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AIRWAY PROTECTION AND COORDINATION OF BREATHING AND SWALLOWING

IN HEALTH AND ANESTHESIA

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M.D.

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To Curiosity

ABSTRACT

Swallowing and breathing is coordinated to ensure that the airway is protected from aspiration. During the pharyngeal phase of swallowing, breathing is interrupted to allow safe passage of bolus. However, details on the complex coordination of breathing and swallowing and their precise temporal relationship are not fully understood. Respiratory complications are common in the postoperative period and drugs used in anesthesia impair pharyngeal function and airway protection in young adults. The aims of this thesis were first to characterize key mechanisms for airway protection, *i.e.* pharyngeal function and coordination of breathing and swallowing and secondly to describe the impact of age and drugs used in anesthesia.

A newly developed airflow discriminator was validated by comparisons with spirometry, diaphragmal and abdominal EMG and integrated with pharyngeal manometry and videoradiography into a multimodal platform recording swallowing and breathing simultaneously with high temporal resolution. Normal coordination of breathing and swallowing was studied in young volunteers, while swallowing different bolus types, changing body position and during hypercapnia. Moreover, effects of morphine and midazolam were studied at two occasions during spontaneous decay of drug concentration. Effects of partial neuromuscular block were examined during rocuronium infusion in elderly volunteers (>65 years) at steady state adductor pollicis train-of-four ratios of 0.70, 0.80 and >0.90.

The airflow discriminator proved highly reliable and provided detailed information on timing of respiratory airflow unambiguously in relation to pharyngeal and diaphragmatic activity. The diaphragm was activated in the apneic period during swallowing, presumably a mechanism for preserving respiratory volume and to promote expiratory airflow after swallowing. This finding has to our knowledge not been described in humans before. Coordination between breathing and swallowing remained mostly unchanged regardless of age, body position, bolus characteristics, respiratory drive or partial neuromuscular block. In contrast, morphine and midazolam dys-coordinated breathing and swallowing, increasing the incidence of inspiration immediately after swallowing.
Moreover, clinically relevant doses of morphine and midazolam caused pharyngeal dysfunction and impaired airway protection in young adults. Partial neuromuscular block profoundly aggravated age-dependent pharyngeal dysfunction by predominantly impairing mechanical properties of the pharynx.

In conclusion, swallowing occurs during expiration in young and elderly individuals and drugs used in anesthesia cause pharyngeal dysfunction and dys-coordination of breathing and swallowing, ultimately compromising the protection of the airway against aspiration.

Keywords: *pharynx, control of respiration, breathing, swallowing, deglutition, anesthesia, morphine, opioids, midazolam, benzodiazepines, partial neuromuscular block, partial paralysis, rocuronium, airway protection, aspiration, sedation, postoperative pulmonary complications*

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. Hårdemark Cedborg AI, Sundman E, Bodén K, Kuylenstierna R, Ekberg O, Witt Hedström H, Eriksson LI.
Coordination of spontaneous swallowing with respiratory airflow and diaphragmatic and abdominal muscle activity in healthy adults
Experimental Physiology 2009, Apr; 94 (4): 459-468

II. Hårdemark Cedborg AI, Bodén K, Witt Hedström H, Kuylenstierna R, Ekberg O, Eriksson LI. Sundman E.
Breathing and swallowing in normal man--effects of changes in body position, bolus types, and respiratory drive
Neurogastroenterology and Motility 2010, Nov; 22 (11): 1201-1208

III. Hårdemark Cedborg AI, Sundman E, Bodén K, Witt Hedström H, Kuylenstierna R, Ekberg O, Eriksson LI.
Effects of morphine and midazolam on pharyngeal function, airway protection and coordination of breathing and swallowing in healthy adults
Manuscript 2013.

IV. Hårdemark Cedborg AI, Sundman E, Bodén K, Kuylenstierna R, Ekberg O, Witt Hedström H, Eriksson LI.
Pharyngeal function and breathing pattern during partial neuromuscular block in the elderly. Effects on airway protection.
Anesthesiology 2013. In press.

CONTENTS

ABBREVIATIONS

Bolus in mouth	Bolus in mouth measured as the interval between the times at which the bolus is first seen in the mouth and onset of pharyngeal swallowing (TB-start)
Coordination	Coordination of pharyngeal swallowing calculated as the interval between onset of muscle contraction at Ph Low (Ph Low-start) and the start of UES relaxation (UES-relaxation start) (negative value)
COPD	Chronic obstructive pulmonary disease
CPG	Central pattern generator, functional group of brainstem neurons for swallowing and respiration
Degree of pharyngeal dysfunction	Calculating *Degree of pharyngeal dysfunction*, by adding the number of signs (0 to 3) of pharyngeal dysfunction category A to C (see below) found in each of three swallows. The individual sum (0 to 9) was thereafter divided by the maximal outcome (*i.e.* 9), yielding degree of pharyngeal dysfunction (%).
E-E	Expiration-swallow-expiration
E-I	Expiration-swallow-inspiration
I-E	Inspiration-swallow-expiration
I-I	Inspiration-swallow-inspiration
EMG	Electromyography
GERD	Gastro esophageal reflux disease
Hypercapnia	Fractional concentration of inspired carbon dioxide ($FiCO_2$) 5%
Initiation	Initiation of the pharyngeal phase of swallowing calculated as the interval between the times at which the head of the bolus passes the anterior faucial arches and the hyoid bone starts to move forward
MAC	Minimal alveolar concentration
Mi	Midazolam
ml	Milliliters
Mo	Morphine
ms	Milliseconds
NMBA	Neuromuscular blocking agent
OSA	Obstructive sleep apnea
PAS	Penetration-aspiration scale (1-8)
Percentage of swallows showing signs of pharyngeal dysfunction	Calculating *Percentage of swallows showing signs of pharyngeal dysfunctio*n by adding the number of swallows showing one or more signs of pharyngeal dysfunction category A to C (see below) found in each of three swallows. The individual sum (0 to 3) was thereafter divided by the maximal outcome (*i.e.* 3), yielding percentage of swallows showing signs of pharyngeal dysfunction (%).

Ph Low	Pharynx lower level, part of oro/hypo pharynx, approximately at the lower pharyngeal constrictor muscle
Ph Up	Pharynx upper level, part of oro/hypo pharynx, approximately at the middle pharyngeal constrictor muscle
Ph Up/Low - start	Pharynx upper and lower level-start, onset of pressure rise at Ph Up and Low due to muscle contractions (relative to TB-start)
Pharyngeal bolus transit time	Pharyngeal bolus transit time measured as the interval between the times at which the head of the bolus passes the anterior faucial arches and the tail passes the UES
Post-swallow apnea	Time from end of pharyngeal swallowing (UES-start) to end of swallow apnea
Pre-swallow apnea	Time from beginning of swallow apnea to beginning of pharyngeal swallowing (TB-start)
Premature leakage of bolus (A)	Premature bolus leakage from the mouth to the pharynx before initiating swallowing, *i.e.* failure of oral coordination
Penetration of bolus to laryngeal inlet (B)	Penetration of contrast medium into the laryngeal vestibule or the trachea, *i.e.* failure of laryngeal protection
Retention of pharyngeal residues after swallowing (C)	Retention of contrast medium in the pharynx after completion of swallowing, *i.e.* impaired pharyngeal clearance
Pharyngeal dysfunction	Combining A to C (above), presented as either 1) Degree of pharyngeal dysfunction or 2) Percentage of swallows showing signs of pharyngeal dysfunction (see above).
PRS	Pyriform sinus residue scale (1-3)
SAD	Swallow apnea duration
SD	Standard deviation
TB	Tongue base, part of the oropharynx behind the base of the tongue
TB-start	Tongue base–start, onset of pressure rise at TB due to muscle contractions and *onset of pharyngeal swallowing* (t=0 ms)
TOF ratio	Train-of-four ratio (T4/T1)
UES	Upper esophageal sphincter muscle
UES- relaxation start	Upper esophageal sphincter relaxation –start, onset of pressure decrease following muscle relaxation (relative to TB-start)
UES-start	Upper esophageal sphincter –start, onset of pressure rise at UES due to muscle contractions (relative to TB-start) and *offset of pharyngeal swallowing*
VAS-sedation	Sedation scoring by visual analogue scale (10 to 0)
VRS	Valleculae residue scale (1-3)

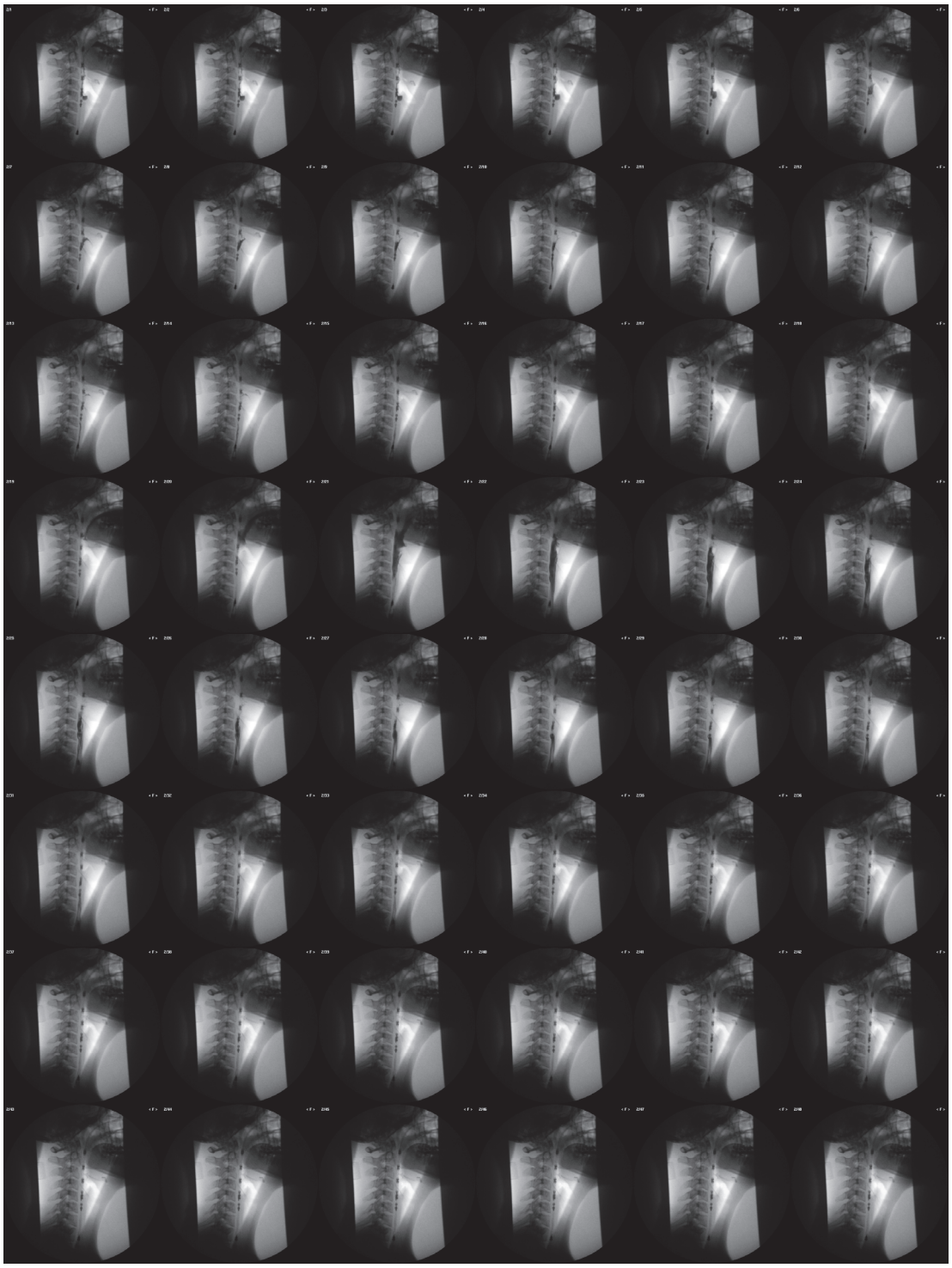

Illustration *A sequence of original fluoroscopic images, illustrating a swallow of 10 ml of contrast medium given to a young volunteer sitting in the upright position. When the bolus was given, oral bolus control was not sufficient which produced a premature leakage into the pharynx- as can be seen in pictures 1 to 4. This minute volume of contrast medium oases through the pharynx first and results in a minute supraglottic penetration during swallowing in picture 7. The actual swallowing of the bolus proper begins in picture 18 and the bolus has passed well below the upper esophageal sphincter into the esophagus in picture 39. Bolus clearance is good with no pharyngeal residues after swallowing. Pictures are ordered left to right in each row.*

INTRODUCTION

SWALLOWING

In 1883 the two German physiologists Hugo Kronecker and Samuel Meltzer published a classical report on swallowing, "Der Schluckmechanismus, seine Erregung und seine Hemmung" [1]. In this landmark paper, the authors used a balloon in the pharynx and esophagus, *i.e.* manometry, and studied human physiology of swallowing describing a variety of swallowing parameters including velocity of the muscle contraction wave in the pharynx and the esophagus. Since then, peripheral mechanisms of normal oro-, pharyngeal- and esophageal- swallowing have been extensively described in humans utilizing a wide array of technologies including manometry, fluoroscopy, endoscopy and electromyography. In contrast, central mechanisms of swallowing and breathing have mainly been studied in animals utilizing neurophysiological and imaging techniques.

The pharynx constitutes an intersection between the upper airway and the route for saliva, liquids and solid food from the mouth to the esophagus. Because of this anatomical challenge, swallowing and breathing is delicately coordinated to ensure normal airway control and safe bolus transfer to the esophagus. In humans, swallowing can be divided into three separate phases, the oral preparatory phase, the pharyngeal phase and finally the esophageal phase. During the pharyngeal phase, pharyngeal muscles contract to form a coordinated wave that moves the bolus onwards to reach the esophagus and the gastrointestinal destination. In addition, muscles providing protective sphincter functions at the resting state in-between swallows relax to allow passage of bolus. Moreover, movement of the tongue, the hyoid bone and the larynx facilitates normal swallowing. While voluntary control influences the oral phase, the pharyngeal and esophageal phases are considered increasingly reflexive.

The pharyngeal constrictor muscles have a major role in the formation and function of the pharynx. Uniting in the midline raphe structure of the posterior pharynx, the superior, middle and inferior laryngeal constrictor forms the muscle tube that constitutes the pharyngeal cavity (Fig. 1). During the dynamic pharyngeal phase of swallowing, the pharyngeal constrictor muscles propel the bolus along a rostral-caudal axis within the pharyngeal cavity targeting the esophageal inlet region in a typical wave pattern. The constrictors are complemented by longitudinal muscles, among them the stylopharyngeus and the palatopharyngeus that aid in elevation of the laryngeal complex during swallowing (Fig. 1). Also important for normal swallowing are the muscles of the tongue, forming and propelling the bolus into the oropharynx. The larynx then aids in protection of the trachea through closure of the vocal cords.

The upper esophageal sphincter or segment (UES) is a muscle complex which consists of the most distal part of the inferior pharyngeal constrictor muscle, the cricoid cartilage and according to some authors, also muscle fibers from the upper part of the esophagus (Fig. 1).

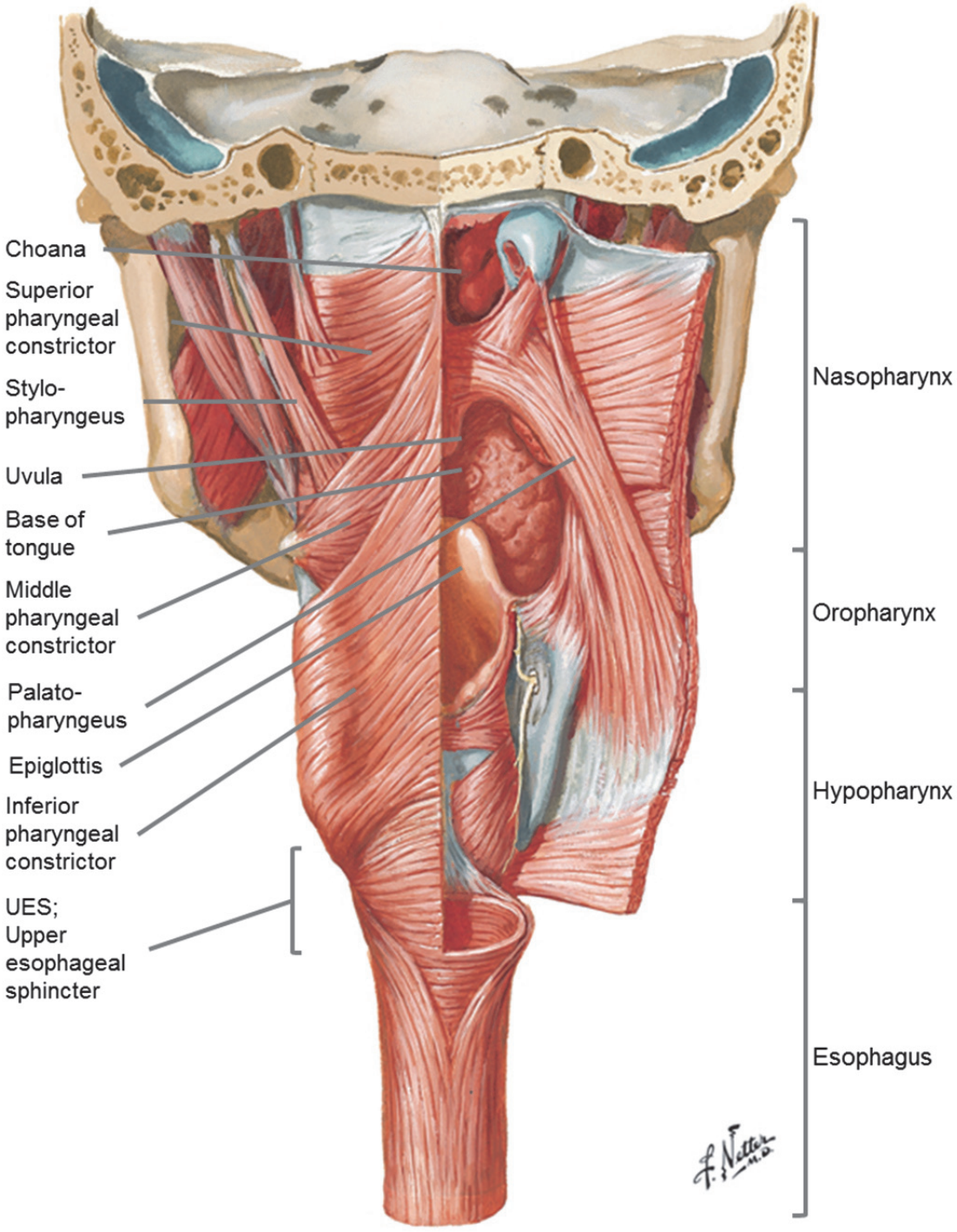

Figure 1 *The muscles of the pharynx*

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It functions as a sphincter in the most distal part of the pharynx where the pharynx transitions into the upper part of the esophagus. It is tonically active in between swallows and relaxes during swallowing to allow passage of bolus. The upper part of the UES, called the cricopharyngeus is structurally unique since it has no median raphe posteriorly. Relaxation of the UES is essential for bolus passage, and incomplete relaxation can result in bolus residues remaining in the pharynx after swallowing. Moreover, tonic contraction of the UES during breathing protects against aerophagia, regurgitation and aspiration.

The triggering and subsequent sequential motor drive that control the interplay between the pharyngeal muscle groups during a swallowing maneuver are dependent on multiple key sensory afferents from the periphery relaying signals to motor efferents via interneurons in the brainstem. In brief, the neuronal control of swallowing have been described as a sequence of five critical components: A) input from sensory afferents, B) input from cortical and midbrain fibers, C) swallowing centers in the brainstem receiving this input and relaying it to D) motor neurons triggering output in efferent motor fibers to E) muscles and other end organs. Sensory input from the periphery affects brainstem neurons and adaptation of muscle function is made accordingly to ensure normal swallowing. Separate reflex motor responses, *i.e.* swallowing, coughing or gaging are elicited depending on the anatomical location of the trigger zone stimulated, and are furthermore modulated by chemical contents, temperature and structure of bolus. Neurons controlling all stages of swallowing are located rostro-caudally in the brainstem reflecting their anatomical distribution within the swallowing apparatus, *i.e.* the neurons controlling the oral stage are located more cranially than the neurons governing the esophageal phase [2]. In addition, neurons within each motor nuclei of the brainstem are organized according to this myotopic map [2]. Here, neurons firing during initiation of pharyngeal swallowing (triggered by peripheral or central input) start a serial reaction of stimulation and inhibition of more caudal neurons that parallels the rostral-caudal anatomy of the swallowing tract in order to form the classical contraction wave pattern [2].

