicited by tartaric acid has been found to consist of early and simultaneous activation of accessory and respiratory muscles, volitional cough, in contrast was characterised by sequential activation of muscle groups (Lasserson et al., 2006). The fact that humans can both induce and inhibit cough voluntarily has led to the suggestion that there is a cortical component to the cough (Widdicombe et al., 2006). The fact that stroke patients without brainstem involvement often have an impaired cough, both reflexive and voluntary, strongly indicates diencephalic involvement (Widdicombe et al., 2006). Asymmetry in site of lesion has been found to impact upon voluntary cough, with left middle cerebral artery infarcts resulting in weakness or loss of voluntary cough, but no comparable effect found for right sided lesions (Stephens, Addington & Widdicombe, 2003).

A study by Raj, Bakshi, Tiwara, Anand & Paintal (2005) found that when comatose patients were administered Lobeline, an alkaloid drug which stimulates dopamine release, a cough was still triggered, showing that regardless of whether or not a voluntary/cognitive element is present, it is not the only factor involved in reflex cough (Widdicombe et al., 2006). Interestingly, Pinto, Yanai, Sezikawa, Aikawa & Sasaki (1995) managed to condition a 'learned' cough response in guinea pigs, by pairing a tussigenic agent with a neutral one for a time period, and then removing the tussigen.

Whilst it is obvious that there are cortical components to the cough, to date very little is known about the exact neural pathways involved (Mazzone, 2005). A recent study (Mazzone, McGovern, Koo & Farrell, 2008) using fMRI to look at the supramedullary pathways involved in

cough production, found activations in the cerebellum and primary motor cortices. However, the authors admit that these findings shed no light on the way in which the supramedullary regions interact with the brainstem neuronal network to produce either voluntary or reflexive coughing. Thus, the exact way in which the cortical pathways are involved remains a mystery.

2.4 Suppressed cough

Leow, Huckabee & Anderson (2006) demonstrated significantly higher cough thresholds when individuals were instructed to try to suppress cough, again pointing to a voluntary constituent to the cough. Similarly, Lee, Cotterrill-Jones & Eccles (2002) found that cough resulting from upper respiratory tract infection can be suppressed for between 3-20 minutes. Another study by Hutchings et al., (1993) found that the extent to which cough was able to be suppressed was related to psychological factors, the effect being greater in patients with obsessional symptoms. The findings prompted these authors to conclude that the mechanism for suppression of human cough is similar to that of placebo effect. Indeed, for the majority of over-the-counter cough medicines, 85% of effectiveness is due to placebo alone (Widdicombe et al., 2006; Schroeder & Fahey, 2002). Placebo effects are based upon psychological factors, such as belief in the efficacy of treatment and patient attitude to health professionals, processes which are presumed to occur at cortical, or close sub-cortical level (Widdicombe et al., 2006). On the basis of what is known from studies such as those mentioned above, it would appear that voluntary suppression of cough is contributed to by areas in (or close to) the cerebral cortex (Widdicombe et al., 2006). Mazzone (2005) postulates that chemoceptor-elicited cough is distinct from coughing initiated by direct activation of mechanoceptors, and queries whether there even exists an ability to suppress cough when triggered by aspiration (as opposed to a chemo-irritant stimulation).

2.5 Adaptations in cough

2.5.1 Medical Covariates

Studies have shown that the cough afferent receptors have a high degree of plasticity and are able to adapt their response to various changes in environmental stimuli (Sitkauskine, Stravinskaite, Sakalauskas & Dicpinigaitis, 2007; Bonham, Sekizawa, Chen & Joad, 2006).

There are a number of factors which alter sensitivity of the cough reflex. These include spinal cord injuries (Lin, Lai, Wu, Wang & Wang, 1999), gastro-esophageal reflux, allergic rhinitis, atopic dermatitis (Pecova, Javorkorva, Kudlicka & Tatar, 2007), taking angiotensin-converting enzyme inhibitors (ACE inhibitors) (Yamaya, Yanai, Ohrui, Arai & Sasaki, 2001), pneumonia (Nimi et al., 2003), central nervous system disorders such as stroke (Addington et al., 2005), sleep and general anaesthesia (Widdicombe & Singe, 2006; Nishino, Tagaito & Isono, 1996), diabetes with autonomic neuropathy (Vianna, Gilbey, Barnes, Guy & Gray, 1988), upper respiratory tract infections (Nimmi et al. 2003) and smoking (Lin et al., 1999). Somewhat surprisingly, 'cough variant asthma' has been found to exert no affect on cough reflex sensitivity (Mokry & Nosalova, 2007).

2.5.2 Gender

There appear to be gender differences in cough sensitivity, with females having heightened sensitivity compared with males (Dicpinigaitis & Rauf, 1998; Kastelik et al., 2002). However a previous study from this laboratory failed to note any significant gender effect (Leow et al., 2006).

2.5.3 Age

There appears to be no alteration of the cough reflex with age (Katsumata, Sekizawa, Ebihara, & Sasaki, 1995; Sams, Truncale & Brooks, 2005; Chang & Widdicombe, 2007). An interesting study by Watando et al. (2004) demonstrated that cough reflex sensitivity in elderly nursing home subjects was significantly improved by intensive daily oral care and hygiene.

