Effects of Remifentanil on Esophageal Sphincters and Swallowing Function
To Martin and Kajsa
Effects of Remifentanil on Esophageal Sphincters and Swallowing Function
Abstract


Pulmonary complications like pneumonia are common perioperatively, and one potentially important cause is thought to be silent aspiration. There are several levels of defence against pulmonary aspiration that can be affected by drugs in anaesthesia. Competence of esophageal sphincters prevents regurgitation of gastric content, and complex reflex systems (with or without coincident swallowing) guard direct entrance into the airway. Furthermore, in our previous studies healthy volunteers spontaneously complained about swallowing difficulties when they received remifentanil, and difficult swallowing may be a poorly recognized side effect of remifentanil. The aim of this thesis was to study the effect of remifentanil on different components of airway protection with and without coincident swallowing, and to explore whether remifentanil increases the risk of pulmonary aspiration. The purpose was also to determine to what extent remifentanil induces subjective swallowing difficulties.

The competence of the esophagogastric junction and esophageal peristalsis were studied using high resolution manometry. Pharyngeal swallowing was evaluated using a novel method called automated impedance manometry analysis. Infusion of a tracer infusion into the nasopharynx and subsequent lung scans was employed to detect remifentanil-induced aspiration, and subjective swallowing difficulties were evaluated on a four-point scale.

This thesis found that, at doses used in clinical settings, remifentanil increases the incidence of aspiration in healthy volunteers. Remifentanil influences several mechanisms that protect the airway towards greater dysfunction, which may increase the risk of pulmonary aspiration. Remifentanil also appears to induce subjective swallowing difficulties when dry swallows are performed, although no association between aspiration and swallowing difficulties was observed. These findings may improve clinical practice toward cautious use of the drug, especially regarding spontaneously breathing patients in the monitored anaesthesia care setting.

Keywords: Pulmonary aspiration, postoperative lung complications, silent aspiration, defence against pulmonary aspiration, remifentanil, competence of esophageal sphincters, esophageal peristalsis, pharyngeal swallowing, high resolution manometry

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<td>Integrated relaxation pressure</td>
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<td>AIM analysis</td>
<td>Automated impedance manometry analysis</td>
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<td>ARDS</td>
<td>Acute respiratory distress syndrome</td>
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<td>CD</td>
<td>Crural diaphragm</td>
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<tr>
<td>CDP</td>
<td>Contractile deceleration point</td>
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<td>CFV</td>
<td>Contractile front velocity</td>
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<td>CP</td>
<td>Cricopharyngeus muscle</td>
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<tr>
<td>DCI</td>
<td>Distal contractile integral</td>
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<td>DL</td>
<td>Distal latency</td>
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<td>EGJ</td>
<td>Esophagogastric junction</td>
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<td>EPT</td>
<td>Esophageal pressure topography</td>
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<td>HRIM</td>
<td>High resolution impedance manometry</td>
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<td>HRM</td>
<td>High resolution manometry</td>
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<tr>
<td>IBP</td>
<td>Intrabolus pressure</td>
</tr>
<tr>
<td>iZn/Z</td>
<td>Ratio of nadir impedance to post-swallow impedance</td>
</tr>
<tr>
<td>LES</td>
<td>Lower esophageal sphincter</td>
</tr>
<tr>
<td>PeakP</td>
<td>Peak pharyngeal pressure</td>
</tr>
<tr>
<td>PZn</td>
<td>Pressure at pharyngeal nadir impedance</td>
</tr>
<tr>
<td>SRI</td>
<td>Swallow risk index</td>
</tr>
<tr>
<td>TCI</td>
<td>Target controlled infusion</td>
</tr>
<tr>
<td>TZn-PeakP</td>
<td>Time from nadir impedance to peak pressure</td>
</tr>
<tr>
<td>UES</td>
<td>Upper esophageal sphincter</td>
</tr>
<tr>
<td>UES-IBP</td>
<td>Intrabolus pressure during UES relaxation</td>
</tr>
<tr>
<td>UES-Nad-P</td>
<td>Nadir pressure during UES relaxation</td>
</tr>
<tr>
<td>UES-RI</td>
<td>UES relaxation interval</td>
</tr>
<tr>
<td>VIP</td>
<td>Vasoactive intestinal peptide</td>
</tr>
<tr>
<td>Z</td>
<td>Impedance</td>
</tr>
<tr>
<td>Zn</td>
<td>Nadir impedance</td>
</tr>
</tbody>
</table>
List of original studies

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

I. Savilampi J, Ahlstrand R, Magnuson A, Wattwil M.
   Effects of remifentanil on the esophagogastric junction and swallowing

   Acta Anaesthesiologica Scandinavica 2013; 57: 1002–1009

II. Savilampi J, Ahlstrand A, Magnuson A, Geijer H, Wattwil M

   Aspiration induced by remifentanil: a double-blind, randomized, crossover study in healthy volunteers.

   Anesthesiology. 2014 Jul; 121(1):52-8

III. Savilampi J, Magnuson A, Ahlstrand R

   Effects of remifentanil on esophageal motility: A double blind, randomized, cross-over study in healthy volunteers

   Accepted for publication in ACTA Anesthesiologica Scandinavica.

IV. Savilampi J, Magnuson A, Ahlstrand R

   Effects of Remifentanil and Morphine on Pharyngeal Swallowing: A Double Blind Randomized Cross-over Study in Healthy Volunteers

   Submitted for publication

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Introduction

Background
Pulmonary aspiration in anaesthesia patients was first described by Mendelson in 1946 (1), and is defined as the inhalation of oropharyngeal or gastric contents into the lower respiratory tract (2). Fulminant aspiration is an infrequent perioperative event; an incidence of 3 to 5 events per 10 000 general anaesthetics has been suggested (3-6). In contrast, postoperative lung complications such as pneumonia are common, with incidence from 5% to 20% depending on different risk factors (e.g. advanced age), and result in both longer hospital stays and increased morbidity and mortality (7, 8). One potentially important cause is thought to be silent, unwitnessed pulmonary aspiration that occurs perioperatively (9), for instance during the immediate postoperative period or other circumstances when the patient is breathing spontaneously and the requirement for analgesia is high. Furthermore, aspiration pneumonia is the leading cause of pneumonia in the intensive care unit (9).