Neuronal sensory afferent input, from the naso- oro and hypo-pharynx, is relayed through the cranial nerves V, IX, X. In the pharynx the glossopharyngeal (IX) and vagal (X) nerves innervate the pharyngeal plexus located in the upper posterior wall of the oro-pharynx providing the main sensory trigger area for the pharyngeal phase of swallowing. In animal models the pharyngeal phase of swallowing can be triggered by artificial mechanical stimulation of the superior laryngeal nerve [3]. Swallowing can also be triggered more distally *i.e.* in the hypo-pharynx. This is more common during sleep [4] and during sequential repetitive swallowing [5]. Efferent innervation of pharyngeal muscles is complex, however the most important motor nerves originate from the vagal nerve (X) and the accessory nerve (XI).

For safe handling of oral content the following key functions need to be intact during swallowing:
A) oral bolus control, preventing leakage of bolus into the pharynx before initiation of swallowing,
B) laryngeal protection, preventing penetration of bolus to the laryngeal inlet and subsequent aspiration and
C) pharyngeal bolus clearance, ensuring no residuals after completion of swallowing.
Moreover, a key factor in normal swallowing is correct timing of events, *i.e.* triggering the separate events in the right order [6,7]. This is often referred to as the "fixed pattern" of swallowing [2]. Critical events included in this pattern are onset of ventral movement of the hyoid bone, laryngeal elevation, folding of the epiglottis, closing and opening of the laryngeal vestibule and vocal cords and onset of muscle contractions and relaxations [8]. Finally, adequate muscle activation with sufficient contractions and relaxations, are important for the swallowing maneuver [9].

COORDINATION OF SWALLOWING AND BREATHING

From an evolutionary perspective, the oral cavity has invariably been an important part of normal gas exchange and breathing, either in combination with gills or later on also with lungs. For example, in subaquatic animals where oral breathing is a part of the respiratory system, pumping water into the oral cavity and over gills was sufficient to guarantee adequate gas exchange. However, during transition to terrestrial life, development of other pumping systems was a prerequisite [10] to sustain organs that consume high amounts of oxygen such as a large brain (Fig. 2). The developmental stages of the frog, from larvae (tadpole) into an adult frog, provide a model to understand this phylogenetic evolution. In the larva stage, during buccal ventilation, *i.e.* sequential buccal dilation and constriction, water is moved in and out of the oropharynx and over the gills. In contrast, the adult frog displays two separate ventilatory patterns, buccal and lung ventilation. In buccal ventilation, air is moved in and out of the oropharynx with nares open and larynx closed. In contrast, during lung ventilation, buccal dilation moving air into the oropharynx is followed by a second step of buccal constriction where the nares close, larynx opens and air is squeezed into the lung. Translating the mechanisms for control of breathing and swallowing in the adult frog into mechanisms in mammals, the neurons active during lung ventilation are proposed to be the equivalent of inspiratory neurons in mammals and subsequently humans [11]. Moreover, the neuronal brainstem pattern generator for buccal ventilation is proposed to have developed into the pre- inspiratory center containing neurons active during late expiration. These neuronal predecessors are important when considering and interpreting muscle activity in the oro-pharyngeal cavity during breathing and swallowing.

Figure 2 *Evolution of coordination of breathing and swallowing*

The origin of the mammalian respiratory rhythm is still debated, however the idea that groups of neurons in the brainstem act as coupled oscillators is generally accepted [10,12-14]. Here, neurons active before inspiration (*i.e.* during late expiration), during inspiration and during expiration generate a continuous rhythm, through collateral neuronal inhibition and stimulation from the first breath until the last. The coupled oscillators are separate functional and anatomical groups of neurons perceived as rhythm generators. This complex neuronal network controlling breathing is located in close proximity to neurons controlling other activities involving the respiratory tract such as coughing, hiccups, belching, gagging, vomiting and swallowing. Moreover, the respiratory rhythm generators are embedded in a plexus of sensory feedback sources

and thereby modulated by peripheral input from mechanoreceptors, nociceptors and chemoreceptors [14] as well as central input from cortical areas [2].

Current knowledge in respiratory rhythm generation was recently comprehensively described in a review article by Smith *et al* [14]. In the CPG for respiration, located in the brainstem, circuits of interneurons, both excitatory and inhibitory, interact and ultimately generate the output to motor neurons.
At onset of inspiration, neurons located in the pre-Bötzinger complex in the ventral respiratory group (column) are active. Within this complex, three types of inspiratory neurons can be distinguished:
A) pre-inspiratory/inspiratory neurons, believed to be involved in initiation of inspiration and also to activate oro-pharyngeal muscles through the hypoglossal nerve, thereby preventing airway obstruction;
B) early-inspiratory neurons and
C) augmenting-inspiratory neurons, driving phrenic motor neurons and ultimately the diaphragm.
At onset of expiration, neurons located in the Bötzinger complex in the ventral respiratory group (column) are active. Here two types of expiratory neurons can be distinguished:
D) post-inspiratory neurons, believed to be critical to termination of inspiration and through vagal afferents adducting vocal cords during early expiration thereby initially preventing airflow;
E) augmenting-expiratory neurons, producing internal intercostal and abdominal muscle activity during late expiration.
Notably, these neuronal activities overlap, mirroring a biological successive transition from inspiration to expiration and back, highlighting the difficulty in defining clear cut phases.

Chemoreception, *i.e.* neuronal activation from elevated levels of CO_2 in the blood or brain (hypercapnia), stimulates both inspiratory and expiratory neurons, through activation of excitatory neurons in the retrotrapezoid nucleus/ parafacial respiratory group and the raphé nucleus, thereby increasing respiratory drive [14]. During breathing at rest or during load (*e.g.* hypercapnia), pharyngeal muscle groups are typically activated in order to stabilize airway dynamics and prevent the upper airway from collapsing during inspiration. This integration between breathing and pharyngeal activation, with increased tonic muscle contraction within the pharynx, is negatively impacted by a majority of commonly used anesthetic drugs in a dose-dependent fashion [15].

Respiratory phase patterns

During the pharyngeal phase of swallowing breathing is stopped and simultaneously the pharyngeal cavity undergo early conformational changes primarily in the epiglottic and laryngeal inlet region to ensure safe bolus passage and avoid aspiration [7,16]. In 1920, Clark demonstrated that most swallows occurred during expiration. Today, four respiratory phase patterns during swallowing have been described [6], that is E-E (inspiration-expiration-swallow apnea-expiration), I-E (inspiration-swallow apnea-expiration), E-I (inspiration-expiration-swallow apnea-inspiration) and I-I (inspiration-

swallow apnea-inspiration). Many investigators have found E-E to be the preferred pattern in humans [6,7,17-21], although the proportional part varies between studies. Moreover, it has been proposed that the E-E pattern is preventive of aspiration [22]. The least common pattern is swallowing during inspiration, *i.e.* I-I. E-I and I-I are often considered risk patterns since inspiratory airflow directly after swallowing is associated with increased risk for aspiration [23]. In animal models, artificial stimulation of the superior laryngeal nerve (X) triggers pharyngeal swallowing, however and interestingly, not during inspiration, but immediately before or after [3]. This suggests inhibition of the CPG for swallowing during inspiration.

Swallow apnea

Swallow apnea, *i.e.* breath holding during swallowing, was defined and described by Clark [24]. While the current definition of swallow apnea depends on the type of methodology used to record breathing and swallowing, it is most commonly described as either zero airflow or airway pressure or lack of respiratory movements during swallowing. Basically, swallow apnea consists of three phases 1) pre-swallow apnea; the period between onset of apnea and onset of pharyngeal swallowing, 2) apnea during pharyngeal swallowing and 3) post-swallow apnea; the period between end of pharyngeal swallowing and end of apnea.

Notably, exact definitions depend on the methodology used and may differ between studies utilizing manometry, fluoroscopy, electromyography, sound or endoscopy to record swallowing and whether indirect or direct methods have been used to assess breathing.

Studies of the phases of swallow apnea has shown that duration of pre-swallow apnea is highly variable in length even in young healthy adults compared to the duration of other events of the stable "fixed pattern" of pharyngeal swallowing [6]. Initially, swallow apnea was thought to be a result of prevented airflow due to laryngeal closure as a part of the normal swallow mechanism. However, more recent data suggests that onset of swallow apnea precedes closing of the laryngeal vestibule and vocal cords and therefore should be regarded as a separate entity [7,25,26] with unique central control mechanisms. Many have investigated possible mechanisms involved in onset of swallow apnea. Interestingly, already at the turn of the previous century, researchers observed a negative deflection in esophageal pressure, *i.e.* decreased intrathoracic pressure, during swallowing and introduced the term "Schluckathembewegung" (transl. swallow-breath) [1,27,28]. In 1957, Atkinson and co-workers discovered negative esophageal and positive intragastric pressures during pharyngeal swallowing and speculated that this could either depend on laryngeal elevation or an inspiratory effort *i.e.* "Schluck Atmung or swallow-breath" [29]. Further exploration of swallow breath were made in 1992 by Grelot *et al* who in the cat [30] described phrenic motor neuron depolarization during swallowing, a finding that confirmed a previously described phrenic nerve activation during swallowing by Nishino *et al* in 1985 [31]. Moreover, in 1994 Oku *et al* revealed a transient activity in inspiratory neurons during swallowing [32]. Using even more complex neurophysiological methodology in studies of the rat, Saito *et al* through intracellular recordings in addition to phrenic and hypoglossal nerve recordings (Fig. 3) concluded bilateral monosynaptic connections

between respiratory related neurons and neurons involved in swallowing [3]. The mechanical significance of "swallow breath" is however still unclear.

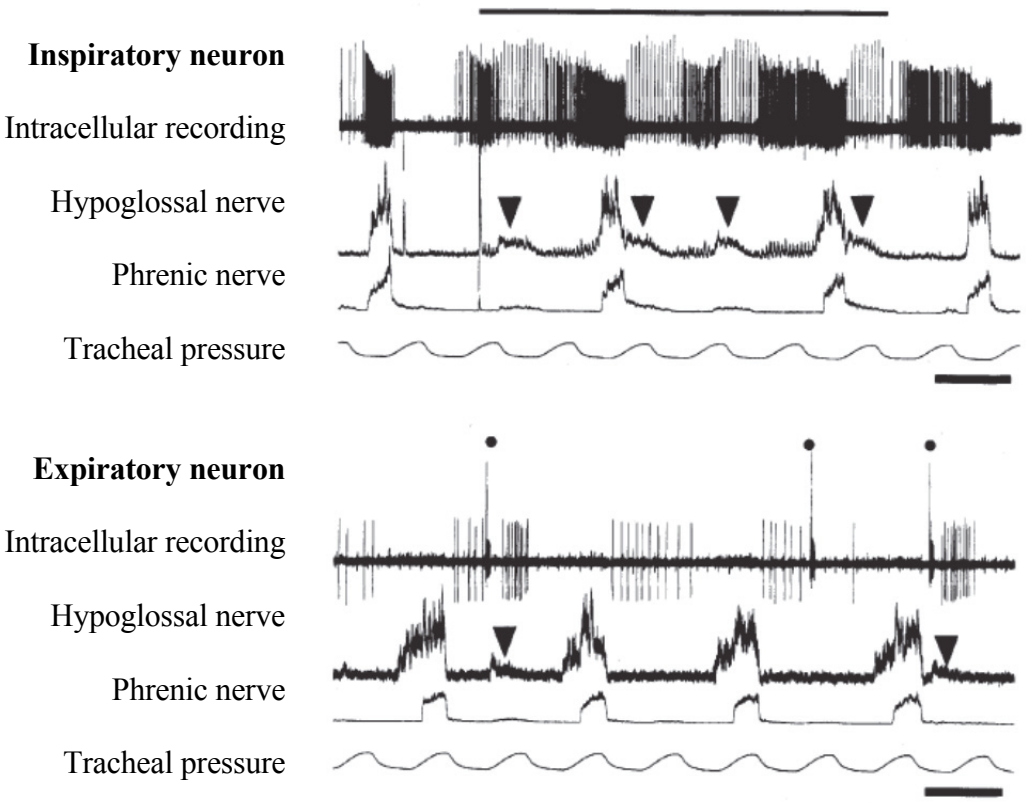

Figure 3 *Respiratory neuron activity in the rat brainstem during swallowing, through artificial triggering by stimulation of the ipsilateral superior laryngeal nerve [3]. Bar= continuous stimulation, Dot= single stimulation. "Swallow breath" activity in the phrenic nerve can be seen during fictive swallowing (▼). Tracheal pressure variation is due to artificial ventilation. Modified from original recordings [3].*

In contrast, compared to pre-swallow apnea, duration of post-swallow apnea is generally shorter, displays a lower degree of variability and the end of swallow apnea is correlated in time to the end of pharyngeal swallowing [6,17]. Moreover, the end of swallow apnea correlates to opening of the laryngeal vestibule and the vocal folds [6,17]. During swallowing, subglottic pressure is normally positive, however this depends on the lung volume being above the functional residual capacity [33]. During breathing at rest, expiration is mostly passive, *i.e.* expiratory airflow depends on lung volume and elastic recoiling forces. However, during stress, *i.e.* exercise or hypercapnia, expiratory muscles are activated increasingly towards late expiration. Evidently, opening of the vocal folds over a positive subglottic pressure would produce expiratory airflow after swallowing, and this is believed to be a protective mechanism reducing the risk for post-swallow aspirations [33].

The mechanism of central integration of breathing and swallowing has recently been proposed to consist of neurons involved primarily in swallowing reconfiguring the

respiratory neural network, thereby recruiting elements ordinarily used in breathing to participate during swallowing [34]. Evidence suggests that non respiratory neurons, activated before and during swallowing, reconfigures the respiratory CPG thereby prolonging the expiratory phase, inhibiting inspiration and generating swallow apnea [34]. This theory is supported by evidence of brainstem co-localization of non-respiratory neurons active during swallowing with neurons active in synchrony with respiration [3]. Moreover, the respiratory phase interrupted by swallowing is prolonged, a phenomenon called respiratory phase resetting [21], which supports the theory that the swallowing CPG reconfigures respiration [22,35]. However, due to the complex nature of these interactions, extensive future studies, both *in vivo*, *in vitro* and *in silico* (computer simulations) will be needed.

In summary, essential activities of the pharynx, such as swallowing, need to coordinate with breathing to ensure normal airway protection to avoid complications ranging from discomfort, choking, overt and silent aspiration to potential asphyxiation and subsequent death. Apparently, several components and details on how swallowing and breathing coordinates are still uncharted.

FACTORS INTERACTING WITH PHARYNGEAL FUNCTION AND COORDINATION OF BREATHING AND SWALLOWING

Physiological factors

Respiratory drive

Since respiration and swallowing are closely integrated, changes in respiratory drive during hypoxia or hypercapnia may influence coordination of breathing and swallowing. Moreover, since brainstem interneurons involved in respiration are active during swallowing [34], peripheral pharyngeal function may also be affected.

There is evidence that hypoxia depresses the reflex to swallow [36,37]. In a study of decerebrated, vagotomized and ventilated cats, as expected, hypoxia increased the respiratory drive, *i.e.* respiratory activity in the phrenic and hypoglossal nerves [36]. In contrast, hypoxia was associated with a dose dependent decrease in swallow frequency, however no effect on latency to swallow could be detected [36].

Hypercapnia also decreases swallow frequency [20]. While a direct inhibitory effect of hypercapnia on brainstem swallowing neurons would be a plausible causality, augmented pulmonary pressure reflexes by larger tidal volumes has also been suggested to inhibit swallowing [37]. Another explanation could be inhibition of swallow related neurons by CO_2 stimulated inspiratory neurons [3,11,34,38]. Moreover, Nishino *et al* detected dys-coordination of breathing and swallowing during hypercapnia, where swallowing during expiration decreased in favor of risk respiratory phase patterns as E-I and even I-I. Notably, swallows occurring more frequently during inspiration at hypercapnia were dose-dependently associated with increased signs of laryngeal irritation, suggesting higher risks for aspiration [20]. Interestingly, during temporary

airway occlusion the effect of normo- and hypercapnia was similar [37], suggesting that pulmonary pressure have a major impact on coordination of breathing and swallowing. In agreement, hypocapnia due to voluntary hyperpnea is associated with decreased swallow frequency [39], again suggesting inhibition by pulmonary pressure reflexes. Moreover during hyperpnea, dys-coordination of breathing and swallowing occurred with increasing risk patterns [39]. Thus, there are evidence of hypoxia and hypercapnia interfering with swallowing and breathing, however the mechanisms are still not fully understood.

Lung volume and airway pressure

Swallowing is facilitated by a large lung volume, and it has been shown that swallows initiated at the residual lung volume exhibits prolonged pharyngeal motor activity compared to those initiated at vital capacity or at functional residual capacity [40]. Moreover, during normal swallowing the subglottic air pressure is positive [33]. Furthermore, subglottic air pressure depends on the recoil of the respiratory system, *i.e.* on lung volume [41]. It has been postulated that subglottic mechanoreceptors, registering subglottic airway pressure provides respiratory-related afferent input to the brainstem CPG for swallowing and that efferent output is modified accordingly [41]. Correspondingly, artificially triggered swallows in the rat often coincides with periods of lung inflation [3] again suggesting that a positive subglottic pressure via vagal afference affects CPGs for swallowing and breathing. This was further investigated by Wheeler-Hegland *et al* in a study of lung volume at initiation of swallowing in healthy human volunteers [42]. Interestingly, they found that regardless of the respiratory phase when swallowing was initiated, the amount of air in the lung was consistent at around 51-56% of vital capacity and 95% of swallows were initiated at 43-64% of vital capacity [42]. Notably, this volume is above the functional residual capacity (normally approximately 35% of vital capacity).

Studies of respiratory phase patterns during artificial manipulations of respiratory loads have revealed that during restriction of respiratory airflow, patterns change from the normal E-E to I-E and during elastic loads the E-I pattern becomes more frequent [43]. Notably, the E-I pattern was also associated with more signs of laryngeal irritation, suggesting increased risk for aspiration. During flow restriction, respiratory rate decreased and tidal volumes increased. In contrast, elastic load had the opposite effect, and moreover swallow frequency increased. Suggested mechanism for these changes have been either that respiratory rate and swallowing are coupled mechanisms and that increased respiratory rate would in parallel increase swallow frequency, or, that there exists an active mechanism contributing to swallowing occurring during the expiratory phase [43]. Fascinatingly, it has also recently been confirmed that the pleasurable feeling created by a sigh is not imaginative. Evidence suggests that removal of an inspiratory load, *i.e.* removal of dyspnea in combination with a large tidal volume generates pleasure [44]. Again this could indicate how brainstem mechanisms strive to maintain a positive subglottic airway pressure.

Another important factor influencing the pharynx and breathing is addition of a continuous positive airway pressure, where an increased end expiratory airway pressure progressively decreased swallow frequency and increased latency to swallow in healthy

volunteers [45]. However, in patients with progressive neuromuscular disease a positive airway pressure and assisted ventilation has been shown to improve swallowing significantly [46] (see section on impact of assisted ventilation, page 21). Notably, airway protective mechanisms elicited by tracheal irritation were mostly unaltered by continuous positive airway pressure, indicating that this mechanism for protection is separate from mechanisms elicited from pulmonary pressure reflexes [47].

Thus, evidence suggests that subglottic air pressure plays a significant role in swallowing and coordination with breathing, and a higher subglottic pressure seems preferable.

Posture, bolus type and administration

Most swallows in life contain about one milliliter of isotonic saliva at oral normothermia. Swallowing can be performed in almost any posture, however the upright position is for obvious reasons easier for drinking. Feeding nursing home residents in the upright position has furthermore proven to be effective in reducing the risk for pneumonia [48].

Notably, most patients during intensive care or in the immediate perioperative period are in a supine or lateral position. During feeding and drinking the oral sensory system is challenged by larger boluses, different temperatures, hyper- or hypotonicity, differences in viscosity and a wide variety of textures. By triggering separate peripheral sensory receptors the afference received by neurons in the CPGs for respiration and swallowing is altered and thus the efferent output is modified accordingly.