Langmore et al. (1998) showed that an important risk factor for pneumonia was oral hygiene, and that if this was maintained, chances of developing pneumonia were reduced. This suggests that the cough reflex may be a possible mechanism by which improved oral care reduces incidence of pneumonia.

2.5.4 Smoking

Cough reflex sensitivity has been found to be enhanced within 2 weeks of smoking cessation, and upon resumption of smoking, diminishes again (Sitkauskine et al., 2006). The mechanism by which smoking inhibits cough reflex remains unelucidated, but it has been proposed that long term tobacco use has the effect of desensitising the actual cough receptors (Dicpinigaitis, 2003). This may also account for the transient increase in coughing following smoking cessation (Dicpinigaitis, 2003).

2.6 The inhalation cough challenge

2.6.1 Clinical application of the inhalation cough challenge

In the decades since the first citric acid inhalation cough challenge, articles pertaining to the test feature widely within the respiratory literature (Ricciardolo, 2001; Bevan & Geppetti, 1994; Ramsay, Wright, Thompson, Hull & Morice, 2008; Kenia, Houghton & Beardsmore, 2008; Moreaux, Nemmar, Beerens & Gustin, 2000). However, it was not until relatively recently that researchers have attempted to apply the inhalation cough challenge to the dysphagic population as a way of identifying patients at risk of silent aspiration and pneumonia (Smith & Wyles, 1998; Addington et al., 1999).

One of the first studies to look at cough responsiveness in the neurogenic dysphagia population was by Smith and Wyles (1998). They looked at 28 patients with neurogenic disorders and categorised them, on the basis of the water swallow test into 2 groups: ‘normal swallow’ and ‘abnormal’, both of which were then administered a capsaicin inhalation test. Their findings were

that the cough thresholds in the 'abnormal' group were actually lower than in the 'normal' group. However, as the authors freely admit, this study is flawed in a number of ways. Firstly, the lack of any true 'normal' subjects for comparison is a significant limitation in this study. All of the individuals under investigation had a neurological diagnosis. Secondly, the only indicator of "normal" swallow was a water swallow test. Without any form of instrumentation (videoflouroscopy, videoendoscopy, etc…) the researchers had no ability to look at the pharyngeal phase of the swallow. Thus, the essential presence of a true 'control' group was absent, as was a method to accurately assess the swallow, both of which would infer that their overall conclusion that 'these patients with neurogenic dysphagia do not have a reduced sensitivity of cough triggering' was possibly invalid. In addition, the act of swallowing water itself is a largely motor response, as opposed to cough reflex, which is a sensory response.

Addington, Stephens & Gilliland (1999) utilised a tartaric acid reflex cough test with 400 stroke patients, with a control group of 204 stroke patients from a sister hospital. Decisions on whether or not to feed the patients were based on outcomes of the inhalation cough challenge and measures of overall 'success' of the cough challenge was based upon incidence of pneumonia. This study was significantly flawed in a number of ways, including no control for site of lesion or co-morbidities, and no consistent instrumental assessment of swallowing status as this was only undertaken when staff believed it to be necessary.

Addington, Stephens, Widdicombe & Rekab, (2005) examined acute stroke patients and the risks of developing pneumonia when comparing site of lesion and status of laryngeal cough reflex. This group used tartaric acid as the tussigenic agent, and delivery was via a mouthpiece with 'exhalation-inhalation' technique. The result of a normal inhalation cough challenge finding was 'an immediate series of forceful coughs' and an abnormal finding was characterised by "an absence of coughing, a diminished cough or coughing not immediately after administration of the test stimulus" (p.2). Thus, the measures of cough implemented in this study were subjective only, with

loosely defined variables regarding factors such as numbers of coughs elicited, and what constituted a 'diminished cough'. Of the 818 patients, 736 (90%) had a normal RCT and 26 (3.5%) went on to develop pneumonia. Sixty-nine (84%) patients had a 'weak' cough response and seven of these developed pneumonia (10%). Thirteen had an absent RCT and two went on to develop pneumonia (15%). Any findings from this study with respect to evaluating the actual performance of the inhalation cough challenge are difficult to interpret, given that the management of the individual patients was modified based on the outcomes of the RCT, which as the authors point out, will have affected the patients' chances of developing pneumonia. A potential weakness in this study is also the lack of diagnostic specificity with respect to the pneumonia that patients were documented to develop. It is not clearly described whether or not these patients had developed specifically aspiration pneumonia, a general pneumonia unrelated to dysphagia, or even a pneumonitis. As Langmore et al. (1998) highlight, there are numerous factors which increase pneumonia risk, other than dysphagia. However the authors were also correlating pneumonia risks with site of lesion, and hence in this respect were possibly not looking to separate out aspiration related to dysphagia from general pneumonia. All of the above factors make it difficult to draw conclusions from this particular study about the efficacy of this test in identifying patients at risk of developing aspiration pneumonia.