Dual action of the upper aerodigestive tract
The common conduit for gases during breathing and for swallowed or vomited material is the pharynx. This anatomical challenge requires intact physiological defence mechanisms against aspiration during anterograde and retrograde flow of fluids and solids through the pharynx.

Levels of defence against aspiration
There are several levels of physiological defense against pulmonary aspiration that can fail in the event of aspiration. Competence of the esophageal sphincters and intact esophageal peristalsis prevent retrograde regurgitation of already swallowed material, while the direct entrance to the airway is protected by several pharyngeal and laryngeal mechanisms, with or without coincident swallowing.

Esophagogastric junction
The main barrier against the reflux of gastric content into the esophagus is the esophagogastric junction (EGJ). The unique anatomical configuration of the EGJ and two sphincters contribute to EGJ competence: the intrinsic lower esophageal sphincter (LES), and the crural diaphragm (CD), which
externally embraces the LES. The CD has both respiratory and gastrointestinal functions; it contracts during inspiration and makes the greatest contribution to inspiratory EGJ pressure, while tone in the LES is the main cause of expiratory EGJ pressure. The clinical assessment of EGJ competence has mainly focused on EGJ pressure during expiration, when it is lowest, and this moment has been considered the most likely time for reflux (10). However, agreement has not been widely established how to measure EGJ pressure (11, 12), and only inspiratory EGJ augmentation is independently significantly associated with objectively confirmed gastro-esophageal reflux (12). Inspiratory EGJ augmentation, the difference between inspiratory and expiratory EGJ pressures, is thought to compensate for the increased abdominothoracic pressure gradient during inspiration, and is an indicator of the functional integrity of the CD (12).

The LES is thicker than rest of the smooth muscles of the distal esophagus, with properties that distinguish it from the esophageal body. The LES exhibits constant myogenic tone (13), which is augmented and reduced by cholinergic excitatory respective nitrergic inhibitory myenteric neurons (14, 15). The LES is centrally innervated by cholinergic vagal efferents from the preganglionic fibers of the dorsal motor nucleus of the vagus; these efferents relay through postganglionic neurons in the myenteric plexus. Although the vagus exerts the main regulatory action on the LES, the postganglionic myenteric neurons receive input from esophageal and gastric intrinsic sensory neurons and from preganglionic sympathetic fibres. In addition to acetylcholine, nitric oxide, and vasoactive intestinal peptide (VIP), various other excitatory and inhibitory transmitters are present in the LES; however, their physiologic importance is unclear (16, 17).

The CD is comprised of skeletal muscle. It is innervated by branches of the phrenic nerve, as well as by inhibitory motor fibers from esophageal nitrergic myenteric neurons. It exhibits phasic contractions along respiration; in addition, it contracts reflexively with manoeuvres that increase intra-abdominal pressure (18). Swallowing, transient LES relaxations, and esophageal distension each induce the relaxation of the CD (19, 20).

**Upper esophageal sphincter**
The upper esophageal sphincter (UES) is a region of high pressure located at the junction between the pharynx and the cervical esophagus. Tonic contraction of the UES prevents air from entering the esophagus and guards against aspiration of gastric reflux. The UES opens to allow the
passage of swallowed food and relaxes or contracts during various reflexes and physiological states. Of most relevance to the UES function are the intrinsic cricopharyngeus muscle (CP) and the extrinsic suprahyoid muscles, which are all striated muscles mechanically coupled to the UES (21). The highly elastic CP is attached to the cricoid cartilage and is the most important contributor to the basal tone of the UES. The pharyngoesophageal nerve, which is a branch of the vagus nerve with motor neuron in the nucleus ambiguus, is the main innervator of the CP. In contrast to many other sphincters, the UES does not appear to exhibit a constant, active tone. Indeed its tone falls to very low levels during anaesthesia or sleep (21). However, changes in posture or arousal can cause very large increases in UES tone (21). The extrinsic suprahyoid muscles are the opening muscles of the UES and are innervated by several cranial nerves and the first cervical spinal nerves. The only neurotransmitter found to mediate contraction of the UES is acetylcholine acting through nicotinic cholinergic receptors, although several neuropeptides have been found in the CP (neuropeptide Y, calcitonin gene-related peptide, tyrosine hydroxylase, substance P, VIP, and galanin)(22).

**Pharyngeal and laryngeal reflexes**
Several pharyngeal and laryngeal reflexes protect the direct entrance to the airway, with or without coincident swallowing. The pharyngoglottal closure reflex, pharyngo-UES contractile reflex, and reflexive pharyngeal swallow are triggered by pharyngeal water stimulation and thought to protect the airway during both anterograde and retrograde flow of fluids through the pharynx (23, 24). The deglutitive glottic closure with vocal cord closure, aryepiglottic adduction, and epiglottal descent are actions of the intrinsic laryngeal muscles and provide a sealed barrier against laryngeal penetration of the bolus during swallowing (25). The pharyngeal plexus, which is mainly formed by the glossopharyngeal and vagus nerves, mediates the sensory innervation of the pharyngeal wall. Branches of the vagus nerve also innervate the larynx. The Efferent motor supply to the laryngeal and pharyngeal muscles originates from the vagus and accessory nerves.

**Swallowing**
Swallowing is a complex process consisting of three stages (oral, pharyngeal, and esophageal) and employing several dozen paired muscle groups and a neural control mechanism that extends from the cortex to the spinal
cord. A central pattern generator located within the medulla oblongata directs the sequential and rhythmic patterns of swallowing. This pattern generator is intimately associated with the nucleus tractus solitaries. The motor neurons that control all stages of swallowing are located in the brainstem and the cervical part of the spinal cord, according to their anatomical distribution within the swallowing apparatus (26). The oral stage of swallowing is a voluntary action, while the pharyngeal and esophageal stages are automatic.

**Pharyngeal swallowing**
All of the muscles involved in the oropharyngeal stage of swallowing are striated muscle. After the initial oral preparation, delivery of the bolus to the pharynx triggers the pharyngeal swallow reflex; the tongue base propels the bolus against the posterior pharyngeal wall and further backwards, the soft palate closes the nasopharynx, the larynx elevates, the vocal folds close, and the UES relaxes and opens to allow the bolus to pass. Simultaneously, the pharyngeal constrictor muscles begin to contract in a descending manner, elevating and widening the pharynx to first engulf the bolus and then clear the bolus residue from the pharynx in a typical wave pattern. The neural control of pharyngeal swallowing involves five major components: 1) sensory afferents contained in cranial nerves; 2) input from cerebral and midbrain fibers; 3) swallowing centers in the brainstem which relay these inputs; 4) motor efferents contained in cranial nerves; and 5) muscles and other end organs.