In more detail, changing body position from upright to supine affects pharyngeal function and UES relaxation pressures and timing, however the time course of the pharyngeal contraction wave or spontaneous swallow frequency remains stable [17,49,50]. Moreover, no effect of posture on swallow frequency could be detected [50]. Data are scarce regarding effects of posture on coordination of breathing, however in the upright position swallows have been reported to occur later during the expiratory phase as compared to when volunteers were swallowing standing on all fours [51]. In contrast, Bodén *et al* could not detect an effect of posture on coordination of breathing and swallowing comparing the upright- to the left lateral (decubitus) position [17].

A more upright position, extension of the head, mandible advancement, closing of the mouth and a lateral position as opposed to supine when lying down, have all been shown to decrease airway collapsibility and thereby improve airway patency [52-57]. However, other positions have been shown to affect swallowing and the coordination with breathing. Here, a more reclined position, flexion of the head (chin-tuck position), mouth opening and mandible advancement have been associated with prolonged swallowing (EMG), longer durations of swallow apnea and increased frequency of E-I swallows [52,58].

In studies of effects of larger bolus sizes on pharyngeal swallowing and breathing, prolonged and earlier UES relaxations [26,49], higher pressure in the UES during UES relaxation [49], increased pharyngeal peak pressures [59], longer total swallow apnea

duration [19,25,60], prolonged pre-swallow apnea duration [60,61], increased duration of activity in laryngeal muscles [62] and laryngeal closure [63] and finally more inspirations before [60] and after [16,64] swallowing have been noted. Notably, some of these studies could not detect effects of bolus size on duration of swallow apnea [21,64] or on respiratory phase patterns [19]. Differences in methodologies and methods of administering the bolus have been suggested as explanations for these conflicting results and also that study sample sizes have been small [19].
Swallows of saliva have been associated with earlier onset of activity in oral muscles and in some studies shorter periods of pre-swallow apnea as compared to bolus swallows of water and contrast medium [22,50,62,65]. Moreover, swallows of pure water have been found to more effectively stimulate swallowing than water with added anions [66] and also to be associated with fewer inspirations after swallowing [50]. Notably, studies on differences comparing boluses of water to contrast medium of the same volume are scarce.

The method for administering the bolus affects the pharynx and breathing. For example, i) self-administered drinking from a cup [19,60] as compared to a syringe [16,64], spoon [67] or straw [5], ii) volunteer being administered the bolus from another source or person [16,67], iii) swallowing spontaneously [22,50] or iiii) on instruction [22] has been associated with changes in coordination of breathing and swallowing, for example prolonged pre swallow apnea, prolonged duration of the breathing cycle around swallowing, initiating swallowing at a higher residual lung volume and more frequent inspirations after swallowing.

Other important factors to consider when studying swallowing and coordination with breathing are bolus temperature [26,68], viscosity [18,21,59,61,64,65,67], texture [64,69,70], taste [71,72] and chemical properties [73,74]. Furthermore, interesting differences in swallowing and coordination of swallowing and breathing have also been seen comparing single bolus swallows to repetitive/sequential swallowing. During sequential swallowing, respiratory phase patterns are more variable where swallowing in proximity to inspiration occurs more often [5,75]. Moreover, swallowing is initiated at a higher lung volume compared to single swallows [5]. Also, sequential swallows are triggered in the hypopharynx [76], *i.e.* bolus is closer to the laryngeal inlet at onset of pharyngeal swallowing. In line with this, sequential swallowing has also been associated with higher penetration-aspiration scores [77] and more so in the elderly [78].

Gender

Gender effects on swallowing are generally small and, if present, can often be explained by size differences of the pharynx [59]. Furthermore, no major differences in coordination of breathing and swallowing have been attributed to gender [6,7,16,19], except for one study where females were reported to have longer durations of swallow apnea [19].

Sleep or arousal

During natural sleep, frequency of spontaneous swallowing is markedly reduced [79]. Moreover, during sleep, pharyngo-esophageal reflexes are attenuated and oropharyngeal muscle activities are reduced [80,81]. In contrast, during arousal and when

subjects are in a negative emotional state the rate of spontaneous swallowing is increased as compared to a relaxed, neutral or positive state [82,83]. Moreover, patients disorientated or unable to follow simple verbal commands suffer a profoundly increased risk for aspiration [84].

In conclusion, many physiological factors influence normal swallowing and coordination with breathing and these effects should be taken into consideration when interpreting impact of disease or anesthesia on pharyngeal function, airway protection and coordination between respiration and swallowing.

Impact of aging

Swallowing and airway protection changes with age. Change related to aging *per se*, without interference from disease, is called presbyphagia. The fact that presbyphagia is generally associated with good health [85] has led to speculations that changes during aging are mostly compensatory and protective.

Signs and results of presbyphagia, are extensively described [85]. In brief, the following changes of pharyngeal function occur during aging; 1) oro-pharyngeal swallowing is slower, especially the oral phase [85], 2) pharyngeal bolus transit is prolonged where bolus remains in close proximity to the laryngeal inlet longer [85] and 3) lingual pressures are reduced [85,86]. Moreover, 4) laryngeal penetrations are more common [87,88]. In addition, 5) relaxation of the UES is delayed, slower and incomplete, in turn making bolus passage slower [85,89], 6) hyoid movement and laryngeal elevation is reduced [85,89] and 7) pharyngeal clearance is more inefficient [90]. Many of these changes are believed to be an effect of sarcopenia [91,92], *i.e.* progressive loss of muscle fibers with increasing age. Sarcopenia weakens muscles and depletes reserves that are important during challenging situations where more muscle strength or speed is needed [91,92]. Moreover, alterations in the peripheral nervous system with reduced pharyngeal sensitivity [93] make thresholds for triggering responses to volume, pressures etc. more difficult to reach, *i.e.* more pharyngeal content needs to accumulate before a swallow can be triggered.

Notably, if compensatory mechanisms occurring during aging can no longer provide functional swallowing and ensure airway protection [94], the normal process of presbyphagia can turn into dysphagia [85]. This is evident in patients with a very high biological age, *i.e.* residents of nursing homes, where inability or difficulty to swallow is the most important risk factor for pneumonia and a major cause for morbidity and mortality [95,96]. Moreover, additional risk factors for aspiration pneumonia are more common in the elderly, for example reduced saliva production, impaired dental status and increased oral bacterial colonization [97], further increasing their vulnerability.

Also, the loss of reserve capacity in the elderly could during physiological or pharmacological challenges, promptly but reversibly transfer presbyphagia into dysphagia with compromised airway protection. This vulnerability is illustrated in many conditions such as: inserting a nasogastric tube [88], increasing and decreasing bolus volumes and delivering bolus through a straw rather than drinking [98,99], where more penetrations and aspirations are seen.

Table 1 *Studies of changes in coordination of breathing and swallowing with ageing.*

			Signs of dys-coordination with breathing						
Age	n	Young control group	Inspiration before swallow	Pre-swallow apnea	SAD	Post-swallow apnea	Inspiration after swallow	Inspirations after swallow	Technique (swallow & breathing)
60-90	18	yes	0		+		0	5%	microphone & nasal pressure [100]
60-90	18	yes	0				0		microphone & nasal pressure [101]
63-83	11	yes	0		0		+		submental EMG & thorax belt [50]
73+/-2	10	yes		0-	0				endoscopy (vocal cord closure) [26]
50-70	~45	yes			+				manometry & oral-nasal flow [102]
70-81	10	hist.		0	0				endoscopy (vocal cord closure) [103]
76+/-5	53	hist.					+	30%	movement sensor & nasal flow [104]
40-59	20	yes	0		0		0		submental EMG & nasal flow [19]
60-83	20	yes	0		+		0		submental EMG & nasal flow [19]
73+/-6	29	n.a.						9%	manometry & nasal pressure [105]
48+/-6	14	yes	0		0		0		fluoroscopy & nasal flow [7]
63-79	20	yes		+					manometry & oral-nasal flow [61]
81-97	20	yes			0+	+	+	>7%	fluoroscopy & nasal flow [6]
20-78	50	yes			+		0	2%	microphone & nasal pressure [106]
50-97	~35	yes		0+					fluoroscopy & nasal flow [107]
60+	40	yes			+				fluoroscopy & nasal flow [71]
60+	40	yes			+				fluoroscopy & nasal flow [74]
62-87	40	yes			+				fluoroscopy & nasal flow [72]
66-85	26	hist.	0	0	0	0+	0	5%	fluoroscopy & oral-nasal flow **

*Data on respiratory phase patterns, in particular inspiration before swallowing and the risk pattern of inspiration after swallowing, duration of apnea before onset of pharyngeal swallowing (pre-swallow apnea), duration of swallow apnea (SAD) and duration of apnea after completion of pharyngeal swallowing (post-swallow apnea) is presented. Age, mean +/- SD or range; n= number of elderly volunteers; n.a. = not available; hist.= historical control group; 0 = unchanged; + = increased or prolonged; % = percentage of swallows followed by inspiration;0+ = tendency for prolonged or increased;0- = tendency for shorter or decreased; ** Bodén et al, submitted manuscript, part of [8].*

Moreover, during sleep, the elderly are at an increased risk for a collapsed pharyngeal airway [108], again with an increased risk for regurgitation, aspiration and hypoxia. Also, during forced repetitive swallowing, elderly are more prone to swallowing impairment compared to the young [104], showing that elderly are less able to adapt to load and stress and therefore more vulnerable to detrimental outcomes.

In contrast to our knowledge of the pharyngeal aspects of presbyphagia, effects of aging on coordination of breathing and swallowing have been studied less extensively. While in some studies aging has been shown to affect coordination of breathing and swallowing, others have not been able to detect a difference due to age *per se*. Current information on coordination of breathing and swallowing in the elderly are summarized

in table 1. The apparent diverging results might be explained by differences in methodology or in variations in the definition of elderly, *i.e.* the actual age intervals of subjects included (Table 1).

Recently, Bodén *et al* [8] demonstrated differences between young and elderly volunteers regarding pharyngeal function and coordination of breathing and swallowing by application of the same methodology as in study II-IV. However, since this study provides a radiological perspective, only swallows of contrast medium were included and analyzed. Comparing 26 elderly volunteers*[1] to 32 young [17], pharyngeal dysfunction were more frequent in the elderly and was primarily due to premature leakage of bolus and laryngeal penetrations of the bolus. Regarding coordination of breathing and swallowing, duration of expiration after swallowing was shorter in the elderly while no difference in respiratory phase patterns or SAD was detected comparing young and elderly.

Effects of disease and other conditions

Artificial airway devices and assisted ventilation

While indwelling airway devices such as laryngeal masks and endotracheal tubes are often placed to maintain and protect the airway, they also disrupt both sensory and mechanical mechanisms for normal pharyngeal and laryngeal function [109]. Suggested mechanisms are mucosal sensory impairment, mucosal swelling and progressive muscle stiffening and weakness [109]. Moreover, a temporal adaptation of the protective reflexes seems to occur. After placing an airway device the initial responses are forceful expiration and cough [110,111]. However, over time, with airway devices present, the response pattern changes and repeated swallowing becomes more common [110]. Furthermore following extubation, laryngeal reflexes are not immediately restored as evidenced by an increased frequency of pulmonary aspirations after removal of the airway device [111-114]. This sensory and mechanical impairment usually improves over time [113]. Notably, many aspirations detected after extubation were silent, *i.e.* did not produce any cough [114,115].

There is an ongoing debate on the effect of tracheostomies on airway protection. In a prospective study of patients before and after tracheotomy, no causality between tracheostomy and risk for aspiration could be found [116]. In contrast, others have described a profoundly impaired pharyngeal function with increased aspirations in patients after tracheostomies [117,118] and more so with inflated cuffs [119]. Notably, it is difficult to study single causalities in this group of patients as the reason for the tracheostomy and any medical complications occurring due to underlying disease or during intensive care will impact results.

*[1] The 17 elderly volunteers in study IV are a subset of 26 elderly in the radiological study by Bodén *et al* investigating effects of body position, currently a submitted manuscript under revision.

A paradoxical improvement in swallowing has been described after tracheotomy and mechanical ventilation in patients with Duchennes Muscular Dystrophy [120]. Moreover, using an expiratory valve improves swallowing and reduces the risk for aspiration [117,121-123]. These findings suggest that the patient's ability to swallow and protect the airway may actually benefit from adequate use of tracheostomies, expiratory valves and mechanical ventilation and thereby challenges the traditional belief that mechanical ventilation and a positive end-expiratory pressure (speaking valve, ventilator etc.) impair pharyngeal function and coordination of breathing and swallowing. However notably other studies have not been able to detect these effects [124,125]. A positive effect on swallowing performance [41] and coordination of breathing and swallowing with the use of a valve [125] and during mechanically assisted ventilation in patients with neuromuscular diseases [46], is in line with previously described effects of a positive subglottic air pressure and large lung volume [41]. Finally, coordination of breathing and swallowing including swallow apnea is preserved in patients after a laryngectomy [25,126], even after complete separation of peripheral pathways, which strongly points towards that coordination of breathing and swallowing is a mechanism centrally controlled by the brainstem.

Disease

Pharyngeal function, coordination of breathing and swallowing and airway protection are affected by diseases, in particular neurological [127,128] and pulmonary conditions. Interestingly, the effects of many neurological diseases on the pharynx and breathing are strikingly similar, whether it be acute stroke [23] or progressive neurological and neuromuscular diseases such as amyotrophic lateral sclerosis [129,130], Duchennes muscular dystrophy [120], Parkinson's disease [131,132] and Alzheimer's disease [127]. Key features of impairment of pharyngeal function in these diseases are prolonged bolus dwelling durations, increased frequency of penetrations and aspirations and multiple smaller swallows (piecemeal swallowing). Notably in patients with Parkinson's disease, physiological stress makes them even more prone to swallowing impairments as seen during forced repetitive swallowing [133]. Moreover, disruption of the coordination of breathing and swallowing are frequent in neurological conditions, where more inspirations follow after swallowing and duration and timing of swallow apnea is altered [23,120,132].

In chronic obstructive pulmonary disease (COPD) increased baseline respiratory rate and lower baseline oxygen saturation is associated with increased risk for penetration/aspiration [134] and COPD is a risk factor for pneumonia in residents of nursing homes [96]. Moreover, aspiration can aggravate existing COPD by triggering exacerbations, a potential vicious circle. Also in COPD patients, larger bolus volumes are associated with aspiration [134] and alterations in respiratory phase patterns [50], suggesting vulnerability to challenges. It is still unclear whether pharyngeal dysfunction in COPD is a primary effect on the pharynx or if it is secondary to increased respiratory efforts or a combination of both. In favor of primary pharyngeal effects are findings of decreased hyoid elevation [134]. However, in favor of a respiratory causality are findings of alterations in lung volumes at onset of swallowing and increased incidence of swallowing associated with inspiratory air flow [50,134,135]. Notably, duration of swallow apnea seems unaltered in COPD [134,135].

The role of the pharynx is central in obstructive sleep apnea (OSA) with nocturnal disturbed breathing, snoring, frequently occurring apneas and hypoxia. Swallowing is

impaired in patients with OSA where oro-pharyngeal sensory decline, impaired oral bolus control, latency to swallow and decreased pharyngeal clearance [136-138] has been described. Notably, data on effects of OSA on coordination of breathing and swallowing are few, however a shorter post-swallow apnea has been recorded [137,138]. However, respiratory phase patterns during swallowing seem to be unaltered in patients with OSA[134,135] .

Gastroesophageal reflux disease (GERD), although primarily a disorder of the lower esophageal sphincter and the esophagus, is associated with impairment of oro-pharyngeal swallowing [8,139]. Reduced oro-pharyngeal muscle forces found in GERD patients could explain findings of increased laryngeal penetrations and impaired pharyngeal clearance [8,139,140]. However a paradoxical shorter pharyngeal transit time has also been noted [8,140]. Data on coordination of breathing and swallowing in GERD patients is scarce, however changes seems to be minor [8,140].

Pharyngeal function and airway protection can also be affected by systemic diseases and structural impairments, due to surgery [141], trauma, malignancy or developmental anomalies. In these situations, coordination with breathing has been only sparsely studied.

Drugs in anesthesia

Anesthesia airway research has focused on physiology and pharmacological effects on i) laryngeal reflexes, *i.e.* laryngospasm and protective reflexes preventing aspiration and ii) airway patency and its tendency to collapse during anesthesia. Recently published comprehensive reviews on upper airway physiology during anesthesia and sleep presents current knowledge on pharmacologic modulation of pharyngeal collapsibility [142-145].

In this thesis, the primary focus was set on the effects of drugs used in anesthesia and intensive care on the coordination of breathing and swallowing, pharyngeal function and airway protection. While effects of such drugs have previously been studied and described, the map is still far from complete, and notably, effects of drugs in anesthesia on coordination of breathing and swallowing are still mostly unknown.

Nitrous oxide

In the late 1980s, Nishino and co-workers found a dose-dependent increased latency to swallow in human volunteers during anesthesia produced by nitrous oxide (N_2O) [146]. This has also been confirmed in studies of the cat where N_2O increased latency to swallow and at high doses abolished swallowing, notably with maintained respiratory activity. Furthermore, N_2O increased the threshold stimulus needed to trigger reflex swallowing, however once triggered, the amplitude of the hypoglossal nerve remained stable compared to control conditions [31]. Moreover, nitrous oxide anesthesia increases the incidence of pulmonary aspirations in healthy patients during dental procedures [147]. In the early 1990s the first study investigating effects of anesthesia on coordination of breathing and swallowing was published. Intubated surgical patients were investigated under the influence of residual effects of enflurane and nitrous oxide combined with pentazocine (opioid) at the end of surgery. Here, respiratory phase patterns were found to be disrupted, with swallowing occurring during inspiration and expiration, without

the normal preference for the expiratory phase [148]. Moreover, 10-15% of all swallows that occurred during expiration were followed by a brief inspiratory effort. No difference in duration of upper airway closure between swallows coinciding with inspiration or expiration could be found. These discoveries set off the exploration of anesthetic effects on airway protection and the search for ways to avoid respiratory complications.

Volatile anesthetics

Videoradiography, *i.e.* manometry in combination with fluoroscopy, was introduced into the research field of airway protective mechanisms and anesthesia in the late 1990s by Eriksson and co-workers [149]. They showed a six- to seven-fold increase in the incidence of pharyngeal dysfunction, even at sub-hypnotic doses of isoflurane and sevoflurane in young healthy volunteers [150]. Here, pharyngeal dysfunction at 0.50 MAC_{awake} was mainly due to penetrations of bolus to the larynx [150]. Moreover, Nishino and co-workers have shown that enflurane at 1.0 to 1.8 MAC and sevoflurane at 1 and 2 % cause a dose dependent attenuation of airway protective reflexes such as cough, forceful expirations, panting, apnea and laryngospasm, provoked by local laryngeal stimulation in humans [151,152], potentially worsening pharyngeal function and increasing the risk for laryngeal penetrations. However, during anesthesia with sevoflurane at 1.2 and 1.8 MAC airway protective reflexes elicited in adults were not attenuated [153]. Suggested explanations for these conflicting findings are different depths of anesthesia, drugs or ages studied [151]. Isoflurane also abolishes upper airway dilator muscle activity, increasing the risk for impaired upper airway integrity and collapse [154,155]. Interestingly, halothane has been shown to impair sensory afferent input from mechanoreceptors [156]. Furthermore, in the cat, halothane and enflurane depress hypoglossal activity to a larger extent than they affect phrenic discharge, *i.e.* swallowing is more affected than breathing at equal depth of anesthesia [157,158]. This is important to consider during emergence form anesthesia, when spontaneous breathing may have resumed, however pharyngeal protective mechanisms are still impaired. There is to our knowledge no data on effects of volatile anesthetics on coordination of breathing and swallowing.