All of the above mentioned studies have utilised mouthpiece as the option of delivery of citric acid from the nebuliser, and an exhalation-inhalation technique to inhale the citric acid, and this is typical of the majority of work done in the field. A high proportion of dysphagic patients will have a coexisting aphasia (language impairment) and subsequently experience difficulties following instructions to use the mouthpiece method. Additionally, many stroke patients will exhibit apraxic characteristics, meaning that, even if they do comprehend the task, they will be unable to volitionally coordinate their breathing to the extent required by the exhalation-inhalation method. Significant oral-motor impairments are another example of neurological sequelae

following stroke, and these patients may not be able to form a lip seal around the mouthpiece. These issues may impact on the ability to administer the test. In the studies by Addington and colleagues, above, the subjects were asked to "exhale, then insert the mouthpiece and take a sharp, deep inhalation" (Addington et al., 2005, p.2). Leakage around the mouthpiece and puffing the nebuliser were not considered effective inhalations. The authors do not mention whether or not the individual was included in the study if they were unable to take 'effective' inhalations. This would immediately exclude a large proportion of dysphagic patients with neurogenic aetiology.

Recently, a group of researchers looked at administering citric acid via a facemask, in conjunction with a modified water swallow test, to a group of dysphagic patients as a means of developing a screening assessment for silent aspiration (Wakasugi et al. 2008). By use of facemask, the patients simply had to breathe passively in order for the tussigen to be administered. The results of the cough test were then compared with videofluoroscopy or videoendoscopy. The concentration of citric acid used was 1.0 w/v% and the criteria for threshold sensitivity was over 5 coughs considered normal and less than 5 abnormal. Of the 204 subjects, 97 presented with no aspiration, of which 84 tested negative on the inhalation cough challenge (i.e. 13 positive). 52 subjects demonstrated silent aspiration, and of these 45 (86%) tested positive on the cough test. 18 patients were found to exhibit 'silent aspiration by little aspiration' (i.e. patients who silently aspirated small amounts of food/liquid, but coughed with larger amounts) and 2 tested positive with the cough test (11%).

One common flaw in the above mentioned studies of inhalation cough challenge as applied to the dysphagic population is the fact that none have referenced or collected any normative data with respect to cough sensitivity, and in particular with respect to facemask method as opposed to mouthpiece. This poses a major limitation when trying to apply the test to a clinical setting. It is acknowledged that there is wide inter-individual variation with respect to cough sensitivities

(Morice et al., 2007), and before the inhalation cough challenge can be added to the clinician's set of assessment tools for aspiration, normative data needs to be gathered.

2.6.2 Methods for eliciting cough

"The only way to test the status of the airway protection mechanism is to stimulate the reflex." (Addington, 1999, p.1203). As mentioned above, the cough reflex can be experimentally evoked either via mechanical or chemical stimulation. Mechanical stimulation involves mechanical probing of the laryngeal mucosa, trachea and large bronchi, or the mucosa of the intrapulmonary bronchi (Lee & Undem, 2008). Chemical stimulation consists of inhalation of a tussigenic compound, otherwise known as the inhalation cough challenge. The more proximal airways are extremely sensitive to mechanical stimulation, whilst the more distal ones are more chemosensitive and less mechanosensitive (Chang, 1999).

2.6.3 Tussigenic agents

Techniques in experimentally inducing cough were first developed over 50 years ago (Bickerman, Barach, Itkin & Drimmer, 1954), utilising citric acid. The inhalation cough challenge involves the delivery of tussive agents as aerosols from either jet or ultrasonic nebulisers, usually through a mouthpiece (Morice, Kastelik & Thompson, 2001). Over the years, a large number of tussigenic agents have been trialled, including sulphur dioxide, ammonia and cigarette smoke (Gravenstein, Devloo & Beecher, 1954). However to date, the most commonly used agents are citric acid, tartaric acid and capsaicin (Morice et al., 2001).

Each of the tussigens are thought to work via different neural pathways to initiate cough (Wong, Matap & Morice, 1999). To further corroborate this, there appears to be little cross sensitivity between individuals to the 3 main tussigens: capsaicin, bradykinin and citric acid (Morice, 1996). There has been found to be no correlation between citric acid and capsaicin cough threshold or cough frequency (Midgren, Hansson, Karlsson, Simonsson & Persson, 1992).

Neither capsaicin nor bradykinin evokes any response in anaesthetized animals or humans, even though mechanical stimulation still results in a cough (Canning et al., 2004). Interestingly, in anaesthetized animals, capsaicin has been found to dampen the cough reflex induced by mechanical stimulation (Canning et al., 2004). It has been suggested that in conscious animals, when chemosensitive stimuli are used, any cough reflex elicited is actually dependent on cortical processing and a behavioural 'urge to cough' sensation (Mazzone, 2005). In humans, capsaicin evoked cough has been found to be able to be voluntarily suppressed (Hutchings et al., 1993), leading Widdicombe et al. (2006) to postulate that any cough induced by chemosensor mediated stimulants may not be a true reflexive cough.

Although citric acid has been widely used as a tussive agent for many years, there is still no certainty as to the exact mechanism by which it induces cough (Wong et al., 1999). In the past, it has been postulated that both capsaicin and citric acid work through a common pathway to induce cough, via stimulating the C-fibres (chemoceptors) (Fox, 1996). In 1999, Wong et al. conducted a study which suggested that stimulation with citric acid was via proton sensitive rapidly adapting stretch receptors- RARs (mechanoceptors). They also found significant correlation between thresholds of both organic and inorganic acid compounds, but none between citric acid and capsaicin cough thresholds in normal individuals, supporting their hypothesis that acids act via a different mechanism from capsaicin and other "non-acid" substances. More recently, a study by Tanaka and Maruyama (2005) indicated that citric acid stimulates both chemosensitive C-Fibres and mechanosensitive A-Fibres. This group found that both citric acid and capsaicin were found to evoke coughs via the C-Fibres exclusively when inhaled. However, when citric acid was injected into the larynx, there were other afferent fibres involved, prompting the authors to suggest the involvement of the low threshold mechanoceptors (A-Fibres) described as 'cough receptors' in the guinea pig.