**Esophageal swallowing**
The esophagus can be simply described as a hollow muscular tube closed by the UES proximally and by the EGJ distally. The cervical esophagus is composed of striated muscle and innervated by lower motor neurons carried within cranial nerves (mainly the vagus nerve). In contrast, the thoracic esophagus is smooth muscle innervated by preganglionic excitatory and inhibitory efferents carried within the vagus nerve (27), as well as by intramural postganglionic neurons located in the myenteric plexus. Preganglionic transmission is mainly cholinergic nicotinic. Nitric oxide is the predominant inhibitory neurotransmitter, and acetylcholine, acting on muscarinic receptors, is the excitatory neurotransmitter in the myenteric plexus. After the bolus has passed through the UES, it shuts rapidly and the esophageal stage of swallowing commences. The peristalsis of the esophagus is the result of sequential inhibition of the esophageal muscles,
which disrupts all activity and finally results in the opening of the EGJ, followed by the excitation and lumen-occluding contraction of the circular muscles of the esophagus (28, 29). This inhibitory wave, also known as deglutitive inhibition, progressively increases in duration distally along the esophagus, and is followed by peristaltic contraction (30).

**The impact of aging**
The efficiency of swallowing and airway protection declines with age, even in healthy older adults (31, 32). In the elderly, several aeroprotective reflexes are impaired (33, 34). Lingual propulsion during swallowing is weakened, and duration of the oropharyngeal stage of swallowing is prolonged (35-39), increasing the time of exposure of the laryngeal vestibule. These multiple subtle reductions in function result in a much smaller margin for error, and may lead to rapid decompensation in swallowing function during illness or during sedation/anaesthesia. For example, patients aged 80 years or older have an almost 10-fold increased risk of pulmonary aspiration compared with 20-year-olds (40). The function of esophageal peristalsis appears to be more preserved with aging; duration may be prolonged and amplitude lessened, but the clinical significance of these findings remains unclear.

**Opioids**
Previous manometric studies investigating the effects of opioids on EGJ pressure (41-48) have mainly focused on end-expiratory EGJ pressure, and have revealed that decreased pressures are induced by opioids. However, the inspiratory EGJ augmentation appears to be more important when examining reflux barrier function of the EGJ (12). There are currently no studies regarding the effects of opioids on inspiratory EGJ augmentation. A few studies have investigated esophageal motility and opioids (44, 49-51), and have found that opioids increase the velocity of esophageal peristalsis and decrease the swallow-induced relaxation of the EGJ. Regarding the effects of opioids on pharyngeal function, case reports describe temporary dysphagia after intrathecal fentanyl (52-55), morphine (55), and sufentanil (56). Furthermore, the impact of other anaesthetic agents on pharyngeal function has been investigated as an indicator of increased aspiration risk (57-60); however, to our knowledge no studies have demonstrated that opioids or other anaesthetic agents directly induce aspiration when administrated at the doses used in sedation.
Remifentanil
The primary focus in this thesis was the effect of remifentanil on the risk of aspiration and on different levels of defence against pulmonary aspiration. Remifentanil is suitable for spontaneously breathing patients in monitored anaesthesia care settings in spontaneously breathing patient because its pharmacokinetic profile features an ultra-rapid onset time and short duration of action. Remifentanil has recently gained field as pain relief among women in labour (61, 62); notably, these patients are rarely fasting. The adverse effect profile of remifentanil is similar to those of other, newer synthetic opioids, and includes muscle rigidity (63). The influence of remifentanil on the risk of aspiration is still mostly unknown. In our previous studies (46, 64), volunteers spontaneously reported subjective swallowing difficulties when they received remifentanil. This possibly unknown side effect of remifentanil and the fact that remifentanil is now widely used in a variety of anaesthesia settings inspired us to examine the potential risks more closely.
Aims

The overall aim of this thesis was to investigate the effects of remifentanil on the physiological defence against pulmonary aspiration.

The specific aims were:

- To determine the effect of remifentanil on the function of the EGJ as a reflux barrier by using the inspiratory EGJ augmentation as an indicator, and to evaluate whether this possible effect is centrally or peripherally mediated.

- To determine through a direct method whether remifentanil results in pulmonary aspiration.

- To evaluate the impact of remifentanil on esophageal motility, and to describe whether there is a dose response associated with remifentanil’s effects and whether they are counteracted by naloxone and/or dopamine.

- To assess the effects of remifentanil on pharyngeal function and UES relaxation during swallowing, to compare these effects with those of morphine, and to describe the impact of age on these effects.

- To describe to what extent remifentanil induces subjective swallowing difficulties.
Volunteers and Methods

Approvals
All studies were approved by the Regional Ethics Committee in Uppsala, Sweden (Dnr 2009/219, Dnr 2011/017, Dnr 2010/220, Dnr 2013/251), and by the Swedish Agency for drugs. All studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practise, and registered in a central database, ClinicalTrials.gov.

Volunteers
After oral and written informed consent, a total of 67 healthy volunteers were included (female: 28, male 39), 60 young (study I-VI) and 7 elderly (study IV). The volunteers lacked any history of swallowing difficulties, upper gastrointestinal conditions including gastro-esophageal reflux disease or previous gastrointestinal surgery. Young volunteers were medication-free and none of the elderly volunteers used any medication known to affect gastrointestinal motility.

High resolution solid-state manometry
High resolution manometry (HRM) has its roots in conventional water-perfused systems and is the current gold standard technique to assess esophageal motility. Its major advantage compared with earlier technologies is the vastly greater number of pressure sensors along the manometric catheter and diminished space between them (65, 66). This feature enables simultaneous dynamic recording of pressure morphology all the way from the pharynx to the stomach without spatial gaps between recording sites and with no need to compensate for the axial motion of (for example) the esophageal sphincters during swallowing. Furthermore, compared with water-perfused systems, solid-state HRM systems are free of hydrostatic influences, consistently measure of rapidly changing pressures (pharynx, UES) and are easily mobile because the pressure sensors are incorporated into the catheter. Pressure transducers underlying the sensors within the catheter convert tactile pressure on the catheter to an electrical signal that is then amplified, and filtered, and then digitised using standard circuitry connected to a personal computer (67).
In this thesis, the HRM system used for pure manometric measurements in studies I and II was the ManoScan 360 (Sierra Scientific Instruments, Inc., Los Angeles, CA). This system uses a solid-state catheter (Ø 4.2 mm) with circumferential pressure sensors at 1-cm intervals over a length of 36 cm.