Intravenous anesthetics

Intravenous anesthetics affect the pharynx and breathing, for example, UES pressure decreased during intravenous induction of anesthesia with thiopentone and midazolam [159] and midazolam decrease swallow frequency [160]. Decreased UES pressure could facilitate regurgitation and reduced swallow frequency could decrease pharyngeal clearance over time, thereby potentially increasing the risk for aspiration. A plausible central mechanism would be anesthetic inhibition of hypoglossal activity, as shown in studies in cats for pentobarbital and ketamine as well as for volatile anesthetics [157]. Moreover, propofol has been shown to increase latency to swallow and decrease upper airway muscle activity [161], again effects that potentially lead to respiratory complications. Decreased upper airway muscle activity (genioglossus) is associated with airway collapse and has been seen with midazolam, pentobarbital and propofol [54,154,162-166], however, pentobarbital and isoflurane has also been shown to increase phasic genioglossus activity, more so during sedation and light anesthesia [154,163,164]. This contradiction in effects of different doses and drugs shows that simple dose-dependent relationships are rare and causalities are multifactorial, as

seen for example when results are influenced by level of consciousness and respiratory drive [157,162]. In 2001, Sundman *et al* presented comprehensive data on effects on airway protective mechanisms during sedation with propofol and inhalational agents [150]. It was concluded that propofol, isoflurane and sevoflurane markedly impair pharyngeal function even at sub-anesthetic levels, increasing frequency of penetrations of boluses to the laryngeal inlet [150]. Interestingly, increased incidence of pharyngeal dysfunction was correlated to volunteers reporting deeper level of sedation as measured using a visual analogue scale.

Ketamine is with regard to effects of anesthesia an exception to the rule of decreased pharyngeal muscle activity. Ketamine is associated with increased upper airway muscle activity [167] and has no significant effect on UES pressure [159]. Moreover, in studies in the cat, ketamine affected hypoglossal activity to a lesser extent than pentobarbital and halothane [157], suggesting a possible beneficial profile for ketamine compared to other anesthetics, however data is limited. Again, there is to our knowledge no data on effects of intravenous anesthetics on coordination of breathing and swallowing.

Neuromuscular blocking agents

Neuromuscular blocking agents are used to abolish oro-pharyngeal and laryngeal muscle activity to facilitate endotracheal intubation. While having a beneficial role during induction of anesthesia, residual effects of neuromuscular blocking agents have, during recovery, been associated with postoperative pulmonary complications [168]. It is well known that spontaneous recovery of muscle function following drug metabolism follows a hierarchical pattern regarding the order of when functionality of different muscle groups are restored (Fig. 4). Here, functionality of laryngeal muscles is restored in a similar time course as the diaphragm [169,171,172] . Moreover, return of laryngeal competency precedes restoration of peripheral muscle function such as in the adductor pollicis muscle of the hand [169,171,172].

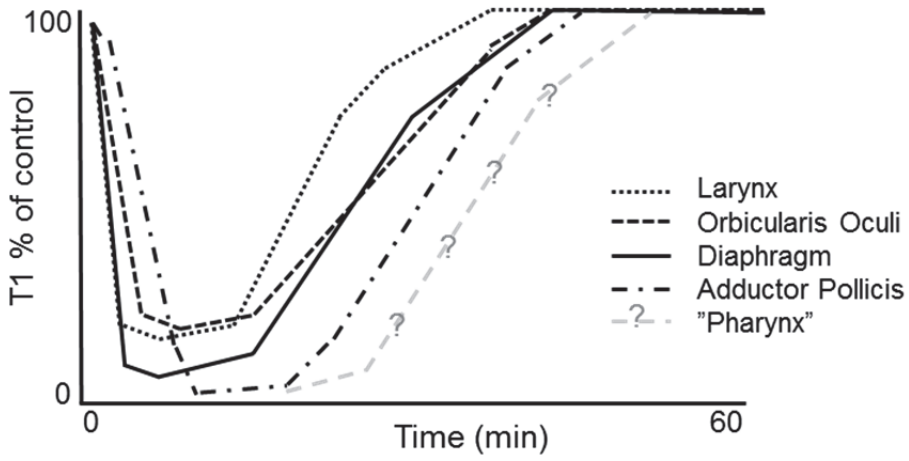

Figure 4 *Schematic picture describing time course of neuromuscular block in different muscle groups, "pharynx" is added for purpose of discussion. Modified after [169-171,149,172]*.

In 1989, Pavlin and co-workers examined effects of partial paralysis (d-tubocurarine) on the pharynx by asking awake volunteers if they could swallow and concluded that,

although normal ventilation was maintained, airway protection with regard to swallowing was still impaired [173]. Since then a growing body of evidence have made it clear that pharyngeal muscles recover considerably later compared to the diaphragm [171] and the adductor pollicis [174]. Notably, no change in latency to swallow has been noted, indicating that neural pathways are mostly unaffected during partial neuromuscular block [174,175].

Up until the late 1990s, an adductor pollicis TOF ratio of 0.70 was considered to indicate adequate recovery of respiratory muscles to allow safe extubation, results that were based on findings on recovered diaphragmatic function. Later, Eriksson, Sundman *et al* found that vecuronium and atracurium caused a significantly increased incidence of pharyngeal dysfunction with penetration of bolus to the laryngeal inlet even at TOF ratio 0.80 [68,92]. Furthermore, TOF ratios between 0.60-0.80 were associated with reduced pharyngeal muscle contraction forces, prolonged bolus transit time and disrupted muscle coordination. Moreover, even after recovery to TOF >0.90, the UES resting tone was still significantly reduced [68,92]. Based on this series of investigations, the authors concluded that an adductor pollicis TOF ratio of >0.90 indicated safe recovery of respiratory muscle function with maintained airway control. Not studied by these authors, NMBAs have also been shown to increase airway collapsibility, reduce airway dimensions and decrease upper airway dilator muscle activity [176-178]. Recent studies have also found residual effects even after recovery to TOF >0.90 regarding pharyngeal muscle functions in young adults [68,92] and upper airway obstruction in postoperative patients [179], suggesting an adductor pollicis TOF ratio of 1.0 as a safe level of recovery [180].

Reversal of neuromuscular block can be used to avoid partial paralysis and thereby decrease the risk for pulmonary complications [181]. This is usually accomplished through administration of an acetylcholinesterase inhibitor (*e.g.* neostigmine) or an encapsulating agent (*e.g.* sugammadex). However, increased airway collapsibility and reduced upper airway muscle forces have been reported in human volunteers after administering neostigmine but not sugammadex, after spontaneous recovery of blockade to TOF ratio >1.0. Notably neostigmine (in the absence of a neuromuscular block) may act directly onto the neuromuscular junction, with subsequent reduction in neuromuscular transmission and TOF fade phenomenon [182]. The mechanism behind this interaction is unclear but could potentially involve direct actions on the acetylcholine receptor ion channel [183]. Effects by an anti-cholinesterase on human swallowing and respiration have, to our knowledge, never been studied. In this context, anti-cholinesterase induced impairment of muscle forces could speculatively impair pharyngeal function and airway protection, therefore unwarranted use of an anti-cholinesterase in the absence of neuromuscular block should be avoided.

Opioids

Opioids have been extensively studied with regard to effects on respiratory rhythm generation [184]. Fentanyl in combination with propofol has been shown to dose dependently impair airway protective mechanisms [185]. Interestingly, opioid induced hypercapnia (spontaneous ventilation) seems to, in part, counteract this depression, preserving the response of apnea with laryngospasm to stimulation of the larynx [185]. In

contrast, at normocapnia (controlled ventilation through laryngeal mask), all airway protective mechanisms were abolished [185]. Regarding effects on pharyngeal function case reports describe findings of difficulty to swallow with intrathecal fentanyl, suggesting a central effect of opioids on the CPG for swallowing [186-188]. Furthermore, subjective difficulty to initiate swallowing has been reported during infusion of remifentanil in young human volunteers [189]. In studies of the frog and rat, it has been shown that opioids act differentially on respiratory oscillators and respiratory neurons with a preference to inhibit lung- as compared to buccal-ventilation [38,190] Moreover, opioids impair the sensitivity of the lung-oscillator to increased levels of CO_2 [38]. To our knowledge, there are currently no studies of humans on opioid effects on coordination of breathing and swallowing.

Topical anesthesia

Topical local mucosal anesthesia, used to ease patient discomfort during procedures involving the nasal cavity and the oro-pharynx, have been speculated to effect airway protection through reduced sensory feedback. Nasal topical anesthesia has recently been shown to increase the risk for laryngeal penetrations [191]. Furthermore, nasal topical anesthesia has been shown to produce faster pharyngeal bolus transit [192]. Mucosal oro-pharyngeal topical anesthesia has been associated with decreased oral bolus control, increased laryngeal aspirations, piecemeal swallowing and increased latency to swallow [193,194]. However, other studies have shown very minor or even no effects on swallowing by topical nasal or oro-pharyngeal anesthesia [195,196].

Clinical anesthesia and intensive care - multiple factors combined

In the perioperative period, complications involving the respiratory system are most frequent [197] and residual effects of drugs used in anesthesia are known to increase risks for postoperative morbidity and mortality [168,181]. Elderly patients are more prone to suffer complications after surgery and anesthesia [198,199] and during and after intensive care [200,201], and age is a risk factor for postoperative pulmonary complications after residual neuromuscular block [168].
Notably, during routine anesthetic practice and intensive care, patients are typically exposed to multiple drugs with poorly described interactions and additive effects on the pharynx and breathing. Moreover, additive effects of age, surgical trauma, co-morbidity, airway management and ventilation with residual effects on the airway mucosa and dimensions need to be taken into account, since they may further increase the risk for respiratory complications.

Clinical data on outcome in patients after anesthesia and intensive care is scarce; however there are a few important studies. Here, frequency of pulmonary aspirations after cardiac surgery and general anesthesia (diazepam, fentanyl, thiopental, enflurane, nitrous oxide and pancuronium) was examined by Burgess and coworkers in 1979 [202]. Using dye to detect aspirations, they found that 33% of patients aspirated directly after extubation, even when fully alert. Interestingly, none of the patients coughed upon aspirating the dye. Notably, they found no association between aspiration and duration of intubation or time after extubation. More recently, Kertz *et al* described postoperative unchanged pharyngeal function with no evidence of dysphagia, aspiration or hypoxia as assessed with fiberscopic endoscopy. They examined patients within 90

minutes after extubation following short neurosurgical procedures under general anesthesia (fentanyl, thiopentone, isoflurane, atracurium).

When considering these data, there is no straight forward statement on when it is safe to resume oral intake after surgery under general anesthesia or after extubation in the intensive care unit (in the absence of sedatives). Moreover, even if the patient is on strict *non per os* or tracheally intubated, micro-aspirations of saliva and regurgitations of gastric content can still be aspirated at all times. Furthermore, micro aspirations cause pneumonias, as oral decontamination with alcohol reduces the incidence of ventilator associated pneumonia (VAP) [203].

While previous studies have provided important information on the impact of some drugs used in anesthesia on pharyngeal function, the effects of many other drugs are still uncharted. Moreover, data on drug effects on the coordination of breathing and swallowing is lacking for most drugs. Even more importantly, in elderly at-risk patients, effects of anesthesia on the pharynx and breathing are unknown.

In conclusion, normal airway protection is a finely tuned mechanism that consists of multiple essential components, including coordination of breathing and swallowing. Increased understanding of these mechanisms for airway protection is essential for patient safety in the perioperative period and during intensive care.
However, in previous studies of young and elderly, definitions of “normal” are disparate and sometimes even contradictory. Therefore, in order to describe changes in key mechanisms caused by physiological challenges, aging, drugs and disease, further details of normal physiology need to be investigated and described.

"The important thing is to not stop questioning.
Curiosity has its own reason for existing."
Albert Einstein

AIMS

The overall aim of this thesis was to characterize key mechanisms for airway protection in healthy adults and thereafter to describe effects of age and drugs used in anesthesia.

The specific aims were:

- To validate the new multimodal high resolution approach for detection of respiratory airflow and swallowing.

- To describe in detail peripheral coordination of breathing and swallowing and their temporal relationship in young healthy adults.

- To determine the impact of changes in body posture, bolus characteristics and increased respiratory drive on coordination of respiration and swallowing.

- To describe pharyngeal function and coordination of breathing and swallowing in healthy elderly individuals.

- To assess the impact of clinically relevant doses of morphine and midazolam on pharyngeal function and integration between breathing and the pharynx in young healthy adults.

- To evaluate the impact of residual neuromuscular block on pharyngeal function and breathing pattern in healthy elderly individuals.

VOLUNTEERS AND METHODS

VOLUNTEERS

All studies conformed to the standards of the Declaration of Helsinki and were approved by the Regional Ethics Committee on Human Research at the Karolinska Institutet, Stockholm, Sweden. A total of 67 healthy volunteers were included (female: male, 33:34), 44 young (study I-III) and 23 elderly (>65 years) (study IV) after oral and written informed consent. Volunteers were without history of dysphagia, gastro esophageal reflux disease or surgery to the pharynx, esophagus or larynx. The studies were stratified with regard to gender. Young volunteers were medication free and none of the elderly volunteers used medication interfering with neuromuscular function or breathing.

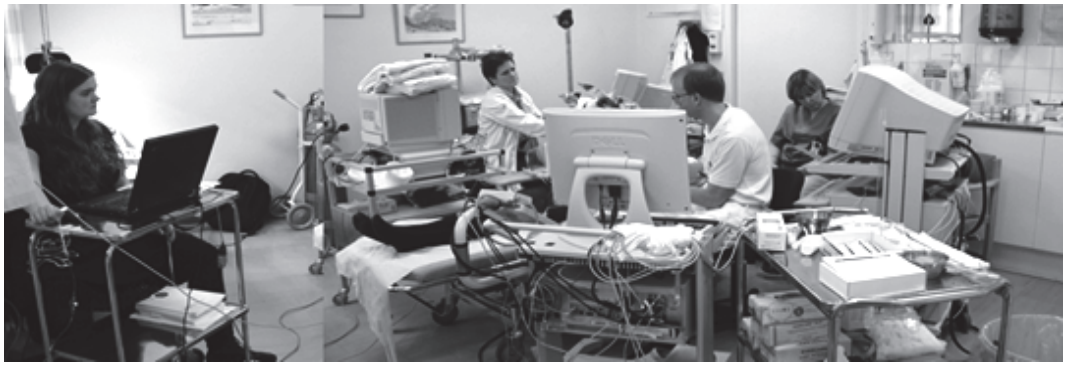

Figure 5 *The lab in study I, from the left, recordings of spirometry; in the middle surrounding the volunteer are two stations recording diaphragmal and abdominal EMG; to the right recordings of pharyngeal and esophageal manometry. All simultaneous recordings were synchronized.*

MULTIMODAL HIGH RESOLUTION TECHNIQUE

To provide a technical platform for in-depth analysis of normal coordination of breathing and swallowing, we first needed to introduce novel methodologies into the already established simultaneous videomanometry set-up. This technical improvement allowed us to perform simultaneous recordings with adequate high resolution in multiple modalities to accurately quantify aspects and timing of pharyngeal swallowing and respiration (Fig. 5).

Videomanometry, *i.e.* pharyngeal manometry and videoradiography recorded simultaneously and superimposed during swallowing of contrast medium provided a comprehensive recording of swallowing and pharyngeal function already in use by the research group. Manometry is suitable for recordings of swallowing and breathing at resting conditions during longer time periods. In contrast, videoradiography is only suitable for shorter recordings (total fluoroscopy time < 5s for recording of approximately 20 swallows in each individual) due to exposure to radiation.

Recordings of respiration needed to be non-invasive and provide a high temporal resolution as described in a review article by Tarrant *et al* [204]. Here, the recommended method was direct measurement of airflow using a nasal cannula, utilizing thermistors or pressure transducers. Moreover, the importance of a short response time for the chosen transducer was emphasized. In order to be able to quantify airflow, detect oral and nasal airflow, discriminate direction of flow and deliver gas (CO_2, anesthetic volatile agents), the option to use a nasal cannula as primary recording was deemed unsuitable and therefore we aimed for a solution including a face mask and a mass flow integrator (ASF1430, Sensirion AG, Staefa, Switzerland) originally designed for industrial purposes.

Bidirectional gas flow discriminator

The mass flow integrator (ASF1430) was remodeled into a bidirectional gas flow discriminator detecting respiratory airflow by dual temperature-compensated thermistors and according to the manufacturer an internal flow integration time of 5 ms (CMOSens®, Sensirion AG, Staefa, Switzerland) (Fig. 6A). The gas flow discriminator was mounted into the breathing circuit using a bypass tube connected to a face mask (Fig. 6B). To achieve simultaneous recordings of breathing and swallowing the signal was first digital-to-analog converted, then digitized and sampled (Polygraph™, SynMed, Stockholm, Sweden) simultaneously with the other modalities (manometry, nasal pressure and EMG recordings). The bidirectional gas flow discriminator detected the presence or absence of respiratory airflow through the mouth and/or nose as well as direction of flow, *i.e.* inspiration and expiration with high resolution. In addition, durations of inspiration, expiration and apnea were measured with this device.

Diaphragmal and abdominal EMG

Electromyography was used to record diaphragmatic and abdominal muscle activity (Study I). For diaphragmatic EMG, a concentric bipolar transcutaneous needle electrode was inserted between the eighth and ninth costae at the left midclavicular line and was manually held in place. Abdominal EMG was recorded by a transcutaneous silver electrode with a small hook at the tip inserted subcostally into the right rectus abdominis muscle and firmly taped to the skin. Surface reference and earth electrodes were placed on the torso. The EMG signals were duplicated, amplified and filtered (5Hz to 10 kHz; Keypoint®, Medtronic, Copenhagen, Denmark). The first pair of analog signals was digitized using the Polygraph™ for analysis in relation to manometry and respiratory airflow using software Polygram® (SynMed, Stockholm, Sweden). The other pair of analog signals was digitized at 5000 Hz and recorded on a computer for analysis of amplitude (Axon Instruments Digidata 1320A, Axon Clampex and Clampfit 8.0, Molecular Devices, Sunnyvale, CA, USA), due to a maximal sampling frequency of 128 Hz of the Polygraph™. During analysis, diaphragmatic EMG amplitude (root mean square) was normalized (ratio) to inspiratory amplitude before swallowing (set to 1.0) and to expiratory amplitude after swallowing (set to 0.0). Abdominal EMG activity was analyzed visually without systematic measurements of amplitude.

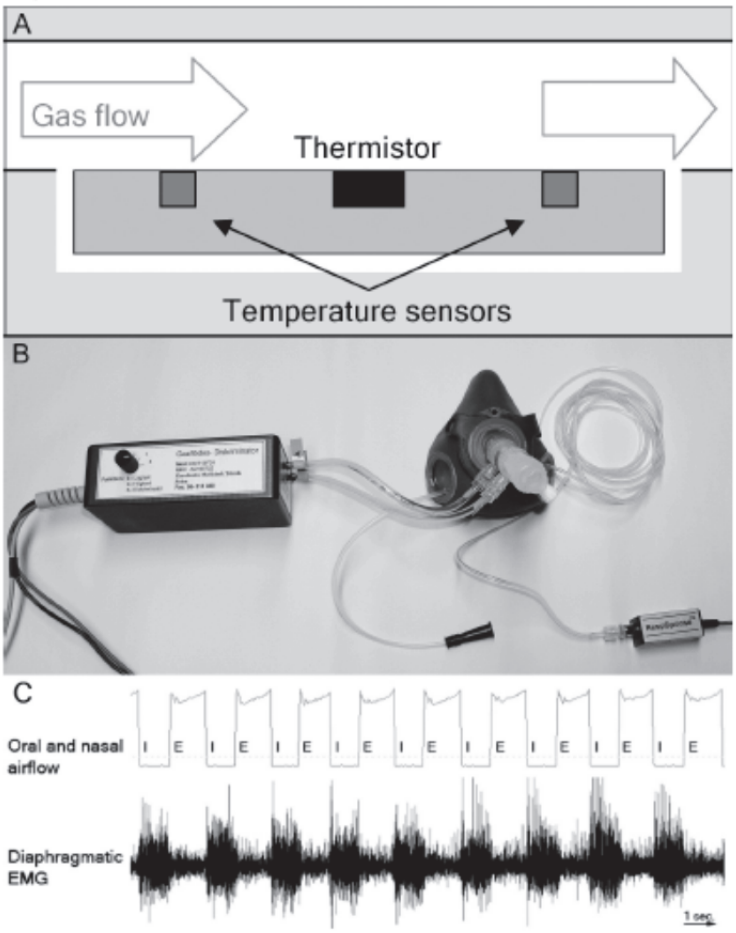

Figure 6 *Bidirectional gas flow meter, schematic illustration of the interior of the bidirectional gas flow discriminator (A). Applied to the face mask (B), oral and nasal airflow was recorded. For method validation, the bidirectional gas flow discriminator readings were compared to diaphragmatic EMG at a voluntary high frequency respiration of 30 breaths/min as directed by a metronome (normocapnia) (C) and during breathing at normo- and hypercapnia. Inspiration (I), expiration (E).*

Validation of the bidirectional flowmeter and diaphragmatic EMG

In study I, the accuracy of the bidirectional gas flow meter was validated when readings were compared with diaphragmatic EMG (Fig. 6C). The time difference between onset of diaphragmatic inspiratory EMG activity and detection of inspiratory airflow by the bidirectional gas flow discriminator was measured in 10 breaths in each volunteer at normocapnia, hypercapnia and a preset respiratory rate of 30 breaths*min^{-1}, where volunteers were asked to breathe as directed by a metronome. The bidirectional gas flow discriminator proved to be accurate and reliable and the median time difference between detection of inspiratory airflow and diaphragmatic EMG activity was 35-42 ms. This time difference between the electrical activation of the diaphragm and the actual airflow results from a combination of biological and methodological delay [205].

