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Does diurnal variation in cough reflex testing exist in healthy young adults?

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ABSTRACT

The aim of this study was to investigate whether diurnal variation in cough reflex sensitivity exists in healthy young adults when a tidal-breathing method is used. Fifty-three participants (19–37 years) underwent cough reflex testing on two occasions: once in the morning (between 9 am – midday) and once in the afternoon (between 2–5 pm). The order of testing was counter-balanced. Within each assessment, participants inhaled successively higher citric acid concentrations via a facemask, with saline solution randomly interspersed to control for a placebo response. The lowest concentration that elicited a reflexive cough response was recorded. Morning cough thresholds (mean = 0.6 mol/L) were not different from afternoon cough thresholds (mean = 0.6 mol/L), $p = 0.16$, $T = 101$, $r = -0.14$. We found no evidence of diurnal variability in cough reflex testing. There was, however, an order effect irrespective of time of day, confirming that healthy participants are able to volitionally modulate their cough response.

1. Introduction

Cough reflex testing (CRT) has been utilised in the field of respiratory medicine for over 50 years, primarily as an outcome measure for antitussive drug therapy studies (Bickerman et al., 1954, 1956, 1957). The test involves stimulation of acid-sensitive, capsaicin-insensitive mechanoreceptors innervating the larynx, trachea and bronchi and/or bronchopulmonary C-fibres via inhalation of a tussive agent (*e.g.* citric acid, capsaicin, tartaric acid), and observing for a cough response.

As a test of airway sensitivity to foreign particles, CRT has obvious applications to dysphagia assessment and management. Addington et al. (Addington et al., 1999) were among the first to apply CRT to a clinical dysphagia population. They reported on the usefulness of CRT for detecting silent aspiration risk at the bedside among patients with acute stroke, citing a 1 % rate of aspiration pneumonia among patients who were managed according to their CRT result. CRT is now increasingly utilised in dysphagia research and clinical practice to assess reflexive cough sensitivity and make inferences about airway protection and silent aspiration risk.

As with many other reflexive physiological features such as heart rate (Kleitman and Ramsaroop, 1948) and temperature (Smolensky et al., 1976), pulmonary function demonstrates a circadian rhythm (Hetzel and Clark, 1980). It is therefore reasonable to suspect that reflexive cough sensitivity may also be subject to diurnal variation. A single study has described a pattern of diurnal variation in cough reflex sensitivity (Pounsford and Saunders, 1985). In this study, healthy adults underwent citric acid cough reflex threshold testing in the morning (9 am – midday) and afternoon (2–5 pm). Citric acid was delivered via a mouth-tube using the 'vital capacity' method and a nebuliser with a flow rate of 10 L/min. The citric acid dose was systematically increased over time, beginning at 0.5 % (≈0.03 mol/L) and continuing to 17.5 % (≈0.9 mol/L). The first dose level that elicited a reflexive cough was considered the participant's cough threshold. Paired *t* test results revealed that seven out of eight participants had significantly lower cough thresholds in the morning compared to the afternoon. The mean morning threshold was 2.84 % (≈0.15 mol/L), compared with 4.44 % (≈0.23 mol/L) in the afternoon.

The authors of this study concluded that airway stimulation via inhaled citric acid is subject to diurnal variation and care should be taken to perform this test in individuals at the same time of day (Pounsford and Saunders, 1985). However, variables such as nebuliser type (Terzano et al., 2007; Wright et al., 2010) and flow rate (Barros et al., 1990), as well as the instruction given to the participant (Hutchings and Eccles, 1994; Leow et al., 2012; Monroe

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et al., 2014) can affect CRT results and may explain why the mean values were different in this study compared to other published norms (Monroe et al., 2014). In addition, by omitting placebo doses, participant blinding was not possible and results may have been confounded.

More recently, research using CRT has focussed on the tidal breathing/face mask method (Miles et al., 2013a,b; Monroe et al., 2014). This method involves presenting a tussive agent via nebulised air using a face mask placed over the subject's nose and mouth. The subject is instructed to continue breathing normally through the mouth throughout the duration of the test. This method is preferable for use in acute clinical settings where concurrent cognitive, language or motor control impairments may be present (Miles et al., 2013a,b) and has been validated as a bedside screening tool of aspiration risk in patients with acute stroke (Miles et al., 2013a,b). However, it remains unknown whether diurnal variation in cough response exists when this method is used.

As a result of the historic study by Pounsford and Saunders (1985), diurnal variation in CRT is considered to be a major confounding variable (Morice et al., 2001; Nakajoh et al., 2000) that is controlled for in experimental research by testing at the same time each day. However, this may not be feasible in acute clinical settings. As diurnal variation in CRT has only been described using a vital-capacity method, our aim was to investigate diurnal variability in healthy young adults using a tidal-breathing method.