**Esophageal pressure topography**

Esophageal pressure topography (EPT) (Fig 1 is a display method in which pressure data acquired by HRM is plotted against both time and distance along the esophagus. The resulting colour-coded topographs are also called Clouse plots, in honour of key innovator Ray Clouse (65). EPT has improved the accuracy and speed with which this form of data is interpreted, compared to conventional line plots. It also enables correct positioning of the manometric catheter and the resolution of technical problems at the time of the recording (67, 68).

The Chicago Classification (69) is a practical classification of esophageal motility disorders based on EPT metrics developed by the HRM Working Group and subsequently updated with intent of improving diagnostic accuracy and clinical utility (70). At present, the Chicago Classification defines swallow induced EGJ relaxation, esophageal contractile activity, and esophageal pressurisation but does not include pharyngeal motility or UES function.

**Analysis procedures**

Manoview analysis software (Sierra Scientific Instruments, Inc.) was used to evaluate the effect of remifentanil on EGJ pressures in study I and on esophageal motility in study III. In study I, in the manner of Pandolfino et al. (12) after identification of the EGJ high pressure zone, inspiratory EGJ pressure was defined as the maximal pressure that occurred during a normal respiratory cycle, and expiratory EGJ pressure was defined as the pressure at the midpoint between adjacent inspiratory EGJ pressures during a normal respiratory cycle. The inspiratory augmentation of the EGJ pressure was the difference between the basal inspiratory pressure and the basal expiratory pressure. In study III, each swallow was examined with the tools provided by the software, and EPT metrics based on the Chicago Classification characterizing the features of esophageal motility were defined (Table 1).
Figure 1. Esophageal pressure topography depicting one swallowing event. EGJ = Esophagogastric junction, I = Inspiration, E = Expiration.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrated relaxation pressure, 4-s-IRP (mmHg)</td>
<td>Mean lowest esophagogastric junction pressure for four continuous or non-continuous seconds in the 10-s window following deglutitive upper esophageal sphincter relaxation, indicative of deglutitive esophagogastric junction relaxation</td>
</tr>
<tr>
<td>Intra bolus pressure, IBP (mmHg)</td>
<td>Greatest pressure obtained for a continuous or non-continuous 3-s period within the same temporal boundaries used to calculate the 4-s IRP, reflects the pressure within the fluid compartmentalised between the EGJ and the esophageal contraction</td>
</tr>
<tr>
<td>Contractile deceleration point, CDP</td>
<td>The inflection point along the 30-mmHg isobaric contour where propagation velocity slows, demarcating the tubular esophagus from the phrenic ampulla</td>
</tr>
<tr>
<td>Distal latency, DL (s)</td>
<td>The time interval between the opening of the upper esophageal sphincter and the CDP</td>
</tr>
<tr>
<td>Contractile front velocity, CFV (cm/s)</td>
<td>The slope of the best-fit tangent approximating the 30 mmHg isobaric contour between the proximal trough and the CDP</td>
</tr>
<tr>
<td>Distal contractile integral, DCI (mmHg-s-cm)</td>
<td>Summarises the vigor of the distal esophageal contraction and is an integral of amplitude, duration, and length of the distal propagation wave from proximal to distal pressure troughs</td>
</tr>
</tbody>
</table>

Table 1. EPT metrics used to assess esophageal motility
High resolution impedance manometry
High resolution impedance manometry (HRIM) combines impedance monitoring segments in the manometric catheter, allowing the simultaneous measurement of impedance and pressures. Intraluminal impedance monitoring is a recent technique to detect the flow of fluids and gases through hollow viscera, such as the esophagus. The physical principle in impedance monitoring is the measurement of resistance to electrical flow in an alternating current circuit that is generated between two ring electrodes (impedance monitoring segments) separated by a nonconductive catheter. Impedance is inversely related to the conductivity of the medium surrounding the catheter: air has high impedance due to very low conductivity, and liquids such as saline or gastric juice have low impedance and high conductivity. In HRIM, several impedance monitoring segments are placed along the catheter to enable the evaluation of the direction and velocity of the flow of medium (71). The method can be used to measure the clearance of a swallowed bolus in the esophagus, to detect gastro-esophageal reflux independent of its acidity, to determine the nature of belches, and to assess pharyngeal swallowing, as described below.

As part of this thesis, a combined solid-state manometric and impedance catheter incorporating 36 circumferential pressure sensors spaced at 1-cm intervals and 18 impedance segments (each 2 cm long) was used to acquire pressure and impedance data in study IV (Sierra Scientific Instruments, Inc., Los Angeles, CA).

Automated impedance manometry analysis
Automated impedance manometry analysis (AIM analysis, AIMplot software copyright, Taher Omari, Adelaide, Australia) is a MATLAB-based analysis program (Mathworks, Natick, MA) that provides a novel technique to assess pharyngeal function (72, 73). Measurements using automated algorithms are derived from pressure and impedance waveforms. These measurements quantify bolus flow, flow resistance, and contractile strength during pharyngeal swallowing and provide objective numerical values for physiological processes associated with pharyngeal swallowing.

Analysis procedure
In this thesis, AIM analysis was used in study IV to evaluate the effects of remifentanil on pharyngeal swallowing. First, the pressure and impedance data was exported from the HRIM recording system as text files, and then the entire pharyngeal region to the proximal UES was displayed as pres-
sure and impedance topographic plots (Fig. 2). Regions of interest encompassing the pharynx, distal pharynx, and UES in these plots were located by placing specific time-space landmarks. Next, the swallow function variables were automatically derived, based on established methodology (72-74) (Table 2).