Spirometry

In study I, a spirometer (D-lite™, Datex-OhmedaAS/3TM, GEMedical Systems, Madison, WI, USA) was used to calculate inspiratory and expiratory tidal volumes (ml). The spirometer measures total pressure and static pressure over a known resistance with laminar flow, calculating dynamic pressure (*i.e.* pressure difference, ΔPressure). Inspiratory and expiratory airflow (L/min) was calculated every 40 ms (Constant *ΔPressure = Flow). Measuring range in technical specifications was 1.5 to 100 l/min. Using manufacturer's software solution (Datex-Ohmeda S/5™ Collect) airflow was recorded at 100 Hz. Offline, flow curves were visually inspected for each breath included and inspiratory and expiratory tidal volumes (ml) were calculated through integration. Accuracy of flow measurements were not stated in technical specifications, however after comparisons to a known volume using a high precision syringe and repeated measures (6-10) over a range of volumes (300, 500, 800 and 1100 ml) accuracy of inspiratory volumes were 96-99% of calibration volume and precision, *i.e.* SD, was +/- 4-7 ml.

Nasal pressure cannula

In order to compare registrations from previous studies using this or similar methodologies, a traditional nasal pressure transducer (RespSponse™; SynMed, Stockholm, Sweden) was inserted into one of the nostrils for respiratory airflow recordings. This non-calibrated pressure transducer delivered an analogue signal that was suitable for monitoring direction of flow but not the exact timing of flow and apnea due to variable built in time delay. The signal was amplified, digitized and sampled at 64-128 Hz (Polygraph™). Owing to frequent disturbances and artifacts, the nasal pressure recordings were difficult to interpret adjacent to swallowing.

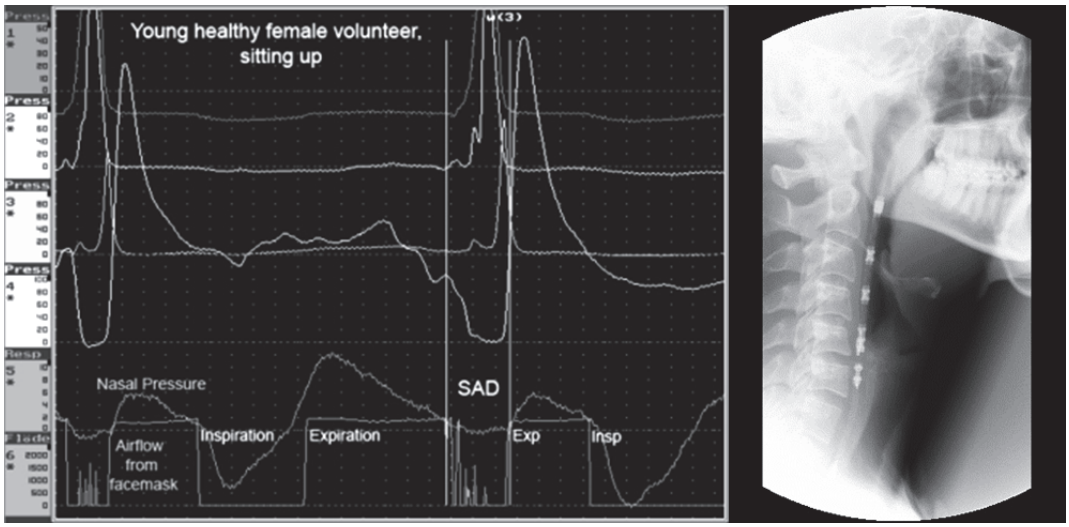

Figure 7 *Manometry and respiratory recordings, left panel, typical registration of pharyngeal manometry (upper four tracings) and respiratory airflow (lower two tracings) and right panel, x-ray picture of catheter in a healthy young volunteer, sitting in the upright position. Two swallows can be seen, first a spontaneous swallow of saliva and second a swallow of contrast medium (marked "w(3)"). SAD, swallow apnea duration.*

Manometry

Solid state pharyngeal manometry, was used to monitor pharyngeal swallowing as previously described [17,149,206] (Fig. 7). The manometry catheter, utilizing four solid-state pressure transducers 2 cm apart (Konigsberg Instruments, Pasadena, CA, USA), was introduced through one nostril and placed in the pharynx with the distal transducer in the UES and the more proximal transducers at the tongue base (TB) and the pharyngeal constrictor muscles (two transducers) at an upper and lower level (Ph Up and Ph Low). No topical anesthesia was applied. Catheter placement was validated using fluoroscopy. In study I, for esophageal manometry, the catheter was placed with the proximal sensor in the UES and the following three placed distally in the upper and mid parts of the esophagus. Manometry signals were amplified, digitized and sampled at 64-128 Hz (Polygraph^{TM}).

Videoradiography

Videoradiography (fluoroscopic imaging) was used as previously described [17] recording swallows of contrast medium (Fig. 8). Registrations of manometry and respiration were superimposed on the fluoroscopic image and recorded simultaneously onto a videotape equivalent to 50 half-frames /s, *i.e.* a response time of 40 ms. Images were interpreted together with an experienced radiologist. No volunteer hade a total exposure time of more than 5 s, and doses of radiation (study III and IV) were approved by the Committee for Protection Against Radiation at Karolinska University Hospital Solna.

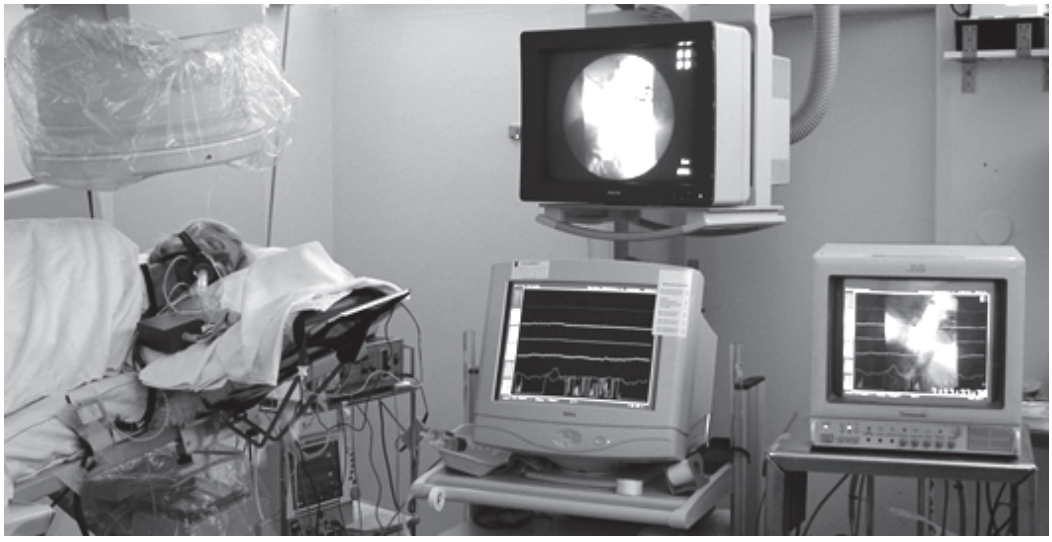

Figure 8 *The lab used in study II, III and IV. Videofluoroscopy (upper screen and lower-right) and pharyngeal manometry including nasal pressure recording and bidirectional gas flow meter (lower-left screen). Volunteer gave permission to publish photo.*

In summary, the multimodal high resolution technique consists of two methods to study swallowing (pharyngeal manometry and videofluoroscopy) and five methods to record breathing (oral and nasal airflow (thermistors), nasal cannula (pressure), spirometry (dynamic pressure), diaphragmal and abdominal EMG) that can be combined and adapted to fit study aims. Response time in comparative measurements is defined by the response times for each modality.

DRUG ADMINISTRATION AND MONITORING

Effects of drugs were evaluated in study III and IV. In study III, morphine 0.1 $mg*kg^{-1}$ or midazolam 0.05 $mg*kg^{-1}$ dissolved in 20 ml of normal saline was administered as an intravenous infusion during 20 min. Plasma concentrations of morphine, morphine-3-glucuronide and morphine-6-glucuronide or midazolam and 1-OH-midazolam were determined. Deep sedation was avoided [166,207] and dosages aimed to be clinically relevant. Due to different pharmacodynamic profiles no effort was made to find equipotency. In study IV, rocuronium (0.5 $mg*mL^{-1}$) was administered as a continuous intravenous infusion that was adjusted to obtain steady state TOF ratios. Neuromuscular transmission was assessed by isometric mechanomyography of the adductor pollicis and was carried out according to international guidelines for neuromuscular research [208]. After a stable twitch response had been obtained (continuous ulnar nerve stimulation of 1 Hz for 15 to 20 min), ulnar nerve TOF stimulation (0.3 ms^2 impulses at 2 Hz for 1.5 s every 12 s) was initiated and calibrated. The volunteers estimated their level of sedation on a visual analogue scale (VAS-sedation), 0 equaling maximal sedation *i.e.* just falling asleep and 10 equaling no sedation. Vital parameters were continuously monitored and recorded in all studies.

MULTIMODAL DATA AQUISTION AND ANALYSIS OF COORDINATION OF BREATHING AND SWALLOWING

Study protocols

Schematic pictures of modalities of the multimodal high resolution technique, bolus types and the study protocols used in study I-IV are presented in Table 2 and 3 and Fig. 9, respectively.

Table 2 *Modalities utilized in study I-IV.*

	Studies			
Modalities	**I**	**II**	**III**	**IV**
Pharyngeal Manometry	x	x	x	x
Videoradiography			x	x
Bidirectional gas flow discriminator	x	x	x	x
Nasal pressure cannula	x	x	x	x
Diaphragmal and Abdominal EMG	x			
Spirometry	x			

Table 3 *Bolus types in study I-IV.*

	Studies			
Bolus types	**I**	**II**	**III**	**IV**
Spontaneous swallows of saliva	x	x	x	x
Swallows of 10 ml water		x		
Swallows of 10ml contrast medium			x	x

In study I volunteers were placed supine with a 45 degree head-up tilt for EMG-needle access to thorax and abdomen. In study II volunteers were first examined in the upright sitting position, followed by the left lateral position, lying down with an 8 degree head up tilt. In study III and IV the left lateral position was utilized. In study III and IV volunteers were allowed solid food until six hours, and liquids until two hours before recordings began. Recordings of swallowing and breathing at rest at normocapnia were made during periods of 10 minutes (Studies I-IV) and at hypercapnia during a 5-minute period (Studies I and II).

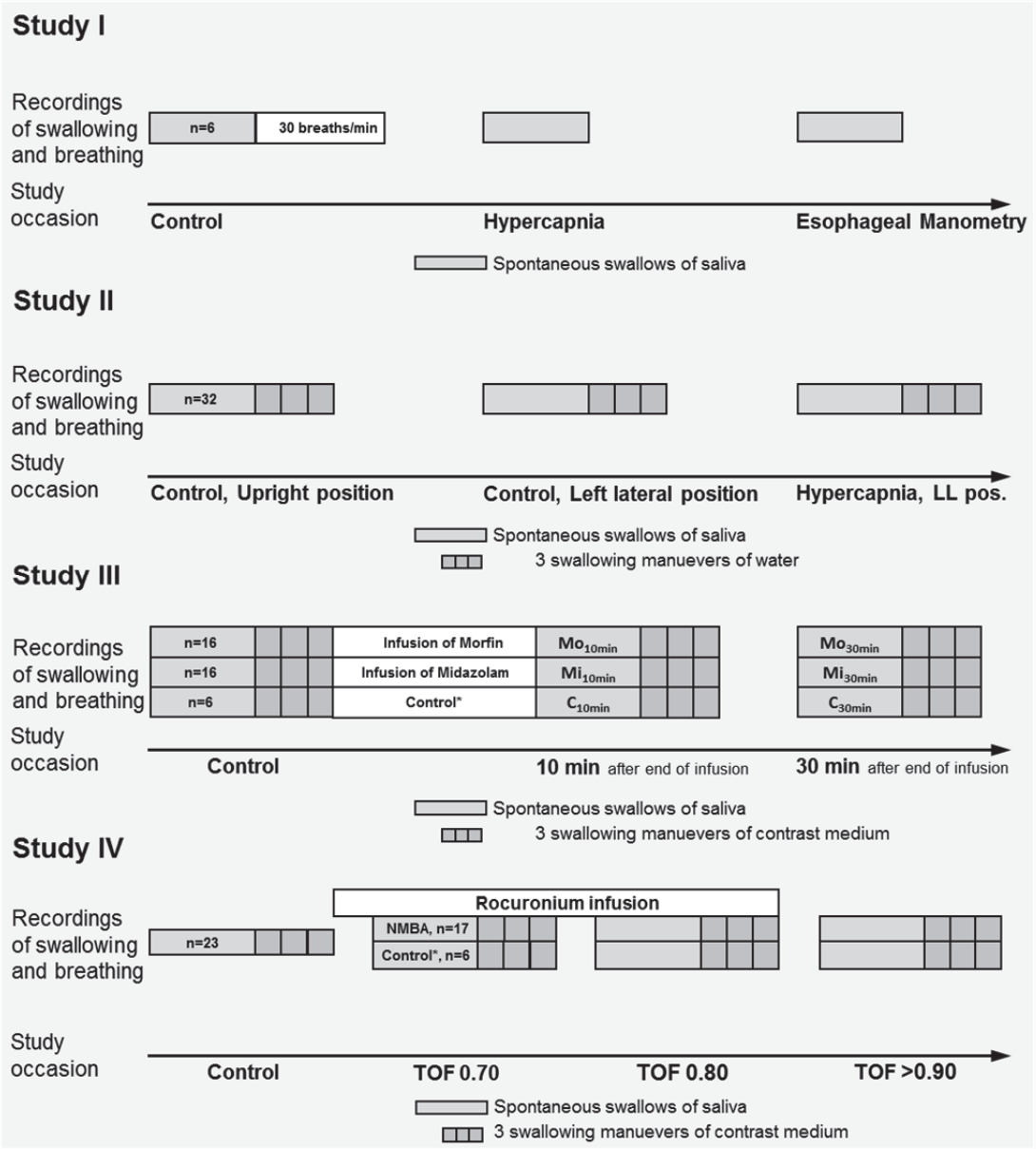

Figure 9 *Schematic presentations of study protocols (Study I-IV). Recordings at control (Control) and in study I and II during challenges with hypercapnia and changing body position, in study III effects of morphine (Mo) or midazolam (Mi) and finally in study IV effects at different levels of partial neuromuscular block (train-of-four (TOF) ratios 0.70, 0.80 and > 0.90). Control* = Control group no drug; n, number of volunteers; NMBA, neuromuscular blocking agent.*

Subjects were allowed to swallow saliva spontaneously without any instructions on how to swallow. Contrast medium and water at room temperature were administered using a syringe at a random phase of the respiratory cycle. Just before administering the bolus, volunteers were instructed "here comes a bolus.", however they were not instructed on when to swallow. Approximate durations of studies were Study I, 2 h and Study II- IV, 2.5 h.

Analysis procedures

In study III and IV, swallows of contrast medium were analyzed as previously described [17,149,150,172]. Signs of pharyngeal dysfunction are presented in table 4. Previous studies have reported percentage of swallows showing at least one sign of pharyngeal dysfunction [149,150,172]. However, since swallows with more than one of the signs of dysfunction were common in the elderly at the resting control state and during partial neuromuscular block, we further explored the severity of pharyngeal dysfunction utilizing in-depth analysis "Degree of pharyngeal dysfunction" presented in table 4. Furthermore, as previously described [209], three validated scales, the penetration-aspiration scale, the valleculae residue scale and pyriform residue scale were used to determine risk for aspiration and pharyngeal clearance (Table 4).

Table 4 *Definitions of degree of pharyngeal dysfunction, risk of aspiration and efficiency of bolus clearance.*

Signs of pharyngeal dysfunction	
A) Premature leakage of bolus from the mouth to the pharynx B) Penetration of contrast medium to the laryngeal inlet or the trachea C) Retention of contrast medium in the pharynx after completion of swallowing	
Degree of pharyngeal dysfunction	Adding the number of signs (0 to 3) of pharyngeal dysfunction category A to C found in each of the three swallows. The individual sum (0 to 9) was thereafter divided by the maximal outcome (*i.e.* 9), yielding the term degree of pharyngeal dysfunction (%).
Penetration Aspiration Scale	Risk for aspiration rated (1-8); *No risk,* 1 = no airway invasion; 2 = bolus enters into airway with clearing; *Risk of Aspiration,* 3 = bolus enters into airway without clearing; 4 = bolus contacts vocal cords with airway clearing; 5 = bolus contacts vocal cords without airway clearing; *Positive Aspiration,* 6 = bolus enters trachea and is cleared into larynx or out of airway; 7 = bolus enters trachea and is not cleared despite attempts; 8 = bolus enters trachea and no attempt is made to clear.
Valleculae Residue Scale	Bolus clearance rated (1-3); 1 = no residual to mild bolus retention; 2 = moderate residual with up to half the recess filled with material post swallow; 3 = severe residual with more than half the recess filled with material post swallow.
Pyriform Sinus Residue Scale	Bolus clearance rated (1-3); 1 = no residual to mild bolus retention; 2 = moderate residual with up to half the recess filled with material post swallow; 3 = severe residual with more than half the recess filled with material post swallow.

Using manometry the following was analyzed in swallows of contrast medium:
A) pharyngeal muscle contraction forces (maximum contraction pressure, contraction rate and contraction duration, illustrated in Fig. 10 A),
B) the time course of the pharyngeal muscle contraction wave (TB-start, Ph Up-start, Ph Low-start and UES-start, illustrated in Fig. 10 B),
C) the resting tension in the UES in-between swallows, *i.e.* mean UES pressure during 10 sec at resting conditions,
D) coordination, measured as the time between the start of pressure rise at the lower part of the pharyngeal constrictor (Ph Low-start) and the start of upper esophageal sphincter (UES) relaxation (UES relaxation-start) (Fig. 10 A).

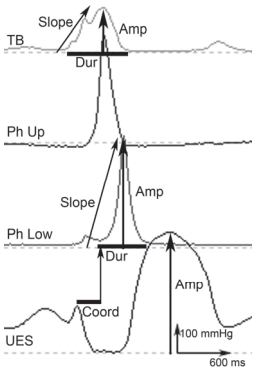

Figure 10 A

Using videoradiography:
E) initiation, of the pharyngeal phase of swallowing, the interval between the times at which the head of the bolus passed the anterior faucial arches and the hyoid bone started to move forward
F) bolus transit time (pharyngeal), the interval between the times at which the bolus head passed the anterior faucial arches and the tail of the bolus passed the UES
G) bolus in mouth, the interval between when the bolus was first seen in the mouth and onset of pharyngeal swallowing (TB-start).

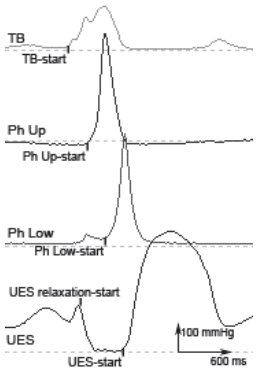

Figure 10 B
Illustrations of manometric measurements.

When studying pharyngeal function, focus during analysis was at swallows of contrast medium including videoradiographic data.

In contrast, when studying coordination of breathing and swallowing, spontaneous swallows of saliva were included. This made it possible to 1) study more rarely occurring respiratory phase patterns, 2) study resting conditions and 3) study swallows of saliva and 4) minimize subjects' dose of radiation. Because of this, definitions of onset and offset of pharyngeal swallowing was derived from manometry as opposed to videoradiography [17]. The start of pharyngeal swallowing was defined as the beginning of pressure rise at the tongue base (TB-start), while the end as the beginning of UES contraction (UES-start) (Fig. 10 B and 11). TB-start was chosen because it represented the first sign of pharyngeal swallowing that was easily detected visually and showed stability in the temporal order of events. All events describing timing of swallow apnea and pharyngeal manometry were referenced in time to TB-start (TB-start = 0 ms).