The main form of delivery of the tussive agent is via either jet or ultrasonic nebuliser (Morice, 1996). Jet nebulisers generate particles as a result of the impact between gas and liquid in a given area, and whilst they produce less volume than ultrasonic nebulisers they are the preferred option for use with dosimeters where nebulisation is initiated by timed bursts of gas passing into the jet chambers (Morice, 1996). Due to the fact that variations in inspiratory rate can affect cough response, dosimeter controlled nebulisers have become the option of choice to control for this variable (Morice et al., 2001).

Tussive agents can be delivered either in 'single dose' or 'dose-response' fashion (Morice, et al., 2001). Single dose involves the individuals being administered just one concentration, whilst dose response consists of subjects inhaling incrementally increasing concentrations, interspersed with placebo (Morice et al., 2001). The latter of the 2 techniques can then be further categorised into either single breath or fixed time challenges. The disadvantages of the fixed time method include longer inhalation times, and thus decreased accuracy of delivery. Previous studies have utilised both a 15 second and a one minute fixed time inhalation period (Godden, Borland, Lowry & Higgenbottam, 1986).

2.6.4 Application of inhalation cough challenge to dysphagia

For the purpose of applying the inhalation cough challenge to the dysphagic population, the factors above need to be taken into consideration. For instance, whilst the literature suggests that single breath exhalation-inhalation is the most accurate measure of threshold, as described above, this is inappropriate for the dysphagic population. Thus the citric acid inhalation challenge appears to hold much promise for aiding clinicians in identifying patients at risk of silent aspiration. However, before this test can be reliably incorporated into the clinical battery of dysphagia assessments, a normative data set must be established.

2.6.5 Aim of Study

The aim of this project was to gather normative data for the citric acid inhalation cough challenge, as administered by facemask method. Acquisition of this dataset should allow more accurate clinical application of this test. The following hypotheses were also probed:

1. There will be significant differences in cough sensitivity between gender groups with males having higher thresholds then females
2. There will be no difference in cough sensitivity between age groups
3. Cough thresholds will be higher for facemask method, when compared with previous studies using the mouthpiece method

CHAPTER 3

Methodology

3.1 Participants

80 healthy participants were recruited from the community, divided into 2 groups with equal gender representation: youngers (under 60 years of age) and elders (over 60 years of age). Exclusion criteria included a history of neurogenic disorders, gastro-esophageal reflux, and/or respiratory disorders. In addition, participants were excluded if taking ACE inhibitor or codeine based drugs, or had a recent history of smoking in the last 5 years or upper respiratory tract infection in the last 2 weeks. Ethical approval was obtained from the Upper South B Regional Ethics Committee of the Ministry of Health in Christchurch, New Zealand.

3.2 Procedure

Citric acid was diluted in 0.9% sodium chloride, to obtain 10 different concentrations, ranging from 0.8M to 2.6M, increasing in increments of 0.2M. The selected concentrations (see Appendix 1) were based on results from Leow, et al. (2006), which showed that approximately 75% of all subjects had reached Natural Cough Threshold by 0.8M. However, this study used 'single breath' method of administration, and hence higher thresholds were anticipated than those seen in the previous study.

Delivery of citric acid was via facemask method. Participants were instructed to place the mask over their nose and mouth, and to breathe normally through the mouth. The acid was nebulised using a DeVelbiss nebuliser with a constant flow rate of 8L/min. Participants were initially coached on a placebo solution of 0.9% sodium chloride to familiarise them with the procedure. Citric acid was administered in incremental concentrations with 3 placebo vials randomly interspersed to increase challenge blindness. Each dose was administered 3 times, with

the nebuliser running for 15 seconds each trial. There was a 30 second interval between each inhalation to prevent tachyphylaxis, which was based on the protocol proposed by Morice (1996).

Participants were told that each vial contained differing concentrations of citric acid, and that some vials would make them cough whilst others would not. For the Natural Cough Threshold, they were instructed "Cough if you feel the need to cough, don't cough if you don't feel the need to." Natural Cough Threshold was identified when participants coughed on at least 2 out of 3 trials. From this point on, the subjects were informed that the test would continue with new instructions. They were now asked to "Try to suppress the cough as much as you can". The Suppressed Cough Threshold was reached when participants coughed on 2 out of 3 trials. For all trials, the time taken (in seconds) until cough was elicited was noted.

3.3 Statistical Analysis

All statistics were performed using the R statistical package version 2.10.0. A p-value <0.05 was considered to be statistically significant. Mann-Whitney U-tests were conducted to compare concentrations at which natural and suppressed cough occurred in experimental groups: youngers, elders, females and males. Mann Whitney U-tests were also used to compare between-groups cough thresholds. For group comparison purposes, participants who failed to cough at any concentration were coded as coughing at the highest concentration provided, rather than excluded from the study. A Pearson's correlation test was used to look for correlation between time of exposure and cough sensitivity. When tabulating data for time taken to trigger cough thresholds, levels of 2.6M were treated as 'NA', as no coughing was triggered.