2. Material & methods

2.1. Participants

An *a priori* sample size of 53 participants was calculated as sufficient to detect a medium-sized effect ($1-\beta = 0.80$). Fifty-three young, healthy participants (27 males, 26 females) were recruited. The average age of males was 28 years (20–37 years) and the average age of females was 25 years (19–33 years). Participants had no history of respiratory disease (*e.g.* severe asthma, chronic obstructive pulmonary disease), gastroesophageal reflux, neurological disorder (*e.g.* stroke, brain tumour, traumatic brain injury), chest infection within the past eight weeks, or tobacco smoking in the past six months. Participants were not experienced in CRT and none reported having a current dental infection or currently taking antibiotics, painkillers, cough syrup or ACE inhibitor drugs. Informed consent was given prior to commencement of data collection. Ethical approval was obtained from an appropriate regional health ethics review committee.

2.2. Study design

As per the methods described by Pounsford and Saunders (1985), participants provided data in two assessment sessions: one in the morning (between 9 am – midday) and one in the afternoon (between 2–5 pm). The order of testing was randomised and occurred on either the same day (*i.e.* morning, afternoon) or consecutive days (*i.e.* afternoon, morning). There was a minimum of three hours between sessions, with a mean of ten hours. In order to control for the potential confounding effect of oral bacteria on cough reflex thresholds (Watando et al., 2004), participants were instructed to brush their teeth using a toothbrush and water for two minutes at the start of each session.

2.3. Cough reflex threshold testing

Citric acid diluted in 0.9 % sodium chloride was prepared at 12 different doses starting from 0.1 mol/L (1.92 %) and increasing in 0.1 mol/L increments up to 1.2 mol/L (23.06 %). This range was based on normative data (Monroe et al., 2014) which state that 96 % of people reach a suppressed cough threshold by 1.2 mol/L using a tidal breathing/face mask method. This range also includes the doses reported by Pounsford and Saunders (1985) as sensitive enough to detect diurnal variation in airway sensitivity, and reflect current clinical methods (Kallesen et al., 2015; Miles et al., 2014)

CRT was performed using a tidal breathing/face mask method. Citric acid was delivered via a facemask (Hudson Micro Mist Nebuliser Model 41893, Standard Connector & Adult Mask, Hudson RCI, North Carolina, USA) placed over the nose and mouth. The facemask was connected to a nebuliser (Turboneb 2 Nebuliser, Clement Clarke International Limited, Harlow, UK) with an obstructed flow rate of 6.6 L/min. As different aerosols were presented for up to 15 s, participants were instructed to "breathe normally through your mouth. Try not to cough". The suppressed cough threshold (SCT) (as opposed to natural cough threshold) was chosen as the primary outcome measure, as this is method is widely used (Hegland et al., 2012; Hutchings et al., 1993; Kallesen et al., 2015; Kelly et al., 2016; Leow et al., 2012; Mazzone et al., 2011; Miles et al., 2013a,b; Monroe et al., 2014) and considered to more closely approximate a true reflexive cough (Hegland et al., 2012; Monroe et al., 2014). Participants were blinded to the different doses presented.

Initially, a placebo dose of 0.9 % sodium chloride was presented to accommodate the participants to the presenation of nebulised air. Up to 12 citric acid doses were presented in progressively higher concentrations, with placebo doses randomly interspersed throughout testing to increase challenge blindness and prevent tachyphylaxis (Morice, 1996). Each citric acid dose was presented up to three times, with at least 30 s between trials to prevent tachyphylaxis (Morice et al., 2007). Cough response was considered positive if two or more consecutive coughs were triggered (C2 response threshold) on two out of three trials. The lowest concentration of citric acid that elicited a cough response was considered to be the SCT. All testing was video-recorded for reliability purposes.

2.4. Statistical analysis

IBM SPSS Statistics (IBM Corporation, Armonk, New York, USA) was used to analyse the data. Statistical significance was set at a level of $P < 0.05$ (two-tailed). Related-samples Wilcoxon signed-rank tests were conducted to compare SCTs measured in the morning versus the afternoon and to compare participants' first SCT compared to their second SCT. Participants who did not cough at the highest citric acid dose were coded as having a SCT of 1.3 mol/L. A simple linear regression was used to predict SCT based on time of day (coded as hours since midnight). Intra-rater reliability was estimated by the primary researcher re-analysing 20 % ($n = 11$) of participants' recordings. The same 20 % samples were also used to estimate inter-rater reliability by two independent raters. Single measure intraclass correlation coefficient (ICC) was used.

3. Results

Morning SCTs (mean = 0.6 mol/L, 95 % *CI* = 0.5–0.7) were not different from afternoon SCTs [(mean = 0.6 mol/L, 95 % *CI* = 0.4–0.7), $p = 0.16$, $T = 101$, $r = -0.14$, $1-\beta = 0.29$]. Six participants received their morning and afternoon CRTs within 3 h of each other. When these individuals were removed from the dataset, there continued to be no significant difference in morning SCTs (mean = 0.6 mol/L, 95 % *CI* = 0.3–0.8) compared to afternoon SCTs [(mean = 0.5 mol/L, 95 % *CI* = 0.3–0.8), $p = 0.17$.

There were no gender differences, with male participants' morning SCTs (mean = 0.8 mol/L, SD = 0.5) not different from afternoon SCTs [(mean = 0.7 mol/L, SD = 0.5), $p = 0.19$, $T = 27$, $r = -0.18$]. Similarly, female participants' morning SCTs (mean = 0.5 mol/L, SD = 0.5)