In spontaneous swallows of saliva a) timing of events in swallowing and breathing and b) the time course of the pharyngeal muscle contraction wave were analyzed. Moreover, in saliva swallows, c) coordination between onset of UES relaxation and the start of pressure rise at the lower part of the pharyngeal constrictor and d) UES maximum contraction pressure was analyzed, since UES has been shown to be sensitive to bolus properties and volume [26,49].

Respiratory phase patterns were analyzed in all swallows. To assess, in-depth, timing of pharyngeal swallowing events and swallow apnea, all swallowing maneuvers of contrast medium (study III and IV) and water (study II) and three spontaneous saliva swallows (five in study I) with the respiratory phase pattern E-E in each volunteer were analyzed since this pattern was predominant (selection criteria see Statistics).

Pre-swallow apnea was defined as the time from onset of swallow apnea to onset of pharyngeal swallowing (TB-start) (ms) and post-swallow apnea as the time from the end of pharyngeal swallowing (UES-start) to the end of swallow apnea (ms). Moreover, total swallow apnea duration was analyzed to allow comparison with previous literature where this measurement is commonly presented [19,50,106]. Coughing associated with swallowing of contrast medium was noted. Duration of respiratory phases before and after swallowing was analyzed, *i.e.* duration of the inspiration and expiration preceding and following swallowing. In study II-IV, UES pressure oscillations with breathing were analyzed as A) pressure during the inspiratory and expiratory phase and B) timing between onset of inspiration and expiration and UES pressure increase and decrease respectively. Respiratory rate and spontaneous swallow frequency was calculated during recordings in the resting control state.

In study I, inspiratory and expiratory tidal volumes, timing and amplitude of diaphragmal EMG activity, presence of abdominal EMG activity and timing between the end of swallow apnea and esophageal activity were analyzed as described above.

In study II a detailed analysis of respiratory phase patterns was made. Here, data on coordination of breathing and pharyngeal swallowing in the dominant E-E pattern was compared to E-I and E-E swallows with an ultra-short expiration before swallowing.

STATISTICS

For all parameters, a mean value based on two or three measurements from three (five in study I) separate swallows or breaths, was calculated for each volunteer and study condition (*i.e.* measure during exposure to drug or other physiological change).

For in-depth analysis of coordination of breathing and swallowing, swallows with the respiratory phase pattern E-E were chosen, since the number of recorded non-E-E swallows were too small to allow statistical analysis. When studying spontaneous swallows or breaths at rest, measurements were made in the first E-E swallow occurring closest to the start, mid and end of the recording period, to avoid selection bias. For all statistical analyses Statistica™ 7.1 (study I and II) or 10 (study III and IV) (Statsoft ® Inc., Tulsa, OK, USA) and ANOVA repeated measures (one or two within factors) followed by planned comparisons, comparing control to measurements at other study conditions respectively, were used unless otherwise stated. Results are presented as mean +/- standard deviation of the mean (SD) or the 95% confidence interval (CI).

For differences between study conditions in study I and for degree of pharyngeal dysfunction (0 to 100%), PAS, VRS, PRS and VAS-sedation (10 to 0) in study III and IV, planned comparisons were made using Wilcoxon. Percentage of swallows with pharyngeal dysfunction was analyzed using ANOVA repeated measures after rank transformation. Here, results are presented as median values (upper and lower quartile in study IV) and (range). Respiratory phase patterns were analyzed including all available swallows, *i.e.* spontaneous swallows of saliva, water swallows and swallows of contrast medium, using generalized linear models (PROC GENMOD, logistic regression, repeated measures with binary data) using SAS® 9.2, maintenance 2, (SAS® Institute Inc., Cary, NC, USA). Here, a ratio between I-E and the total number of swallows was calculated and analyzed, considering that spontaneous swallows of saliva occurred with varying frequency at each of the study conditions. In study II saliva swallows with the E-I pattern in the upright and left lateral positions and swallows with the pattern E-E ultra-short in the upright position were analyzed and compared with E-E swallows in the same individual using T-test, comparing differences in durations to zero.

In study II, Spearman rank correlation coefficients (r) were calculated for the two time intervals from the start of pharyngeal swallowing (TB-start) to the end of pharyngeal swallowing and the end of swallow apnea and in study III between degree of pharyngeal dysfunction and VAS-sedation.

In study IV, analysis of effects of gender was made using ANOVA repeated measures with a categorical predictor or Mann-Whitney U test. To investigate factors that could be associated with pharyngeal dysfunction, degree of pharyngeal dysfunction at TOF ratio 0.70 was analyzed for effects of gender and respiratory phase patterns (*i.e.* presence of non E-E phase patterns) using Mann-Whitney U test. Family wise Bonferroni corrections for multiple comparisons were made where more than two comparisons were planned (study IV). Exact unadjusted p-values are reported. P-values <0.05 were considered significant.

RESULTS

STUDY I AND II:

In study I and II we evaluated the normal physiology of coordination of breathing and swallowing in young healthy volunteers and the effects of challenges, *i.e.* changes in body position, bolus type or respiratory drive. In a total of 1825 swallows analyzed in study I and II, we demonstrated that a majority of swallows occurred during expiration and that all swallows were preceded by expiratory airflow. Moreover, 93% (study I) and 98% (study II) of swallows were also followed by expiration. Respiratory phase patterns were unchanged regardless of body position or bolus type. During increased respiratory drive, *i.e.* during hypercapnia in study I, there was a tendency towards an increasing number of swallows followed by inspiration. However, in study II we could not detect an effect of hypercapnia on respiratory phase patterns.
Further analyzing rare respiratory phase patterns, in the small fraction of swallows followed by inspiration (2.5 %, study II), the expiratory phase before swallowing and duration of post-swallow apnea were significantly longer. This suggests that in these swallows most of the tidal volume is expired already before swallowing, causing lower lung volume with subsequent low subglottic pressure. Thus, when the larynx is reopened after swallowing, there is no positive pressure to generate expiratory airflow and consequently, swallow apnea does not end until the next inspiration starts and the swallow apnea duration is therefore prolonged. Moreover, in swallows with an ultra-short pre-swallow expiration (mean 70, 30-150 ms), duration of pre-swallow inspiration was shorter, and durations of pre-swallow apnea and expiration after swallowing were longer suggesting the ultra-short expiration to be an adequate adaptive behavior to secure expiratory airflow after swallowing. Notably, none of the 1825 swallows in study I and II were preceded and followed by inspiration (I-I).

During swallow apnea, the non-active state of the diaphragm (passive expiration) was interrupted by static activity, never described in humans before (Fig. 11). This diaphragmatic activation always preceded onset of pharyngeal swallowing. Moreover, peak diaphragmatic activity occurred simultaneously with onset of pharyngeal swallowing. The diaphragmatic EMG activity then gradually decreased throughout pharyngeal swallowing, reaching a minimum at the start of post-swallowing expiration. This phenomenon could be “active breath holding” preserving lung volume to enable post-swallow expiration. The time difference between the start of diaphragmatic EMG activity during swallow apnea and onset of pharyngeal swallowing decreased during hypercapnia. Abdominal EMG increased throughout pre- and post-swallowing expiration, more so during hyper- than normocapnia, possibly to assist expiratory airflow. Notably, duration of pre-swallow apnea was highly variable inter- and intra-individually. In contrast, in swallows followed by expiration, the endpoint of swallow apnea correlated well to the endpoint of pharyngeal swallowing. The pharyngeal phase of swallowing ended before post-swallow respiration had resumed and the esophageal phase of swallowing started approximately 500 ms after the end of swallow apnea.

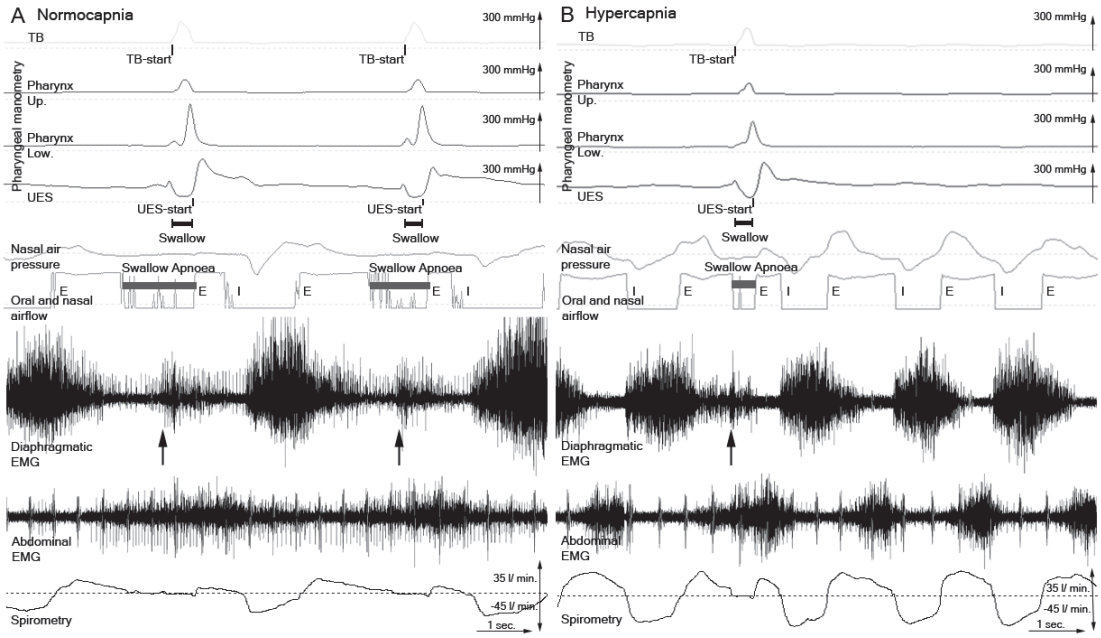

Figure 11 *Diaphragmatic EMG during pharyngeal swallowing (arrow) Registrations of pharyngeal manometry, nasal airpressure, oral and nasal respiratory airflow by the bidirectional gas flow discriminator, diaphragmatic and abdominal electromyography (EMG) and spirometry. Recordings of two swallows at normocapnia (A) and one swallow at hypercapnia (B). Swallows with the respiratory phase pattern E-E (inspiration-expiration-swallow apnea-expiration). The start of pharyngeal swallowing was defined as the start of pressure rise at the tongue base (TB-start) and the end when the upper esophageal sphincter started to contract (UES-start). Duration of pharyngeal swallowing is marked with a horizontal bar (Swallow). Swallow apnea was detected by the respiratory airflow discriminator as an oscillating signal, representing zero airflow. Diaphragmatic activity during swallow apnea is marked with black arrows (↑). Pharyngeal manometry at the tongue base (TB), upper/lower level of the pharynx (Pharynx Up./Low.), and upper esophageal sphincter (UES). Inspiration (I), expiration (E).*

When evaluating effects of bolus types and body position, water swallows had longer duration of pre-swallow apnea and earlier UES-relaxation compared to spontaneous swallows of saliva. Moreover, duration of pharyngeal swallowing was shorter in water swallows and in the upright position compared to the left lateral position.
Increased respiratory drive, *i.e.* hypercapnia, reduced swallowing frequency (study II) and shortened duration of pre-swallow apnea. Moreover during hypercapnia, duration of expiration after swallowing decreased while expired tidal volumes (study I) were unaffected.

UES pressure at rest oscillated in correlation with timing of breathing, where UES pressure increase started earlier and decreased later in relation to onset of inspiratory and expiratory airflow. The increase in upper esophageal sphincter tone during inspiration was affected by body position and respiratory drive where UES pressure increase occurred earlier relative to onset of inspiration lying down and during hypercapnia. Moreover during hypercapnia, UES pressure decrease occurred later relative onset of expiration.

STUDY III

In study III, we evaluated the impact of clinically relevant doses of morphine and midazolam on pharyngeal function and integration between breathing and the pharynx in young healthy adults. We demonstrated that pharyngeal dysfunction increased after morphine infusion to 42% and 44% of swallows, compared to 17% in control recordings. Midazolam increased pharyngeal dysfunction from 16% to 48% and 59%. During drug exposure, swallowing showed a more severe degree of dysfunction as compared to the control state. Morphine and midazolam reduced the frequency of spontaneous swallowing at rest. In contrast, neither morphine nor midazolam had any detectable effect on the time course of the pharyngeal muscle contraction wave. However, the mechanical pharyngeal muscle forces were altered by both morphine and midazolam and morphine exposure was associated with prolonged duration of pharyngeal bolus transit time. Notably, both drugs impaired coordination of breathing and swallowing since morphine prolonged apnea before swallowing and both morphine and midazolam increased the number of swallows followed by inspiration, a pattern that may be associated with higher risk for aspiration. Moreover, respiratory-dependent UES oscillations were affected by morphine and midazolam, where UES pressure increase started earlier and decrease later in relation to onset of inspiratory and expiratory airflow compared to control recordings.

STUDY IV

Age

After in-depth analysis of data from spontaneous swallows of saliva during resting conditions in addition to swallows of contrast medium in healthy elderly volunteers, a comprehensive view on the pharynx and coordination with breathing in healthy elderly was provided which added nicely to the knowledge previously presented [8]. Comparing assessment from 515 swallows of saliva (study II) and 93 swallows of contrast medium (Bodén *et al* [17]) in young adults to 139 swallows of saliva (study IV) and 51 swallows of contrast medium (study IV) in elderly individuals, we could not detect any difference in respiratory phase patterns (Fig. 12) and similarly to the young, no swallows in the elderly were preceded and followed by inspiration (I-I).

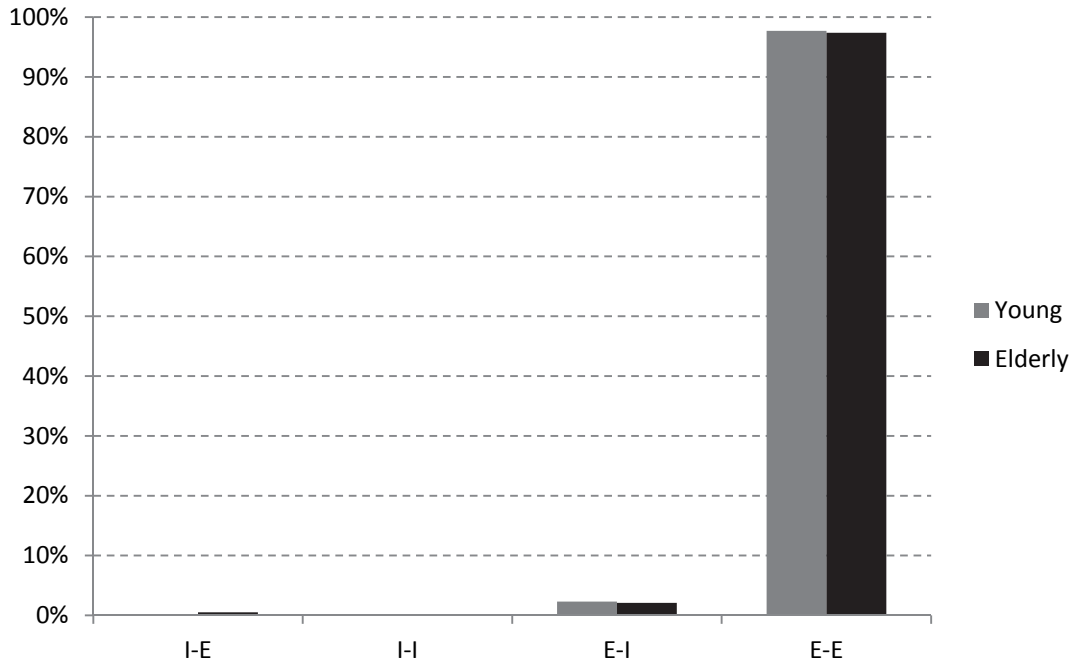

Figure 12 *Respiratory phase patterns in the young and elderly, frequency (%) of respiratory phase patterns (E-E, inspiration- expiration-swallow apnea- expiration; E-I, inspiration- expiration- swallow apnea- inspiration; I-E, inspiration- swallow apnea – expiration and I-I inspiration-swallow apnea-inspiration). The respiratory phase pattern I-I was never detected in study I-IV.*

Moreover, comparing young and elderly, we found no difference in durations of respiratory phases surrounding swallowing (Fig. 13) including durations of pre- and post-swallow apnea (Fig. 14).

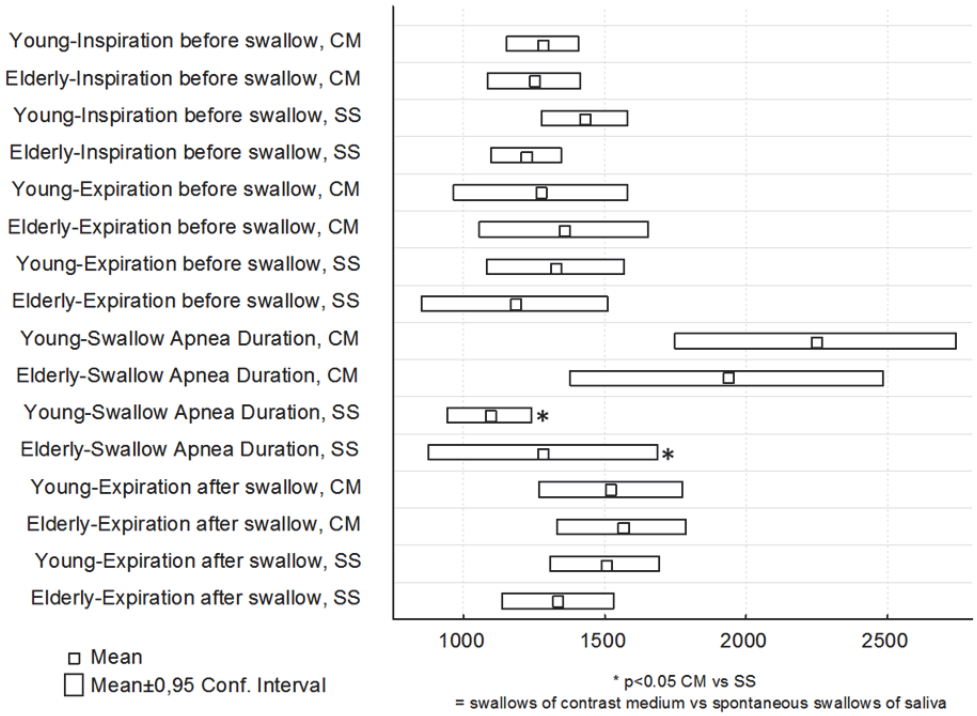

Figure 13 *Durations (ms) of respiratory phases surrounding swallowing in the young and elderly. CM, swallow of contrast medium; SS spontaneous swallow of saliva.*

Nor could we detect any difference in the time course of the pharyngeal muscle contraction wave (Fig. 14). We therefore conclude that coordination of breathing and swallowing and coordination of pharyngeal muscles seems unaltered in the elderly as compared to the young.