.

CHAPTER 4

Results

The majority (92.5%) of subjects in this study triggered Natural Cough Threshold (NCT) at 0.8M, with 70% also triggering Suppressed Cough Threshold (SCT) at 0.8M. Mean thresholds for cough reflex are reported in Table 1.

Table 1.
Mean thresholds for cough reflex between groups

Variable	**n**	**Mean (in Molarity)**
NCT Youngers	40	0.865
NCT Elders	40	0.875
SCT Youngers	40	1.395
SCT Elders	40	1.03
NCT Males	40	0.885
NCT Females	40	0.885
SCT Males	40	1.28
SCT Females	40	1.145

For Youngers, NCT was significantly lower than SCT ($U= 479.5$, $n_1=n_2= 40$, $p=0.001$). However, no such difference was demonstrated for Elders ($U=718.5$, $n_1=n_2= 40$, $p=0.216$). Detailed analysis using a Mann-Whitney U-test to compare Youngers and Elders showed no significant difference for NCT ($U=763$, $n_1=n_2= 40$, $p=0.361$), but a significant difference for SCT ($U=554.5$, $n_1=n_2= 40$, $p=0.009$), with Youngers demonstrating greater ability to suppress the cough (see Figure 2).

Similarly, Mann-Whitney U- Tests were used to analyse differences between males and females. Males demonstrated a significant difference between NCT and SCT ($U=555.5$, $n_1=n_2= 40$, $p=0.009$), whilst statistical analysis failed to identify a statistical difference between NCT and SCT in females ($U=651$, $n_1=n_2= 40$, $p=0.075$). No significant differences were found between gender (see Figure 3) for either NCT ($U=798$, $n_1=n_2= 40$, $p=0.492$) or SCT ($U=704$, $n_1=n_2= 40$, $p=0.178$). Data collected on time (seconds) taken to cough triggering across the different groups is presented in Table 2 and Table 3.

A Pearson product-moment correlation coefficient was computed to assess the relationship between cough sensitivity and time of exposure. There was no significant correlation between Natural Cough Threshold and time of exposure ($r = 0.388$, $df = 3$, $p = 0.519$), or between Suppressed Cough Threshold and time of exposure ($r = 0.635$, $df = 5$, $p = 0.126$).

Figure 2.
Differences in Natural Cough Threshold and Suppressed Cough Threshold between Youngers and Elders.

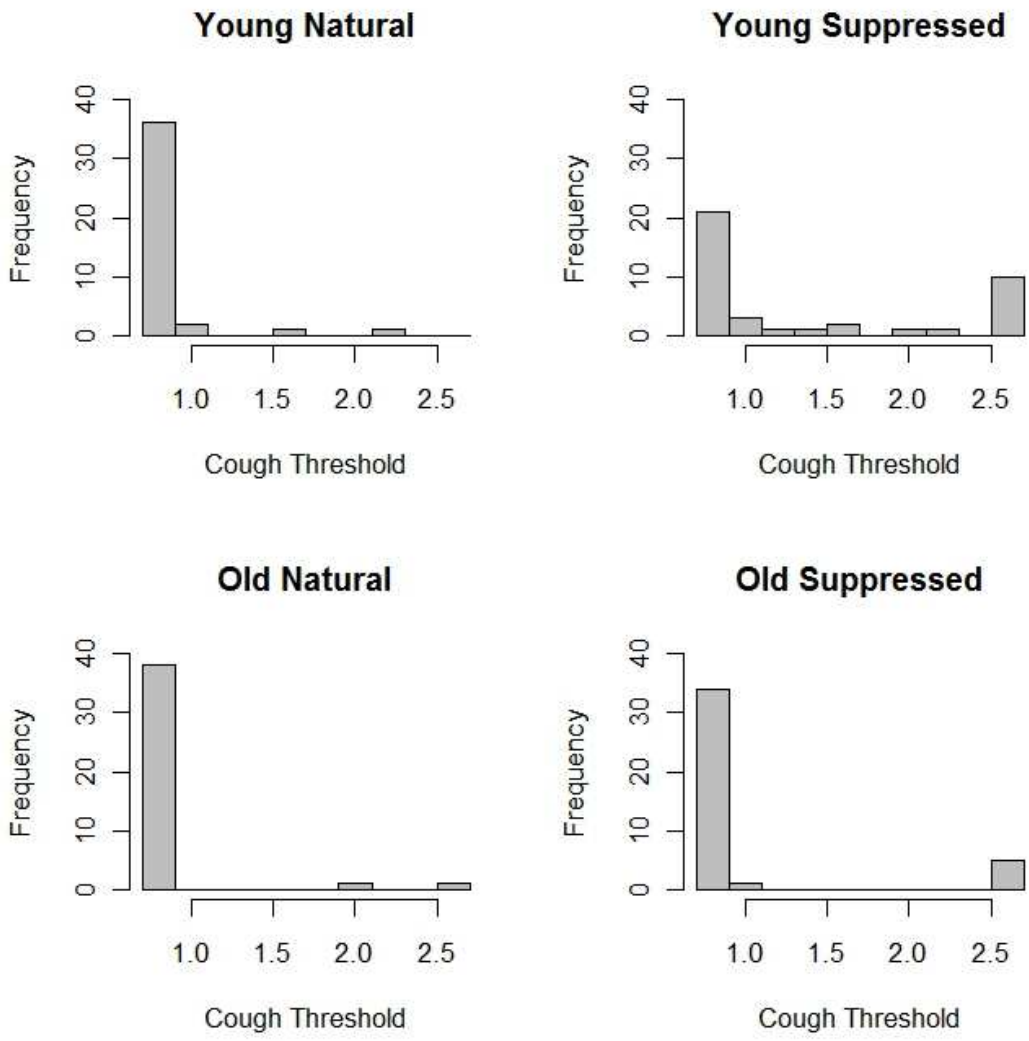


Figure 3.
Differences between Natural Cough Threshold and Suppressed Cough Threshold between genders.