Figure 2. A colour pressure topography plot of a 10ml swallow. The UES high pressure zone (HPZ) is an easily recognisable region of tonic pressure. ROI = region of interest.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Indicates</th>
</tr>
</thead>
<tbody>
<tr>
<td>PeakP, mmHg</td>
<td>Mean pressure of the entire pharyngeal stripping wave</td>
<td>Contractile vigor of the pharyngeal stripping wave. Low = poor capacity to clear bolus residue</td>
</tr>
<tr>
<td>PZn, mmHg</td>
<td>Mean pressure when the pharyngeal impedance is at its nadir</td>
<td>Pharyngeal residual pressure during maximum bolus flow. High = poor compliance/obstruction</td>
</tr>
<tr>
<td>TZn-PeakP, ms</td>
<td>Mean time interval from nadir impedance to peak pressure</td>
<td>Pharyngeal capacity to propel the bolus in advance of the stripping wave. Short = poor bolus propulsion</td>
</tr>
<tr>
<td>Flow Interval, ms</td>
<td>Time interval of the impedance drop</td>
<td>Bolus dwell time during swallow. Long = delayed initiation of and/or failed clearance of bolus residue</td>
</tr>
<tr>
<td>Swallow Risk Index</td>
<td>Flow Interval × PZn × 100 / PeakP × (TZn-PeakP + 1)</td>
<td>Global measure of swallow function. High = swallow dysfunction predisposing to aspiration risk</td>
</tr>
<tr>
<td>UES-Relaxation Interval, s</td>
<td>UES relaxation time</td>
<td>Duration of UES pressure drop</td>
</tr>
<tr>
<td>UES nadir pressure, mmHg</td>
<td>Minimum relaxation pressure</td>
<td>UES residual pressure</td>
</tr>
<tr>
<td>UES intrabolus pressure, mmHg</td>
<td>Median intra-bolus pressure during UES relaxation</td>
<td>UES bolus distension pressure</td>
</tr>
<tr>
<td>UES-Resistance, mmHg/s</td>
<td>UES-IBP/RI</td>
<td>UES bolus distension pressure relative to UES relaxation time</td>
</tr>
<tr>
<td>Ratio of nadir impedance to post-swallow impedance</td>
<td>iZn/Z</td>
<td>Postswallow residue</td>
</tr>
</tbody>
</table>

Table 2. Pressure flow variables and UES relaxation variables derived by AIM Analysis
Radionuclide scans
In study II, a method previously described by Gleeson et al. (75) was used to determine whether remifentanil results in aspiration. Radionuclide tracer solution ($^{99m}$Tc-labeled colloid albumin) was infused into the nasopharynx using a pliable plastic tube (10FR) during one hour parallel to intravenous remifentanil or placebo infusion. Eventually, aspirated tracer was detected in subsequent lung scans. All images were reviewed by the same nuclear medicine physician and scored as positive or negative for aspiration. Aspiration was diagnosed when activity was present in the lung fields on either side of the midline structures, such as the esophagus and trachea (Fig. 3).

Figure 3. Frontal lung scan image showing aspirated tracer in the right lung field.
Assessment of subjective swallowing difficulties
In all four studies, volunteers were asked to perform dry swallows (studies I and II) or wet swallows (studies III and IV) and assess subjective swallowing difficulties on a four-point scale (no difficulty, mild difficulty, moderate difficulty, or severe difficulty).

Drug administration and monitoring
In all four studies, remifentanil was administered as a target controlled intravenous infusion (TCI; Minto Model, Alaris PK syringe pump, Alaris Medical Nordic AB, Sollentuna, Sweden). In studies I and II the effect site target concentration was 3 ng/ml, in study III three target concentrations were used (1, 2, and 3 ng/ml), and in study IV the target concentration was 3 ng/ml for young volunteers and 2 ng/ml for elderly volunteers. In study I, methylnaltrexone 0.15 mg/kg was administered as subcutaneous injection. In study III, naloxone was administered as an intravenous bolus injection of 6 μg/kg with subsequent infusion of 0.1 μg · kg\(^{-1}\) · min\(^{-1}\) and metoclopramide 0.2 mg/kg was given intravenously. In study IV, morphine was administered as a bolus injection of 0.1 mg/kg for young volunteers and 0.07 mg/kg for elderly volunteers. Saline was used as a placebo comparator in equal amounts to respective drug in study I (methylnaltrexone), study II (remifentanil), and study II (naloxone). Grip strength was measured using a Jamar dynamometer in study II. Vital parameters were continuously monitored and recorded in all studies.
**Study protocols**

Schematic pictures of study protocols used in studies I – IV are presented in Fig. 4. In all studies, the volunteers were examined on two different occasions according to crossover design, with intervals of approximately 1 to 2 weeks. Volunteers were placed in supine position in studies I – III and had a 30° head-up tilt in study IV. Randomization was conducted using sealed envelopes in study I, and a random number generator in studies II – IV. In HRM and HRIM studies (studies I, III, and IV), after intravenous access was obtained the manometric catheter was positioned transnasally, with the catheter tip in the stomach and the measuring sensors straddling the pharynx and the entire esophagus. Before and immediately after each investigation, the catheter was calibrated outside the body using the calibration options provided by the software. In study II, the tube for infusion of radionuclide solution was placed in the nasopharynx, approximately 7 cm into the naris.
Figure 4. Schematic presentations of study protocols (Studies I – IV). EGJ = esophagogastric junction; HRM = high resolution manometry; MNTX = methyl-naltrexone; EPT = Esophageal pressure topography; HRIM = high resolution impedance manometry.
Statistics

For all manometric and impedance parameters, a mean value based on several measurements (study I: five consequent respiratory cycles; study III: five swallows; study IV: ten swallows) was calculated for each volunteer and study condition (baseline or during exposure to drug). Data are summarised as mean ± SD and treatment effects were presented together with 95% confidence intervals (CI). Two-way repeated measures ANOVA with one to two within-subject factors were used, followed by planned comparisons between baseline and exposure to drug (factor time) and between treatments (factor agent; e.g., remifentanil versus morphine in study IV). In addition, the linear effect was evaluated for a possible dose-response association of remifentanil in study II, and age was evaluated as a between-subject factor in study IV. If violation from the normal distribution of the parameters was observed with the Shapiro-Wilk test, then it was re-evaluated after logarithmic (log10) transformation. If the Shapiro-Wilk test still indicated non-normality after log transformation, then non-parametric methods were used for sensitivity analysis (study IV).

In all studies, for each study condition the most frequent answer regarding subjective swallowing difficulties was chosen from repeated assessments. The Wilcoxon paired signed-rank test was used to determine statistically significant differences in subjective swallowing experiences at baseline versus during exposure to drug.