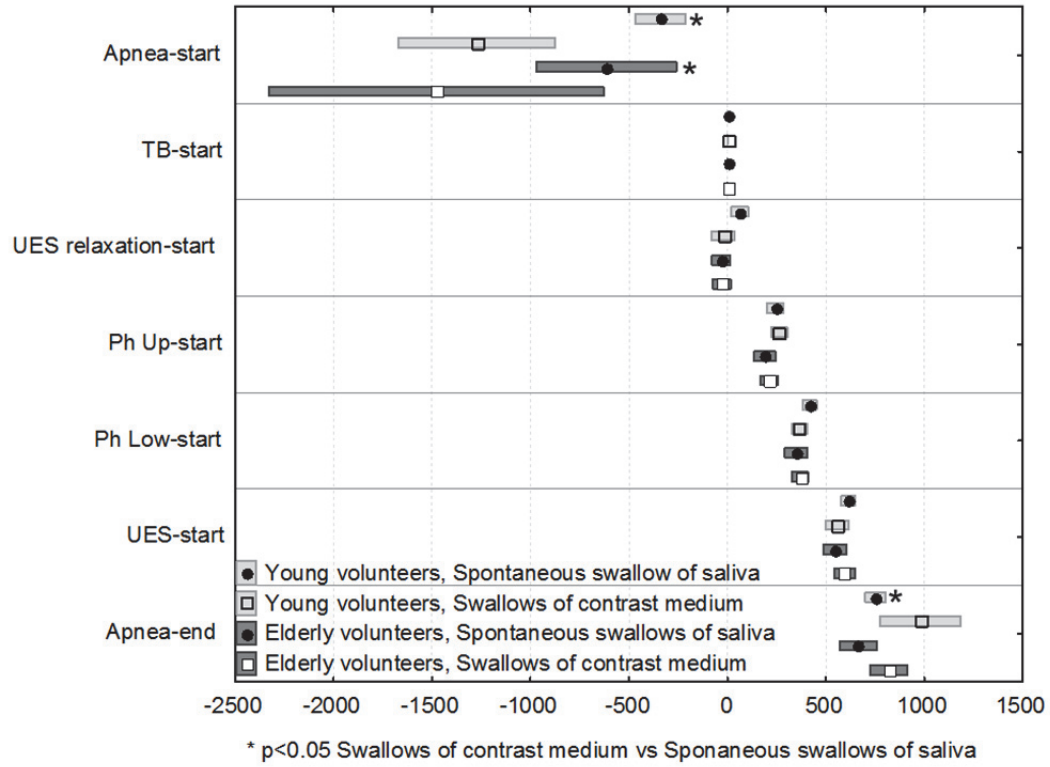

Figure 14 *Time course of the pharyngeal muscle contraction wave in young and elderly volunteers, spontaneous swallows of saliva (S) and swallowing maneuvers of contrast medium (C), with the respiratory phase pattern inspiration-expiration-swallow apnea- expiration (E-E). Onset of pharyngeal swallowing (TB-start). All pharyngeal manometry events as well as start and end of swallow apnea were referenced in time (ms) to TB-start = 0. Start of swallow apnea (Apnea-start) and end of swallow apnea (Apnea-end). Mean (ms) +/- 95% confidence interval. * p<0.05 vs. swallowing maneuvers of contrast medium.*

In contrast, concerning oro-pharyngeal function, in-depth analysis of degree of pharyngeal dysfunction showed significantly higher degree of dysfunction in the elderly compared to the young (p=0.034, Mann Whitney). Moreover, there was a striking difference regarding the interval between when the bolus was first seen in the mouth and onset of pharyngeal swallowing (bolus in mouth) where median duration was significantly shorter in the young compared to the elderly (p<0.001, Mann Whitney).

In addition, comparing data on breathing and spontaneous swallowing at rest in the young and elderly we found a lower swallow frequency in the elderly (p=0.008, T-test). There were no detectable differences regarding UES pressures or timing in oscillations with breathing between the young and elderly except that UES pressure decrease occurred later relative onset of expiration in the young (p=0.017, T-test).

Partial neuromuscular block in the elderly

In study IV we evaluated the impact of residual neuromuscular block on pharyngeal function and breathing pattern in healthy elderly individuals. We demonstrated that during partial neuromuscular block in healthy elderly, the incidence of pharyngeal dysfunction increased significantly at mechanical adductor pollicis TOF ratios of 0.70 and 0.80 to 67% and 71%, respectively compared to 37% at control recordings. Moreover, swallowing showed a more severe degree of dysfunction during partial neuromuscular block, *i.e.* swallows displayed multiple signs of dysfunction. After spontaneous recovery to an adductor pollicis TOF ratio of > 0.90, pharyngeal dysfunction was not significantly different from the control state. Risk of aspiration assessed using PAS and efficiency of bolus clearance using VRS and PRS, revealed an increased VRS score at adductor pollicis TOF ratio of 0.70 compared to the control state. In contrast, partial neuromuscular block had no effect on the time course of the pharyngeal muscle contraction wave, the mechanical pharyngeal muscle forces or duration of pharyngeal bolus flow. However, the tonic pressure of the upper esophageal sphincter at the resting control state was lower at all levels of partial neuromuscular block compared to control recordings while UES pressure oscillation correlating to timing of breathing was unchanged. Finally, we were unable to demonstrate impaired coordination of breathing and swallowing since respiratory phase patterns, durations of respiratory phases before and after swallowing and durations and timing of pre- and post-swallow apnea were not affected at any level of partial neuromuscular block.

Control groups

In study III and IV, control groups of six volunteers were assessed in order to determine the effect of elapsed time. For all included parameters, except VAS-sedation in study III, no significant changes could be detected, confirming the stability of the model over time both in young and in elderly volunteers.

DISCUSSION

In this thesis we strove to provide a more comprehensive understanding of normal physiology of coordination of breathing and swallowing, and thereafter explored how drugs in anesthesia effect airway protection in young and elderly individuals.

Coordination of breathing and swallowing

We have shown that coordination of breathing and swallowing is strictly coordinated during adult life, and in this series of investigations swallowing primarily occurred during expiration. This strict association with expiration contrasts to previous data on respiratory phase patterns, where more swallows were found to be associated with inspiration [6,7,19,24,35,50,64,210,211]. Reasons for this difference may include methodological variations in time resolution, that is, recordings of respiration with either indirect measures of breathing and swallowing such as plethysmography with chest bands [50,64,211] or with lower resolution of direct measurements of respiratory airflow [24,210]. More recent studies have utilized methods with direct measure of airflow and consequently higher resolution of recordings. This was also suggested in a comprehensive review article evaluating methods to study coordination of breathing and swallowing [204]. In summary, more recent studies have progressively found fewer swallows associated with inspiration in healthy individuals [7,17,67].

The multimodal method combine simultaneous recordings of swallowing and breathing with two separate methods to record swallowing and up to five methods to study breathing. Moreover, validation of the bidirectional gas flow meter (Study I), found it reliable with only minor biological and methodological delay in response time as compared to diaphragmal EMG. A high resolution of recordings with sample rates above 100Hz (*i.e.* < 10 ms) are of importance when studying coordination of breathing and swallowing because many events, for example the period of swallow apnea before onset of pharyngeal swallowing have durations in this time-range. Applying this new methodology, we identified swallows with a very brief expiratory airflow before swallowing, E-E ultra-short (study II). We suggest that these swallows would have been classified as associated with inspiration (I-E), if the methodology used had been less sensitive. This observation could explain why our studies have found the lowest frequency of swallows associated with inspiration in the current literature.

Swallows with inspiration after swallowing (E-I) may occur via two separate mechanisms: 1) an inspiration is triggered during, or directly after, swallowing, *i.e.* an inspiratory airflow terminates swallow apnea upon opening of the vocal cords or 2) swallowing occurs exclusively during expiration, however upon opening of the vocal cords subglottic air pressure is low, presumably due to a low residual lung volume [41], and therefore no expiratory airflow occurs, which later is followed by the next inspiration. In favor of the second mechanism, we found a prolonged duration of apnea after swallowing in E-I swallows (study II). Depending on the current definition of respiratory phase patterns, where events of swallowing and breathing studied in the periphery are used to classify swallows into four categories, we suggest that if instead a

definition based on central measurement of brainstem signals was possible, many E-I swallows (type 2, see above) would be re-classified as E-E. Moreover, this suggests that there may be one pathologic and one normal version of E-I, depending on duration of post-swallow apnea. This is important since inspiration after swallowing have been associated with increased risk for aspiration [23].

Swallows occurring during inspiration, *i.e.* both preceded and followed by inspiratory airflow, are the least frequent [7,17,50,67,211]. In the four studies (I-IV) of this thesis, we did not detect any I-I swallows. This is well in line with findings from animal studies, showing that pharyngeal swallowing cannot be artificially triggered during inspiration [3]. Based on these observations, we speculate that during inspiration the CPG for respiration can temporarily "veto" a reconfiguration "requested" from the triggered CPG for swallowing, thereby delaying swallowing to occur during the following expiration.

We have for the first time in humans described diaphragmatic activation during swallowing. Activity in the phrenic nerve during swallowing, as studied in animal models, has previously been described by others [3,30-32]. Moreover, studies in humans have detected a brief negative intrathoracic pressure in the beginning of swallow apnea and swallowing [29,212]. After this finding was published in 2009, diaphragmatic involvement in swallowing has been elegantly verified by Uysal *et al* [213]. The biological and mechanical significance of this diaphragmatic activation is however still unclear. Based on our findings that activation of the diaphragm started at the onset of swallow apnea and the peak was found at onset of pharyngeal swallowing, we speculate that it is a mechanism to facilitate initiation of swallow apnea, preserving air in the lung to assist an expiratory airflow after swallowing. Another mechanism to produce swallow apnea is vocal fold closure. However during swallowing, closure of the vocal folds has been found to be highly correlated to other sequential events of pharyngeal swallowing and moreover to occur in close conjunction to the beginning of pharyngeal swallowing [7,25]. Hence, if swallow apnea would depend on vocal fold closure, the apneic period before swallowing would be short and duration would be stable. In contrast, we and others have found duration of pre swallow apnea to be highly variable in length [7,17], and furthermore independent of vocal fold closure [25,126]. This observation rather suggests a central control mechanism of onset of swallow apnea, separated from, but still connected to, the more fixed pattern of pharyngeal swallowing. We believe this is in line with theories suggested by Davenport and coworkers, that during swallowing, the CPG for swallowing temporarily reconfigures the CPG for respiration [34].

In contrast to the variable pre-swallow apnea period, we found duration of post swallow apnea to be short and stable. In line with the discussion above, this suggests a tight biological coupling to pharyngeal swallowing. Correspondingly and in agreement with previous studies [7,17] we could confirm that pharyngeal swallowing is a highly coordinated "fixed pattern" [2] of sequential events. Moreover and supported by the high resolution when measuring timing between events, we found a strong correlation between the start of the contraction of the UES and the end of swallow apnea. Furthermore, others have found a strong correlation between the end of swallow apnea and the opening of the vocal cords and detection of air in the vestibule [7,17]. This

suggests that, in contrast to the highly variable duration of pre swallow apnea, duration of post swallow apnea is a part of the "fixed pattern" [2] of pharyngeal swallowing. Notably, a positive subglottic air pressure, *i.e.* sufficient preserved lung volume [41] is a prerequisite for vocal fold opening to cause expiratory airflow and thereby terminate swallow apnea.

None of the physiological challenges in this thesis, *i.e.* changes in body posture, bolus type or exposure to hypercapnia affected respiratory phase patterns in young adults. In healthy individuals, coordination of breathing and swallowing is thus highly coordinated to ensure safe swallowing and therefore robust and unyielding to change. While these observations contrast somewhat to previous findings, the dissimilarities in results can most likely be explained by differences in methodology and tested challenges [20,51,64]. Hence, we cannot rule out that more extreme physiological challenges ultimately affect respiratory phase patterns.

In contrast, durations of pre- and post-swallow apnea were affected by physiological challenges. Boluses containing water and contrast medium were associated with longer duration of pre-swallow apnea and duration of post-swallow apnea was shorter during hypercapnia when increased respiratory drive activated abdominal muscles during late expiration. These results are in agreement with findings by others [6,7,107] and suggest that in contrast to the stability of respiratory phase patterns in healthy adults, onset of swallow apnea is modulated by afferent input, thereby adapting to physiological challenges.

Comparing the young and the elderly, we were unable to detect differences in measures of coordination of breathing and swallowing. This contrasts to previous findings of age-dependent impact such as: 1) more frequent inspirations after swallowing and 2) prolonged durations of swallow apnea (Table 1, Introduction). Differences in findings could depend on the definition of "elderly", *i.e.* the actual age-interval investigated as illustrated by Martin- Harris *et al* who found a by age increased number of swallows followed by inspiration and, in the most elderly individuals (>=81years), a prolonged post-swallow apnea [6]. Moreover, type of methodology including the combined resolution of recordings used to study breathing and swallowing influences the number of swallows found to be associated with inspiration as discussed above. Based on these combined data we believe that central coordination of breathing and swallowing is mostly preserved in the elderly.

In conclusion, the multimodal high resolution technique was a prerequisite for our novel findings that under normal conditions in young and elderly healthy humans, swallowing occurs exclusively during expiration, and that the diaphragm facilitates onset of swallow apnea and expiratory airflow after swallowing.

Key factors for airway protection in health

While normal swallowing is essential in avoiding pulmonary aspiration, adequate protection of the airway is complex and depends on several separate mechanisms each contributing to the whole [214-216]. Here the chain of events may only be as strong as its weakest link, and impairment in one or more key factors may lead to a detrimental outcome. While coordination of swallowing with breathing has been suggested as the most important mechanism in airway protection [215], other proposed processes essential in avoiding pulmonary aspiration are glottal closure [217], laryngeal elevation [217], muscle motor control and force and timing and coordination of events in swallowing [6,218]. Because of this complexity and difficulty in identifying a hierarchy between the severities of the separate mechanisms involved, we believe that studying multiple mechanisms will provide the most comprehensive map of normal airway protection in healthy individuals. Moreover, using scales to quantify otherwise subjective findings in fluoroscopy and fiber-endoscopy greatly contributes to the current knowledge of normal swallowing [87,216,219]. Here, the penetration aspiration scale has been widely adopted [87,209]. Notably, this scale takes into account the level of, and response to bolus penetration, including pharyngeal clearance. Because amount and the level of bolus residues were not included, we have added the valleculae and pyriform sinus residue scales [209]. Notably, oral bolus control is not included in these scales but is included in the definition of pharyngeal dysfunction.

With the multimodal approach we could identify seven key mechanisms for airway protection in healthy young and elderly individuals (Fig. 15). Here, affected oral coordination, *i.e.* evidence of premature leakage of bolus from the oral cavity into the pharynx well before initiating swallow apnea and pharyngeal swallowing, frequently contributed to an increased incidence of pharyngeal dysfunction in the elderly. This sign of impaired bolus control is also seen in OSA patients [136-138] and after applying topical mucosal local anesthesia [193,194]. Moreover, evidence suggests reduced pharyngeal sensitivity in both the elderly [93] and patients with OSA [136-138]. Based on these findings we speculate that reduced oro-pharyngeal sensory function contributes to impaired overall pharyngeal function in the elderly. Furthermore in the elderly, we found an increased latency to initiate pharyngeal swallowing after bolus was present in the oral cavity. Again, this could be due to impaired oro-pharyngeal sensitivity, making it more difficult to reach the triggering threshold to swallowing. Another mechanism that would increase latency to swallow is slower brainstem circuits in the elderly. However, slow circuits would probably affect other parts of pharyngeal swallowing and since we could not detect a change in the coordination of the pharyngeal contraction wave or in coordination of breathing and swallowing, this mechanism seems unlikely to have a major role. Moreover, as previously described by Bodén *et al*, pharyngeal muscle contraction forces are altered by age, in particular at the base of the tongue. This is in line with previous data [85] and here age-related sarcopenia has been suggested as an important contributor making muscles weaker, slower and stiffer [92,220]. Also, confirming previous findings in the elderly [85], we found affected laryngeal protection, *i.e.* an increased incidence of penetration of bolus to the laryngeal inlet and reduced pharyngeal clearance with bolus residues remaining in the pharynx after swallowing.

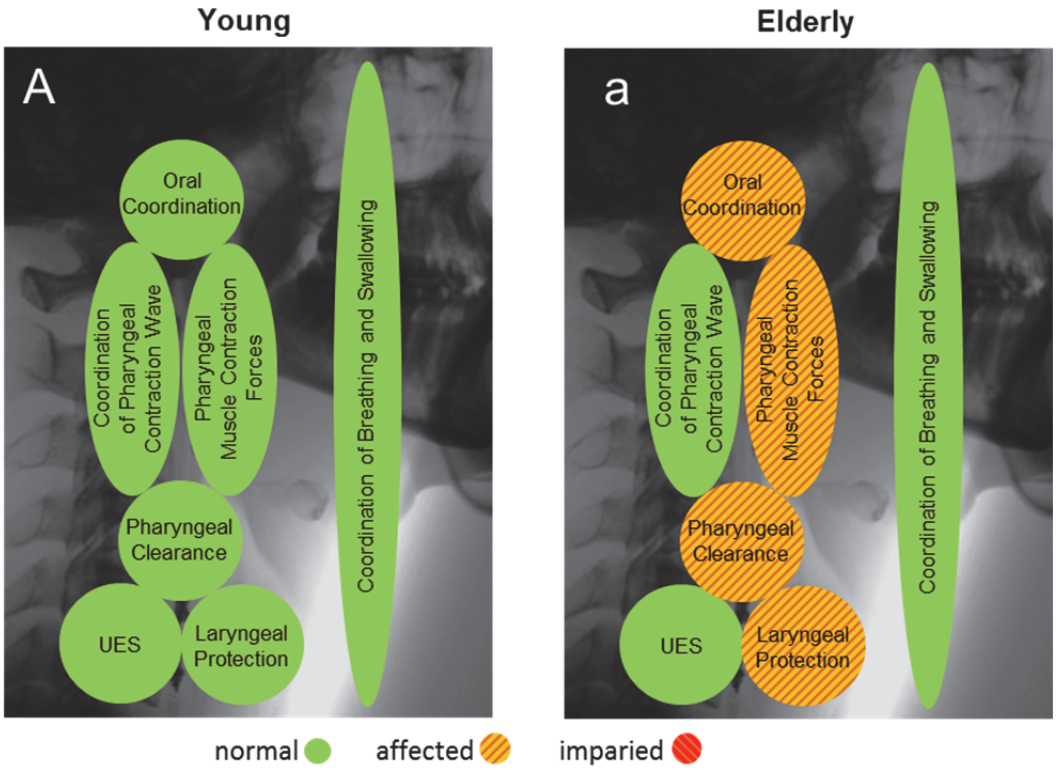

Figure 15 *schematic illustrations of key factors for normal airway protection in healthy young and elderly individuals. oral coordination, (prevents premature leakage of oral contents into the pharynx); coordination of pharyngeal contraction wave (the propagation of contractions in the pharyngeal constrictor muscles into the upper esophageal sphincter (UES)); pharyngeal muscle contraction forces (peak, duration and speed of pharyngeal muscle contractions); pharyngeal clearance, (prevents retention of pharyngeal residue after completion of the pharyngeal contraction wave); UES, (resting pressure in the UES contributes to airway protection by preventing aerophagia and regurgitation); laryngeal protection, (prevents penetration of contents to laryngeal inlet or aspiration); coordination of breathing and swallowing, (swallowing during expiration and normal duration and timing of apnea in relation to the pharyngeal phase of swallowing prevents aspiration); Including data modified from Bodén et al* [8,17].

In contrast, we could not detect a difference in UES resting pressure by age. This is interesting since UES opening is closely involved in laryngeal elevation, both these processes being affected by age [89] . Furthermore, reduced laryngeal elevation and slower and impaired opening of the UES has been associated with increased risk for aspiration [217]. Based on this, we speculate that age affects UES ability to relax but has either none or only minor impact on the resting tension or the contraction forces of the UES. Notably though, presbyphagia is most often associated with maintained health [85], which means that although we can detect changes in pharyngeal function in healthy elderly, protection of the airway is sufficiently preserved to prevent morbidity. On the other hand, when facing a challenge, it has been repeatedly shown that reduced physiological reserve capacity during aging can have detrimental consequences [85,95,96].

Effects of drugs in anesthesia

Morphine and Midazolam

Midazolam caused profound changes in pharyngeal function and subsequent airway protection (Fig. 16). In this way, midazolam had similar effects on the pharynx compared to previously reported effects of sub-anesthetic levels of propofol, isoflurane and sevoflurane [150]. However, more surprisingly, morphine, which primarily is an analgesic drug, had very similar effects to midazolam in clinically relevant doses (Fig. 16). Furthermore, spontaneous swallowing frequency, important for protection against aspiration [214], was reduced by both drugs, which is similar to previous data evaluating effects of nitrous oxide [146] and midazolam [160]. In contrast, neither morphine nor midazolam affected the temporal propagation of the pharyngeal muscle contraction wave, indicating that once pharyngeal swallowing is triggered, the pharyngeal timing is largely unaffected by opioids or sedatives in sedative doses (Fig. 16). However, in agreement with data on other anesthetic agents [150], the mechanical properties as recorded by pharyngeal muscle contraction forces were reduced by morphine and midazolam (Fig. 16). Moreover, for morphine, reduced muscle forces probably contributed to a detected prolonged bolus transit time that could possibly further increase the risk for aspiration.