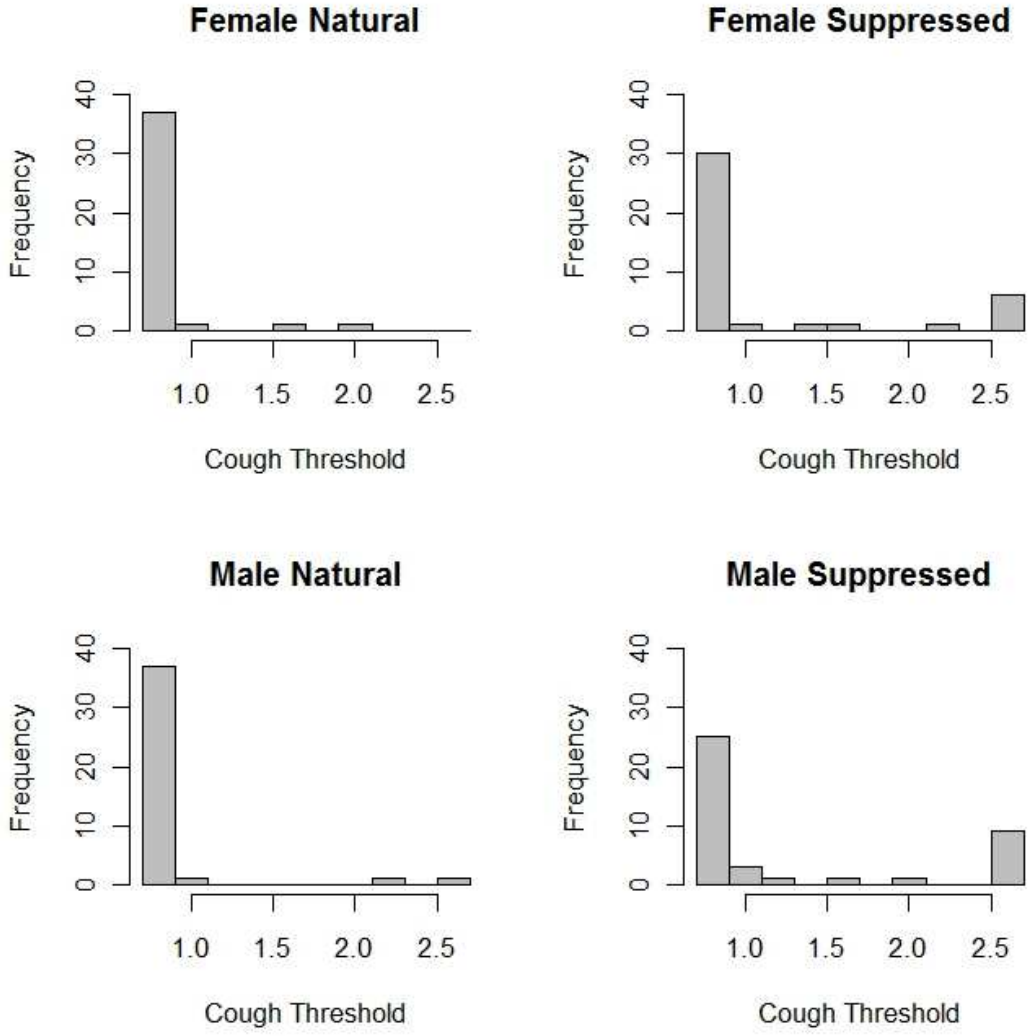


Table 2.

Mean time (seconds) taken to trigger Natural Cough Threshold, across each Molarity triggered.

Threshold	Time to Cough (secs)	Number of Participants
0.8	3.72	74
1	8.75	2
1.6	10	1
2	3.5	1
2.2	12.5	1
2.6	NA	1

Table 3.

Mean time (seconds) taken to trigger Suppressed Cough Threshold, across each Molarity triggered.

Threshold	Time to Cough (secs)	Number of Participants
0.8	5.11	55
1	7.75	4
1.2	10	1
1.4	4.5	1
1.6	7.5	2
2	13	1
2.2	10	1
2.6	NA	15

CHAPTER 5

Discussion

5.1 Natural Cough Threshold

This research established the first normative data set for the citric acid inhalation cough challenge, as administered with facemask method. The majority of subjects (92.5%) triggered Natural Cough Threshold (NCT) at 0.8M. Previous research using the exhalation inhalation method (Leow et al., 2006) reported that 75% of subjects reached NCT by this level. We hypothesised that this study would elicit higher thresholds than exhalation inhalation method. Thus, the decision was made to test at higher concentrations, at the expense of lower ones. For the purposes of clinical application, a delicate balance has to be found. If concentrations are too low, they give rise to false positives, and if too high will result in false negatives. As over 90% of subjects triggered a cough by this point, we can be fairly confident that if a patient fails to respond, this is likely an abnormal response (thus inferring an impaired cough reflex).