In study II, the occurrence of aspiration with remifentanil versus placebo was compared using an exact test for paired proportions, and the results were presented as proportional differences with normal-approximated 95% confidence intervals (CIs). The $\chi^2$ test was used to test the association between swallowing difficulties and aspiration. Two-way repeated measures ANOVA was used to evaluate grip strength in the same way as manometric data in other studies.

Vital parameters were evaluated in the same way as manometric data. P-values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 19 to version 22 (IBM Corp, Armonk, NY, USA) or STATA release 11 (STATACorp., College Station, TX).
Results

Study I
In study I, we examined the effect of remifentanil on the EGJ and showed that remifentanil significantly decreased the inspiratory EGJ augmentation (P < 0.01) (Fig. 5). The decrease was not significant when the volunteers received methylnaltrexone and remifentanil, nor were the differences between placebo/remifentanil and methylnaltrexone/remifentanil statistically significant.

Figure 6 illustrates the swallowing difficulties experienced by volunteers before, during, and after remifentanil infusion. The time during remifentanil infusion was divided into two 15-min periods. With both placebo and methylnaltrexone, the same 7 volunteers reported more difficulty swallowing during remifentanil infusion compared with both before infusion and 15 min after ending remifentanil infusion. Statistically significant increases in swallowing difficulty were observed between pre-remifentanil and the first 15 min of remifentanil infusion with both placebo (P = 0.02) and methylnaltrexone (P = 0.03). Statistically significant differences were also observed between pre-remifentanil and the second 15-min period of remifentanil infusion. After remifentanil infusion was stopped, the swallowing difficulties gradually vanished as the target concentration of remifentanil diminished.
Figure 5. Boxplots showing the inspiratory EGJ augmentation over time in 10 volunteers randomly assigned to receive methylnaltrexone or placebo in addition to remifentanil infusion in study I. T2 = 30 min after methylnaltrexone/placebo injection, T3 = 15 min after remifentanil infusion was started, T4 = 30 min after remifentanil infusion was started, T5 = 15 min after remifentanil infusion was stopped. Boxplot showing the 25th and 75th percentiles as the top and bottom edges of a box and the median as the line inside the box; whiskers represent the minimum and maximum, if no outliers are present.
Subjective Swallowing Difficulties
- Methylnaltrexone

Before Remifentanil

During Remifentanil 15 min

During Remifentanil 30 min

After Remifentanil

Subjective Swallowing Difficulties
- Placebo

Before Remifentanil

During Remifentanil 15 min

During Remifentanil 30 min

After Remifentanil

A = no difficulties  B = mild difficulties  
C = moderate difficulties  D = severe difficulties

Figure 6. Subjective swallowing difficulties experienced by volunteers in study I.
**Study II**

In study II we investigated whether a 1-hour infusion of remifentanil at a target controlled concentration of 3 ng/ml results in pulmonary aspiration. Subjective swallowing difficulties and grip strength were recorded, and the association between eventual aspiration and swallowing difficulties was determined. The occurrence of aspiration in volunteers during the study is presented in Table 3. Ten of 25 study subjects had evident radionuclide tracer in their lung fields, indicating aspiration, after remifentanil treatment but not after placebo. Twelve subjects did not have apparent radionuclide tracer in the lungs after either treatment, two subjects had positive lung scans after both treatments, and one subject had radionuclide in the lungs only after placebo treatment. The difference between remifentanil and placebo treatment was significant; 48% and 12% of volunteers aspirated after remifentanil and placebo, respectively (Difference: 36%; 95%CI 10% to 62%). The location of the tracer in the lung fields demonstrates that the majority of volunteers aspirated into the right lung field (Table 4).

During remifentanil treatment, seven volunteers experienced moderate swallowing difficulties and five volunteers experienced severe swallowing difficulties. None of these 12 volunteers had swallowing difficulties before the infusion, and all returned to normal swallowing after the infusion. The remaining 13 volunteers experienced no difficulty swallowing before, during, or after the infusion. The difference in swallowing difficulty before infusion (0%) versus during infusion (48%) was significant (p = 0.002). None of the volunteers had difficulty swallowing before, during, or after placebo treatment. The difference in swallowing difficulty among the placebo group (0%) versus the remifentanil group (48%) was significant (p = 0.002).

Swallowing difficulties were reported by seven of the 12 volunteers (54%) who aspirated during remifentanil treatment compared with five of the 13 (38%) who did not aspirate during remifentanil treatment. The association between aspiration and swallowing difficulties was not significant (p = 0.32). None of the three volunteers who aspirated during placebo treatment had swallowing difficulties.

No differences in grip strength were observed between the remifentanil and placebo groups.
Table 3. Aspiration in groups that received remifentanil versus placebo in study II. Proportions are presented as percentages, with actual frequency counts in parentheses.

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Remifentanil</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Right Lung</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Right Lung</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>Right Lung</td>
</tr>
<tr>
<td>4</td>
<td>Right and Left Lung</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Right Lung</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Right Lung</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Right and Left Lung</td>
<td>Left Lung</td>
</tr>
<tr>
<td>8</td>
<td>Right Lung</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Right and Left Lung</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Right and Left Lung</td>
<td>Right and Left Lung</td>
</tr>
<tr>
<td>11</td>
<td>Left Lung</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Right Lung</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Right Lung</td>
<td>-</td>
</tr>
</tbody>
</table>

"-" indicates no aspiration.

Table 4. Location of aspirated tracer in study II
Study III
In study III, the impact of remifentanil on esophageal motility was evaluated by analysing five specific EPT metrics based on the Chicago Classification. We also aimed to determine whether the effects of remifentanil were subject to dose-response association and whether the effects could be counteracted by naloxone or metoclopramide. The results are presented in Table 5. The study showed that three of the EPT metrics that define primary esophageal motility were significantly influenced by remifentanil: the latency time of distal swallow-evoked esophageal contraction was shortened, the swallow-induced EGJ relaxation was decreased, and the intrabolus pressure preceding the esophageal contraction was increased. There was a negative association between the latency time of the distal esophageal contraction and the concentration of remifentanil. No statistically significant differences were observed between occasions with or without naloxone. Metoclopramide significantly increased the vigor of the swallow-evoked esophageal contraction on both occasions.