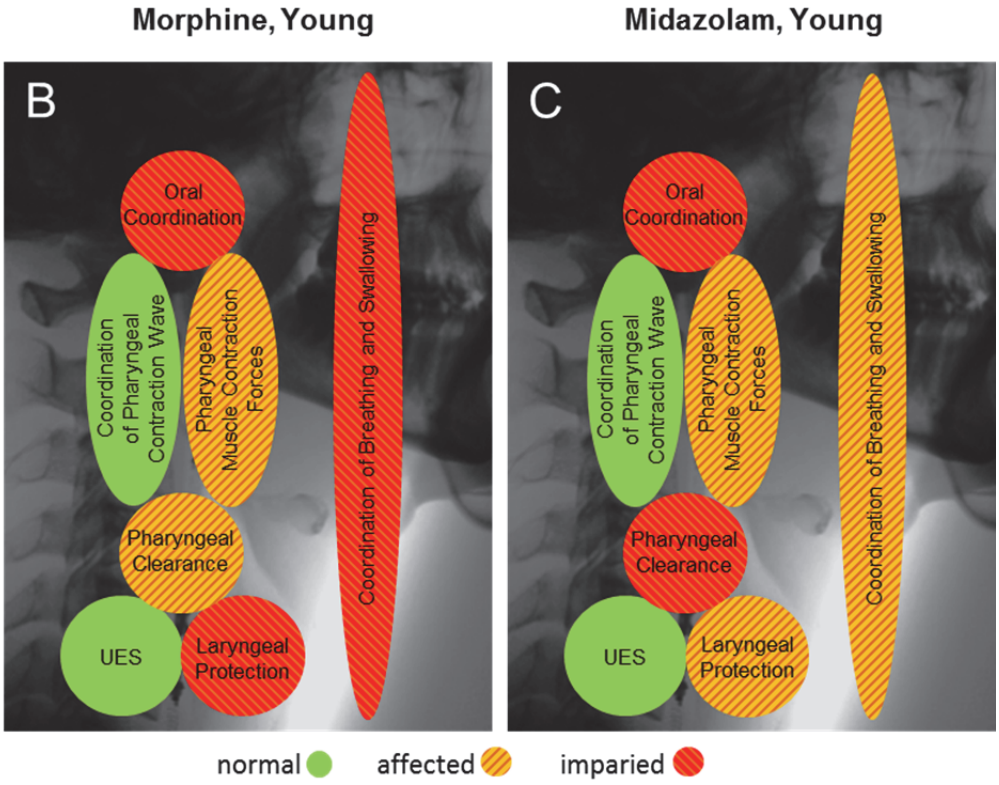

Figure 16 *Schematic illustrations of effects of morphine and midazolam on key factors important for normal airway protection in young adults (study III). For explanations of the key components, see Fig. 15.*

In agreement with one previous study on the effects of anesthesia on coordination of breathing and swallowing [148], morphine and midazolam profoundly altered respiratory phase patterns with an increased number of swallows followed by inspiration (Fig. 16),

a pattern that may be associated with pulmonary aspiration [23]. This confirmed the initial hypothesis that coordination of breathing and swallowing is affected by sedatives and analgesics in concentrations likely to occur in clinical practice. Moreover, since respiratory phase patterns origin from brainstem neurons coordinating breathing and swallowing, we suggest a centrally located target for the drugs. However, we cannot rule out peripheral causes, *i.e.* absence of expiratory airflow after swallowing due to altered muscle forces or lung volumes. Morphine had the most profound effect on coordination of breathing and swallowing, altering not only respiratory phase patterns, but also significantly prolonging the apneic period before swallowing. However this is not surprising, since preclinical data suggest that opioids inhibit the activity of inspiratory neurons [11,190].

Notably, the effects of morphine and midazolam on the pharynx were more prolonged than effects on the respiratory phase patterns. While pharyngeal dysfunction was still apparent in measurements after 30-minutes of spontaneous drug metabolism with decaying drug concentrations, the respiratory phase patterns had returned to baseline after 30 min (study III). Unexpectedly, we found that while pharyngeal dysfunction increased during drug exposure, the incidence of coughing did not. This drug-induced attenuation of coughing could aggravate consequences of impaired airway protection. Moreover, we found no correlation between level of sedation and impairment of airway protection after morphine and midazolam. This observation should be considered in the routine clinical setting where morphine and midazolam are often considered more "safe" than other drugs commonly used in clinical practice and therefore used where there is a lower degree of monitoring and awareness about respiratory complications.

Partial neuromuscular block

Partial neuromuscular block further aggravated the by age impaired protective mechanisms of the upper airway (Fig. 15 "a" and Fig. 17 "b"). As expected from studies in young volunteers during partial neuromuscular block [149,172], the incidence of pharyngeal dysfunction was increased due to deficiencies in oral bolus control, laryngeal protection and pharyngeal clearance. In the elderly, this extended to a majority of swallows showing one or multiple signs of dysfunction, despite the limited depth of neuromuscular block.

Based on knowledge of how ageing affects pharyngeal function and protection of the airway, we believe that an age-induced decline in airway protective mechanisms reduces the margins of safety during swallowing and, by that, accentuates the effects of NMBAs, making the elderly more prone to adverse pharyngeal effects of residual neuromuscular block. Fascinatingly, pharyngeal muscle contraction forces, already decreased by age (Fig. 15 "a") were not further affected during partial neuromuscular block (Fig. 17 "b"). This was surprising since pharyngeal muscle forces were reduced during partial paralysis in previous studies in the young [172]. However, a more profound degree of neuromuscular block was studied in the young. Another possible explanation could be age-related sarcopenia attenuating muscle strength and thereby "masking" a pharmacologically induced reduction in muscle forces. In contrast, but in line with previous findings in young adults, the resting tension in the UES was markedly reduced during all levels of partial neuromuscular block [149,172]

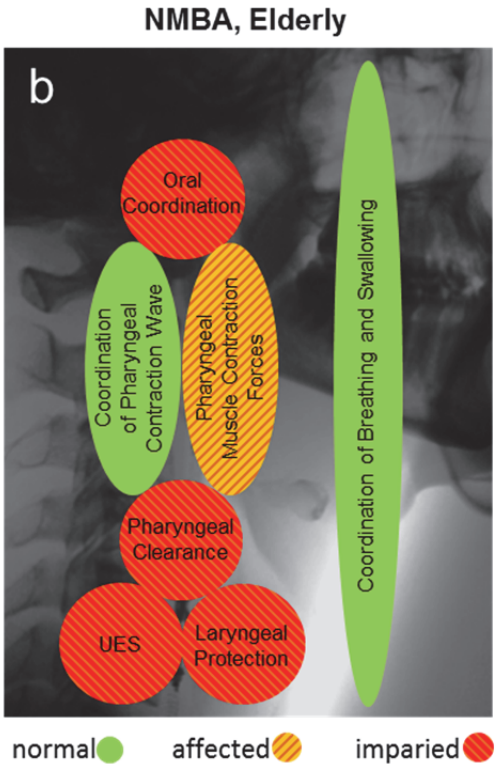

Figure 17 *Schematic illustration of effects of partial neuromuscular block (rocuronium) on important key factors for normal airway protection in the elderly (study IV). For control recordings in elderly and explanations of the key components, see Fig. 15 "a".*

Coordination of the pharyngeal muscle contraction wave as well as coordination of breathing and swallowing were both unaffected by partial neuromuscular block. This implies that central neuronal control circuits within the brainstem were unaffected. This is expected since NMBAs only poorly penetrate the blood brain barrier [221]. Hence, other targets must be responsible for the decline in pharyngeal function during partial neuromuscular block. A plausible explanation would be a cholinergic block within peripheral muscles spindles, attenuating afferent cholinergic signals of proprioception into the brainstem. This idea is supported by the previously presented "attenuation theory" [222] and the observation that visual analogue sedation scoring was increased during partial neuromuscular block, an observation that corresponds to findings of increased level of hypnosis during neuromuscular block [223]. To summarize, we believe that the aggravation of already impaired airway protection in the elderly is due to a combination of 1) reduced margins of safety by aging *per se, i.e.* reduced pharyngeal sensitivity, reduced muscle forces, agility and speed due to sarcopenia and inability to adapt to physiological challenges, 2) reduced sensory afference from muscles and 3) direct muscle weakness, in particular of the UES muscle. Although the frequency of misdirected swallows increased during partial neuromuscular block, frequency of coughing was unchanged. Again, this could aggravate consequences of impaired airway protection, and even more so since other protective mechanisms are reduced in the elderly population (see Introduction).

Historical data on Propofol, Isoflurane, Sevoflurane and Partial neuromuscular block

Because previous studies of effects of sub-anesthetic doses of propofol, isoflurane and sevoflurane [150] as well as of partial neuromuscular block by atracurium [172] and vecuronium [149] were made by the same group of researchers utilizing the same methodology but without respiratory recordings, we here present how these historical data can be categorized into the seven key components of airway protection (Fig. 18). We are aware that the historic nature of data obtained over a time period of more than fifteen years, mandates very cautious interpretation. Moreover, with the exception of the study of anesthetic agents [150], these studies were never designed to *compare* effects between drugs. Thus the categorizing of results should not be considered exact, but as *guidance* for increased understanding and planning of future studies. Yet, we would like to provide these comparisons as an illustration of how the physiology and regulatory control of the pharynx and respiration is being changed by normal aging and drugs used in clinical practice.

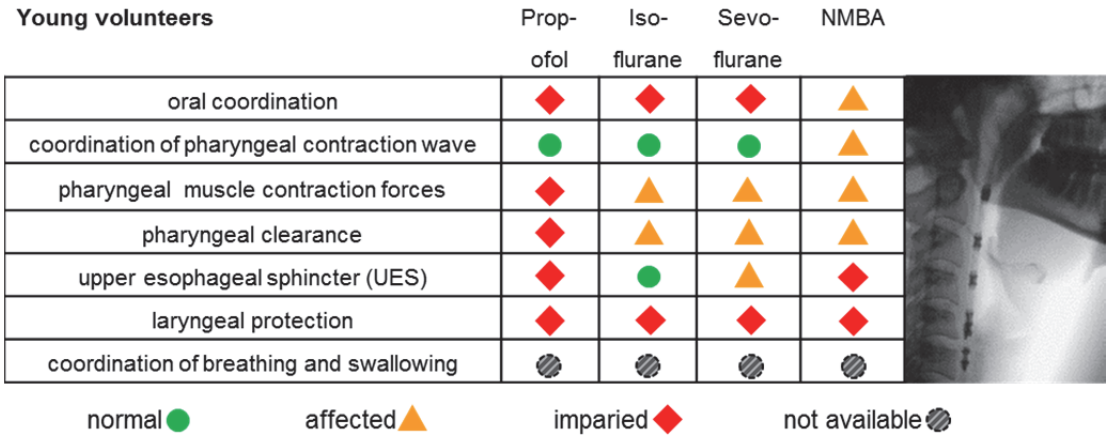

Figure 18 *Illustration of effects on key components in airway protection of sub-anesthetic doses of propofol, isoflurane and sevoflurane and during partial neuromuscular block by NMBAs. For explanations of the key components, see Fig. 15. Modified after* [149,150,172].

Additional methodological considerations

Placing a manometry catheter in the pharynx can influence normal swallowing. However, it has repeatedly been shown that this effect is small and transient [26,59,224]. Therefore, we believe that the manometry catheter has minimal impact on results. However, introducing the catheter were by some young volunteers perceived as uncomfortable, why a transient psychological effect cannot be ruled out [82,83]. Moreover, effects of psychological stress should be considered in study I, since subcostal placement and fixation of diaphragmal EMG needles were reported to be uncomfortable and in some instances painful.

Administration of bolus through a syringe is evidently different from normal drinking and could therefore possibly affect the preparatory stage of swallowing, the incidence of pharyngeal dysfunction and coordination with breathing [16,64]. However, as volunteers adapt to this challenge, the incidence of pharyngeal dysfunction should decrease or

remain stable as in the control groups (study III and IV). As the incidence instead increased during drug exposure we conclude that findings of increased pharyngeal dysfunction, impaired oral bolus control and prolonged apnea before swallowing are not likely overestimated.

The incidence of pharyngeal dysfunction at the resting control state in study III was slightly higher than found in previous groups of young healthy adults [149,150,172]. Though all swallows of contrast medium were analyzed by the same investigators (Bodén and Hårdemark-Cedborg), we cannot rule out increased detection of signs of pharyngeal dysfunction over time. Moreover, no volunteer was excluded due to frequent uncontrolled swallowing during control recordings, as done in previous studies to ensure that only healthy individuals were included before drug exposure [149,150,172].

In study III and IV, the orders of measurements were set to reflect clinical recovery, *i.e.* not randomized, nor were the studies placebo controlled. However, pharyngeal swallowing is considered a "reflexive" mechanism with a fixed pattern, that after being triggered continues without voluntary control [2], therefore placebo effects should be minimal. Moreover, we could not detect an effect over time in either control groups (study II and IV) in any parameter except for VAS-sedation that was affected over time in study III. This was probably an effect of the inactivity of the volunteers while lying down for up to 2.5 hours. All studies (I-IV) were stratified with regard to gender and thereby not designed or powered to examine effects comparing male and female sub-groups.

An evident limitation of studying the phases of respiration through direction of airflow is that it is not possible to distinguish between central and peripheral apnea. Moreover, discriminating between a "passive" or "active" apnea, *i.e.* apnea caused by zero airflow during passive expiration when lung recoil forces have reached FRC (passive apnea) and apnea caused by muscle activity in respiratory muscles or vocal cords (active apnea), cannot be done. Here, by adding modalities progressively closer to the source, *i.e.* recording chest movements (crude), diaphragmal EMG, phrenic nerve activity or brainstem neuronal activity gives a more accurate picture of "apnea", ultimately leading to the question; what is apnea? The answer being that it depends on your definition and your methodology.

A phenomenon frequently noticed in studies of coordination of breathing and swallowing is "the inspiratory spike" [7,225], "swallow inspiration" [7] or SNIF [211,225] (small non inspiratory flow), describing a negative pressure deflection just before the end of swallow apnea. Utilizing the recording of nasal pressure we have noted this phenomenon as well, however since no simultaneous inspiratory airflow was detected by the bidirectional gas flow meter we agree with the current literature [225] that this probably reflects a movement of the soft palate or the larynx at the end of pharyngeal swallowing changing nasal pressure but not resulting in oral and nasal airflow.

Clinical implications

In this thesis we have investigated and defined factors important for airway protection in health and explored deficiencies in airway protection due to aging and drugs used in anesthesia. These findings are essential to consider in clinical practice, in perioperative anesthesia and during intensive care.

We know from previous work that dysphagia and impaired dental health are risk factors for pneumonia in residents of nursing homes [95,96]. Furthermore, dys-coordination of breathing and swallowing has been associated with increased risk for pulmonary aspiration in patients after stroke [23] and silent aspirations are common after extubation in the intensive care unit [114]. In investigations of postoperative complications, respiratory complications have been found to be the most frequent in the first three days after surgery [197]. Furthermore, elderly patients are more vulnerable to suffer postoperative pulmonary complications than young adults [168]. A combination of improved oral hygiene, ambulation, head-of-bed elevation, incentive spirometry and education/awareness have been shown, in clinical studies, to reduce the risk for postoperative pneumonia [226,227]. Moreover, reversal of effects of opioids and partial neuromuscular block reduces the risk for postoperative morbidity and mortality [181,228].

As swallowing is the most important mechanism for keeping the pharynx free from material that otherwise could contaminate the lower airways, a thorough understanding of this process is of utmost clinical value. Increased knowledge of normal coordination of breathing and swallowing can contribute to novel treatment policies, exercises and guidelines, hereby improving airway protection in patients having disrupted patterns. Awareness of the effects of opioids and benzodiazepines on airway protection, and the lack of relation to level of sedation, may improve clinical practice towards cautious use of these drugs. Moreover, based on findings of a profound impairment of airway protection during partial neuromuscular block in the elderly, we believe that appropriate management of neuromuscular function including avoidance of residual neuromuscular block in the postoperative period is of utmost importance. Increased awareness and research regarding mechanisms for airway protection will ultimately improve patient safety.

CONCLUSIONS

Based on this thesis it is concluded that:

- The highly accurate airflow discriminator enabled determining respiratory airflow unambiguously in relation to pharyngeal and diaphragmatic activity in humans. Furthermore, after validation, the discriminator was integrated into a novel multimodal high resolution method that allowed detailed studies of airway protection and coordination of breathing and swallowing.

- Airway protection depends on multiple mechanisms such as oral bolus control, coordination of the pharyngeal muscle contraction wave, pharyngeal muscle contraction forces, pharyngeal clearance, functionality of the upper esophageal sphincter, laryngeal protection and coordination of breathing and swallowing, each contributing to safeguard the airway against aspiration.

- Swallowing normally occurs during the expiratory phase of respiration, and the fraction of swallows preceded and followed by expiration approach 100%. This robust integration between breathing and swallowing remains unchanged despite changes in body position, bolus characteristics or respiratory drive.

- The diaphragm is activated in the apneic period during swallowing, an action which we believe serves to promote expiratory airflow after swallowing. To our knowledge, this activation has not been described in humans before.

- Ageing is associated with increased incidence of pharyngeal dysfunction and impaired airway protection. In contrast, ageing does not change coordination of breathing and swallowing.

- Morphine and midazolam, in clinically relevant sedative doses, cause pharyngeal dysfunction, dys-coordination of breathing and swallowing and impaired airway protection in young healthy adults and these impairments cannot be predicted by monitoring the level of sedation.

- The increased incidence of pharyngeal dysfunction by age *per se* was further profoundly aggravated during partial neuromuscular block, ultimately compromising airway protection. Partial neuromuscular block does not impair coordination of breathing and swallowing.

FUTURE PERSPECTIVES

Physiological mechanisms behind airway protection have been studied for over 130 years and many aspects are known to us. However, there are still many areas that are unexplored. With regard to normal physiology of coordination of breathing and swallowing the exact role of the diaphragm during swallowing still needs further investigation. Moreover, since computer simulations and technical advancements in neurophysiology presents a chance to further venture into how central integration affects the periphery and vice versa, more detailed studies in this field would be fascinating.

This thesis presents further insights into oro-pharyngeal, respiratory and peripheral mechanisms of importance for airway protection. However, since our studies span over hours and not days, months or years, future protocols should be targeted against the link between how and to what extent pharyngeal dysfunction and dys-coordination of breathing and swallowing impair airway protection and cause pulmonary complications.

Prospective clinical outcome studies after the use of potent anesthetics and neuromuscular blocking agents are urgently needed to describe the impact of impaired airway protection on postoperative respiratory complications including pneumonia caused by silent aspirations. Here, the additive effect of multiple drugs combined with stress of surgery should ideally be explored at several time points in the perioperative setting, and including patients exposed to regional anesthesia could elucidate effects of surgical stress *per se*. Perioperative studies should also focus on high risk patients for pulmonary complications, *i.e.* include elderly, stroke-patients, patients with OSA and COPD and patients with degenerative neurological diseases. These patient groups represent challenges with regard to patient safety and need more attention in order for us to improve their perioperative care.

Volunteer studies evaluating the association between impaired airway protection and the risk for pulmonary silent or overt aspirations would also be of clinical value. Here, additional sensitive and reliable methods for detection of pulmonary aspiration would further strengthen our methodology.

Despite the current knowledge on how some of the drugs commonly used in anesthesia affect airway protection, the picture is far from complete. In this context, desflurane, dexmedetomidine, remifentanil and fentanyl need to be characterized. In line with this, knowledge on the effects on integration of breathing and swallowing is lacking for propofol, volatile anesthetics, dexmedetomidine, remifentanil and fentanyl. Drugs used for sedation are of special interest due to an increasing clinical demand to combine local anesthesia and conscious sedation for minor invasive surgery and procedures, and even more so in risk patients due to our reluctance to expose them to risks related to general anesthesia. Furthermore, clinical studies of outcome, *i.e.* following up on respiratory complications, are needed to guide clinical practice and increase patient safety.

Another fascinating field with rapidly expanding knowledge is how coordination of breathing and swallowing affects airway protection in patients dependent on assisted ventilation and artificial access to the airway. Here, studies should also investigate effects of sedative drugs since they are sometimes needed during mechanical ventilation, surgery, diagnostic and therapeutic procedures and intensive care in this vulnerable patient category.

Finally, we strongly believe that increased awareness and research regarding mechanisms for airway protection in the areas mentioned above will improve patient safety.

”Omnia mirari etiam tritissima”
”Förundras över allt, även det vardagliga”
”Find wonder in all things, even the most commonplace”

Carl von Linné

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…..to boldly go where no man has gone before…….

Jean Luc Picard,
Captain of USS Enterprise, Next Generation, Star Trek