Testing at a concentration of 0.8M, does however, lend itself to the possibility of false negatives (screening may imply that subjects have a normal cough reflex when in fact it may be weakened). As we did not test at lower concentrations, there is the possibility that many subjects would have triggered NCTs at concentrations lower than 0.8M. Thus, the potential exists for a patient (with true NCT <0.8) with a slightly desensitised cough reflex to cough with 0.8M. Nevertheless, patients who fail to trigger a cough at this concentration are likely to be the significantly impaired, high risk patients.

5.2 Suppressed Cough Threshold

Given the evidence for voluntary control of cough (Widdicombe et al., 2006; Stephens et al., 2003; Pinto et al., 1995), it could be argued that Suppressed Cough Threshold (SCT) constitutes a better measure of true cough threshold, as this is the point where subjects theoretically no longer have voluntary control over their response. The majority of subjects (70%) in this study demonstrated SCT at 0.8M. However, the clear flooring effect evident in this work may have masked differences between NCT and SCT. An analysis of our results would indicate little difference between NCT and SCT for most subjects. Testing at lower concentrations would likely have shown up larger gaps between NCT and SCT.

The concept of cortical down-regulation is an important one for anyone working with the cough reflex. The cough reflex is extremely susceptible to placebo effect, and it has been suggested that the mechanism whereby suppression of the cough occurs, is similar to this (Hutchings et al., 1993). It is extremely difficult to assess the cough reflex, whilst minimising any placebo-type effects, which are inherent given the instructions for the test, "The substances in these vials may make you cough…". As far as we know, there have been no studies which have looked at cough reflex using a blind study design. Similarly, research could look at cough thresholds in patients with severe dysphasia, and thus comprehension problems. This would constitute a patient group for whom any placebo effect associated with the test instructions may also be minimised. It would be interesting to see if thresholds changed when any cortical component was removed.

5.3 Facemask method versus mouthpiece

Cough thresholds from this study were lower than a previous study which utilised mouthpiece method. Leow et al. (2006) found 75% of their participants coughing by 0.8M (with mouthpiece method), as compared to 92% in this study, with facemask method. Whilst mouthpiece method involves deep, sharp inhalations, facemask method consists of breathing passively (through

the mouth), and thus more shallowly. However, for facemask method, participants are exposed to the tussigen over a longer period of time (on average, 3-5 seconds, and up to 12 seconds) and this may have accounted for the lower threshold. However, there was no correlation found between cough sensitivity and time of exposure for either NCT or SCT. The above findings that cough thresholds differed from those obtained in previous studies is interesting as it means that any normative data for the inhalation cough challenge as administered with a mouthpiece method cannot be directly applied when a facemask is used for inhalation.

5.4 Chemical versus mechanical stimulation

Is there a difference between chemoceptor-elicited cough and mechanoceptor-elicited cough? This question is important if the inhalation cough challenge is to be applied to dysphagia management. Whilst aspiration would probably involve both types of cough receptors to some extent, it would predominantly trigger the mechanoceptors (Mazzone, 2005). Mazzone (2005) hypothesized that cough triggered by aspiration, and hence mechanoreceptor stimulation, is inherently different from cough elicited by chemical stimuli, and may be unable to be suppressed. Widdicombe et al. (2006) went a step further, and postulated that any cough induced by chemosensor mediated stimuli may not be a true cough at all. This raises the question: how appropriate is any test seeking to elicit the cough reflex (such as the one used in this study), which looks only at chemoceptor-elicited cough?

Whilst the cough may differ depending upon the stimulation, the important fact is that both chemoceptors and mechanoceptors are innervated by the vagus nerve (albeit different branches). Thus, any test which stimulates the chemoceptors will inherently be testing the integrity of the vagus nerve, and if the innervation for chemoceptors is functioning normally then the innervation to the mechanoceptors should be intact as well. The only exceptions to this rule would be cases involving lower lesions which occur after the vagus nerve branches into the pulmonary and superior laryngeal nerves. Thus, regardless of any differences between type of stimulus, the inhalation

cough challenge should give us valuable information about a patient's cough reflex and the status of the vagus nerve. Additionally of the major tussigens, citric acid is the only one that has been found to stimulate both machanoceptors and chemoceptors (Tanaka & Maruyama, 2005), further justifying its use in the inhalation cough challenge.

5.5 Age

General trends in the literature show no alteration of the cough reflex with age (Katsumata et al., 1995; Sams et al., 2005; Chang & Widdicombe, 2007). Whilst this study found no significant difference across the age range for NCT, there was a significant difference between the groups for SCT. In keeping with findings from Leow et al. (2006), younger adults (under 60 years) were much better at suppressing the cough than older adults (over 60 years). It has been suggested that the mechanism by which suppression occurs is similar to the placebo effect and may be due to generation of endogenous opioids (Eccles, 2006). Much of the work into placebo effect, has been done in the area of analgesia (Lee et al., 2005). Studies on pain control have yielded evidence that placebo treatments somehow manage to activate the endogenous opioid system and interestingly administration of opioid antagonists can block this placebo analgesic reponse (Benedetti, 1997). If the hypothesis about cough suppression having similarities with the placebo analgesia system is correct, these differences between age groups may reflect age-related declines in the opioid system. There is evidence for this from studies in both humans (Washington, Gibson & Helme, 2000) and animals (Knisely & Ham, 1989).