On the occasion without naloxone, 14% of volunteers experienced mild or more-severe swallowing difficulties at baseline compared with 43% when exposed to remifentanil ($P = 0.096$). On the occasion with naloxone, 43% of volunteers experienced mild or more severe swallowing difficulties both at baseline and when exposed to remifentanil ($P = 0.41$).
Table 5. Esophageal pressure topography metrics in 14 volunteers in study III

Data are means ± SD. Each esophageal pressure topography metric was evaluated with ANOVA for repeated measures, with difference from baseline (T0) as the outcome variable for the interaction P-values (agent × time). Linear dose-response evaluation for remifentanil was done from T0 to T3. Paired t-test was used to compare T3 and T4 for each agent, as well as with an ANOVA interaction test (time × agent) with only T3 and T4 as time factors (metoclopramide effect). 4s-IRP = integrated EGJ relaxation pressure; IBP = intra bolus pressure; DL = distal latency; CFV = contractile front velocity; DCI = distal contractile integral; Nalox = naloxone; T0 = baseline; T1 = 1 ng/ml remifentanil; T2 = 2 ng/ml remifentanil; T3 = 3 ng/ml remifentanil; T4 = after metoclopramide.

<table>
<thead>
<tr>
<th>T0</th>
<th>Placebo</th>
<th>T1</th>
<th>Placebo</th>
<th>T2</th>
<th>Placebo</th>
<th>T3</th>
<th>Placebo</th>
<th>ANOVA</th>
<th>Dose-response (T0-T3)</th>
<th>T-test</th>
<th>Placebo</th>
<th>ANOVA (T3,T4)</th>
<th>T-test (T3 vs T4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.4±3.4</td>
<td>9.7±4.7</td>
<td>11.7±4.7</td>
<td>11.8±5.5</td>
<td>12.0±4.9</td>
<td>12.3±7.7</td>
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<td>11.8±5.5</td>
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<td>0.07</td>
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<td>12.0±3.5</td>
<td>13.4±3.3</td>
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<td>&lt;0.01</td>
<td>0.06</td>
<td>16.4±8.5</td>
<td>17.0±7.3</td>
<td>0.048</td>
<td>0.13</td>
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<tr>
<td>8</td>
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<td>8</td>
<td>7</td>
<td>0</td>
<td>6.7±1.2</td>
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<td>5.0±0.8</td>
<td>&lt;0.01</td>
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<tr>
<td>4.5±2.0</td>
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<td>4.5±1.0</td>
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<td>0.91</td>
<td>4.6±1.0</td>
<td>4.5±1.4</td>
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<tr>
<td>1909</td>
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<td>2395±</td>
<td>1561</td>
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<td>2431±</td>
<td>872</td>
<td>1799±</td>
<td>2430±</td>
<td>1665</td>
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<td>0.01*</td>
<td>0.01</td>
<td>0.01*</td>
<td>0.01*</td>
<td>0.01*</td>
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</tbody>
</table>

Table 5. Esophageal pressure topography metrics in 14 volunteers in study III
Study IV
In study IV, we evaluated the effects of remifentanil on pharyngeal swallowing. We employed HRIM combined with AIM analysis to derive pressure-flow and UES relaxation metrics. We also aimed to compare remifentanil with morphine, and both young and old volunteers were enrolled in the study. The effects of each drug on different swallow function variables are presented as effect ratios in Table 6. Comparisons of the two drugs are presented in Table 7. Most of the pressure flow variables were influenced by remifentanil: the peak pharyngeal pressure (PeakP) was decreased, the time interval from the pharyngeal peak pressure to the nadir impedance (TZn-PeakP) decreased, and the swallow risk index (SRI) was increased. Furthermore, measures of UES flow resistance were increased.

Similar although smaller effects were observed with morphine. One variable in particular exhibited a statistically significant difference between treatments: TZn-PeakP was decreased only with remifentanil. When the volunteers were stratified by age, two variables exhibited significant interaction effect: in young volunteers, pressures during UES relaxation (UES-Nad-P and UES-IBP) increased by a significantly greater degree with remifentanil than with morphine. No differences between remifentanil and morphine were observed among the older volunteers.

At baseline, one subject reported swallowing difficulties with both treatments (remifentanil: male aged 26 years; morphine: male aged 27 years). No subjects reported swallowing difficulties during morphine exposure, while two subjects (both males, aged 22 and 65 years) reported swallowing difficulties during remifentanil exposure. This increase in proportion was not statistically significant (p=0.41).

Vital parameters exhibited some minor but statistically significant differences in studies II-IV during drug exposure compared with baseline or between treatments; however, but no clinically relevant changes were observed.
Table 6. The effects of remifentanil and morphine on pressure flow variables in 18 volunteers in study IV. Each variable was evaluated with repeated measures ANOVA. A mean ratio is a relative mean difference: a mean ratio of 1 indicates no difference, and a mean ratio of 1.40 indicates a 40% greater mean level (at time-points T1, T2) with a specific agent (remifentanil or morphine) versus T0. The P-value for age tests whether treatment effects differ in young versus old volunteers. All = all 18 volunteers; Young = 11 < 30 years; Old = 7 > 65 years; PeakP = peak pharyngeal pressure; Zn = nadir impedance; PZn = pressure at nadir impedance; TZn–PeakP = time interval from nadir impedance to PeakP; UES = upper esophageal sphincter; Zn/Z = ratio of nadir impedance to post swallow impedance T0 = baseline; T1 = 15 min after treatment start; T2 = 30 min after treatment start; CI = confidence interval. Data for which P < 0.05 are presented in bold.
Table 7. The effects of remifentanil versus morphine on pressure flow variables in 18 volunteers in study IV. Each variable was evaluated with repeated measures ANOVA with differences between T0 and mean (T1 T2) as outcomes. A mean ratio is a relative mean difference: a mean ratio of 1 indicates no difference, and a mean ratio of 1.40 indicates a 40% greater mean effect T1, T2 vs. T0 with remifentanil versus morphine. P-value for interaction (age*agent) tests whether the agent effects differ in young versus old volunteers. All = all 18 volunteers; Young = 11 < 30 years, Old = 7 > 65 years; PeakP = peak pharyngeal pressure; Zn = nadir impedance; PZn = pressure at nadir impedance; TZn–PeakP = time Zn to PeakP; CI = Confidence Interval. Data for which P < 0.05 are presented in bold.