From a clinical perspective, this reduced ability to suppress cough in the elderly may have an adaptive purpose. Aging has the effect of reducing capacity of all aspects of pulmonary function (Zaugg & Lucchinetti, 2000) in addition to reducing neuromuscular reserves in swallowing (Ney, Weiss, Kind & Robbins, 2009). In this situation, any cortical input is possibly overridden by the risks posed by chemical or mechanical stimuli to a weakened pulmonary system.

5.6 Gender

Evidence from previous studies shows gender differences for cough sensitivity, with males tending to have higher thresholds than females (Dicpinigaitis & Rauf, 1998; Kastelik et al., 2002). The reason for this is currently unknown. It has been suggested that sex hormones may act upon airway inflammation, which impacts upon the cough (Kastelik et al., 2002). However, this present study further corroborates findings from Leow et al., (2006) that failed to note any difference between males and females for either natural or suppressed cough thresholds. The flooring effect evident in this study may have masked differences between males and females for NCT as we would not have picked up lower thresholds for females. Previous studies which have found gender differences have only looked at NCT (Dicpinigaitis & Rauf, 1998; Kastelik et al., 2002; Dicpinigaitis, Allusson, Baldanti & Nalamati, 2001). Given the general trend that females appear to have more sensitive cough, it is perhaps surprising to find that in this study there was no difference in the ability to suppress the cough. However, as mentioned above, if cough suppression is due to release of endogenous opioids, this finding may suggest no differences in this system between the genders. Currently, the results from studies conducted in this area fail to give conclusive evidence on this hypothesis (Fillingim, King, Ribeiro-Dasila, Rahim-Williams & Riley, 2009).

5.7 Generalising from our sample

As this study focused on normative values for cough thresholds, our exclusion criteria were quite comprehensive including gastro-esophageal reflux, codeine based drugs, asthma and smoking. It is known that many of these factors influence the sensitivity of the cough reflex, and hence had to be eliminated in order to map normative values. However, a number of otherwise healthy volunteers were excluded due to exhibiting one or more of these criteria. Similarly, the typical stroke patient will likely have at least some of these common conditions/factors operating, and it would be interesting to look at how these various conditions, when they coexist in the same subject, influence cough sensitivity. For instance, we know that smoking tends to reduce cough sensitivity

(Pecova et al., 2007), as does diabetes mellitus (Behara, Das, Dash & Jindal, 1995). From a clinical point of view, what is the functional significance of this; is a diabetic smoker automatically at greater risk of aspiration? More work needs to be conducted in this area before we can address these types of questions.

5.8 Rehabilitation potential

Research into cough reflex may also yield important information with respect to rehabilitation of this important airway protective mechanism. The factors which are now known to increase cough sensitivity, such as improving oral care (Watando et al., 2004) could potentially be utilised to increase (or maximise) cough sensitivity in stroke patients. It is known that angiotensin – converting enzyme (ACE) inhibitors have the effect of increasing cough sensitivity and previous studies have shown reduction in pneumonia rates in patients on these drugs (Arai et al., 2005). This has prompted the suggestion that patients with oropharyngeal dysphagia should be treated with ACE- Inhibitors regardless of whether they are hypertensive or not (Marik & Kaplan, 2003).

Another exciting possibility in this field is that repeated inhalation of a tussigenic compound could be used to 'prime' an impaired sensory system, in a way similar to thermal stimulation therapy. Thus if a dysphagic patient with impaired cough reflex was exposed to repeated inhalations of citric acid prior to a meal, it may possibly improve the functioning of the cough receptors in the short term, hence affording better airway protection. When testing this hypothesis, the concept of tachyphylaxis (adaptation to repeated exposure over a short time resulting in reduced response) would need to be considered, making the time in between exposures a crucial factor. Whilst currently, this idea is no more than mere conjecture, only further research into this area will confirm or deny these possibilities.

CHAPTER 6

Conclusions

This study has gathered the first normative data set for the citric acid inhalation cough challenge. This test can now be applied in a clinical setting, where hopefully it will improve dysphagia management and patient outcomes.

This is a new and exciting field of research, as it relates to dysphagia, and there are many areas open to explore. What we know so far is that there is a cortical component to the cough, the cough reflex is malleable to some degree and citric acid will induce a cough in the vast majority of healthy subjects. What is currently unknown is, the amount of cortical input involved in the cough reflex, whether we can manipulate this and other variables to rehabilitate a cough, and whether or not the citric acid inhalation cough challenge is capable of predicting the occurrence of silent aspiration or even aiding in rehabilitation. This field of research is wide open, waiting to reveal the answers to these and many other questions.

> *The known is finite, the unknown infinite; intellectually we stand on an islet in the midst of an illimitable ocean of inexplicability. Our business in every generation is to reclaim a little more land, to add something to the extent and the solidity of our possessions.*
>
> (Thomas Henry Huxley, 1887, as cited in Darwin, F. (ed) 1888).

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Appendix 1.

Concentrations of citric acid used.

Citric acid concentration (M)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6