<table>
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<tr>
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<th>Δ Remifentanil vs. Δ Morphine</th>
<th>Interaction Age*Agent</th>
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<tbody>
<tr>
<td></td>
<td>Mean ratio (95% CI)</td>
<td>P</td>
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<tr>
<td>PeakP, mmHg</td>
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</tr>
<tr>
<td>All</td>
<td>0.95 (0.89 - 1.02)</td>
<td>0.19</td>
</tr>
<tr>
<td>Young</td>
<td>1.07 (0.98 - 1.17)</td>
<td>0.11</td>
</tr>
<tr>
<td>Old</td>
<td>0.85 (0.77 - 0.95)</td>
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<tr>
<td>PZn, mmHg</td>
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<td>All</td>
<td>1.23 (0.72 - 2.10)</td>
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</tr>
<tr>
<td>Young</td>
<td>1.35 (0.69 - 2.64)</td>
<td>0.35</td>
</tr>
<tr>
<td>Old</td>
<td>1.11 (0.48 - 2.58)</td>
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<tr>
<td>TZn-PeakP, ms</td>
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<tr>
<td>All</td>
<td>0.88 (0.79 - 0.97)</td>
<td><strong>0.018</strong></td>
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</tr>
<tr>
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<td>Old</td>
<td>1.55 (0.54 - 4.43)</td>
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<td>UES Relaxation Interval, ms</td>
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<tr>
<td>All</td>
<td>0.86 (0.73 - 1.00)</td>
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<tr>
<td>Old</td>
<td>0.81 (0.63 - 1.04)</td>
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<tr>
<td>UES nadir pressure, mmHg</td>
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<tr>
<td>All</td>
<td>1.07 (0.93 - 1.27)</td>
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<tr>
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<td>1.10 (0.93 - 1.30)</td>
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<tr>
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<tr>
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<td>UES-Resistance, mmHg/s</td>
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<tr>
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<tr>
<td>Old</td>
<td>0.95 (0.82 - 1.35)</td>
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</tbody>
</table>
Discussion

In this thesis, we attempted to explore how drugs used in anaesthesia, and remifentanil in particular, affect defence mechanisms against pulmonary aspiration in individuals. We also surveyed subjective swallowing difficulties induced by remifentanil.

Aspiration induced by remifentanil

We demonstrated in study II that a 1-hour infusion of remifentanil at a target-site concentration of 3 ng/ml resulted in aspiration in healthy volunteers. Aspiration of pharyngeal content mixed with radionuclide tracer was directly demonstrated in scintigraphic lung images, in contrast to other investigations that have used impaired pharyngeal function induced by agents during anaesthesia as an indirect indicator of increased aspiration risk (57-60). No differences in grip strength, which was recorded as a measure of general muscle strength, were observed between remifentanil and placebo; however, the study did not determine the mechanisms underlying aspiration induced by remifentanil in this experimental setting. Several levels of defence against pulmonary aspiration can be impaired. The volunteers might aspirate the tracer detected in lung scans directly after its entrance into the pharynx from the nasopharynx (pharyngeal-to-pulmonary aspiration), or they might first swallow and thereafter regurgitate the tracer before aspiration (gastric-to-pulmonary aspiration). The relevance of this framing of the question is that gastric-to-pulmonary aspiration has been implicated in the development of aspiration pneumonitis, pneumonia, and acute respiratory distress syndrome (ARDS) (76), while the aspiration of oropharyngeal secretions may contribute to the development of pneumonia, but not necessarily pneumonitis or ARDS (77). The direct entrance to the airway is protected by several laryngeal and pharyngeal reflexes, with or without coincident swallowing (24), and disruption of these complex motorsensory neural circuits is one possible cause. Furthermore, the competence of the esophageal sphincters and well-functioning esophageal motility prevent the retrograde regurgitation of already swallowed material, and these mechanisms may also be affected by remifentanil. The remaining studies aimed to investigate the impact of remifentanil on these airway defense mechanisms.
Effects of remifentanil on the esophagogastric junction

In study I, we used HRM to show that remifentanil affected the barrier function of the EGJ by decreasing augmentation of the inspiratory EGJ. Pandolfino et al. reported that the inspiratory augmentation of EGJ pressure is significantly independently associated with gastro-esophageal reflux disease (12). When they analysed inspiratory EGJ augmentation as a predictor of objective reflux disease, they determined an optimal cut-off value of 10 mmHg, with lower values being indicative for reflux. In study I, the inspiratory augmentation of EGJ pressure decreased to 6.0 mmHg with remifentanil, suggesting a risk for reflux when remifentanil is administered.

The augmentation of the inspiratory EGJ is thought to reflect the respiratory function of the CD, which is innervated by the phrenic nerve, and we detected no statistically significant effects of the peripheral opioid antagonist methylnaltrexone on remifentanil-induced changes. However, because of the limited sample size, no conclusion can be made regarding whether remifentanil’s effects on EGJ augmentation and swallowing are centrally or peripherally mediated.

Effects of remifentanil on esophageal motility

In study III, we demonstrated that remifentanil influenced distal esophageal peristalsis by shortening the latency time of distal esophageal contraction, decreasing the swallow-evoked relaxation of the EGJ, and increasing the pressure in the fluid bolus preceding the esophageal contraction. Furthermore, there was a clear negative dose-dependent association between remifentanil and distal latency time. After a swallow, a wave of hyperpolarisation of the esophageal smooth muscle precedes the arrival of the peristaltic contraction that relaxes and prepares the esophageal body to receive the oncoming bolus and open the EGJ (28). This relaxation starts simultaneously throughout the entire distal esophageal body, but lasts progressively longer in progressively more distal segments of the esophagus. Normal esophageal peristalsis is produced by an aborally increasing latency between swallowing and contraction, also called deglutitive inhibition of the esophageal body (28). Shortening of the distal latency time and decreased swallow-evoked EGJ relaxation are both expressions of dysfunctional deglutitive inhibition (28, 78), and the results of study III indicate that remifentanil impairs of deglutitive inhibition of the smooth muscle of the distal esophageal body and the EGJ. Morphine has been...
suggested to have similar effects, although with older manometric methodology; this implies that both older and newer synthetic opioids have a similar influence on esophageal motility. Furthermore, in patients, failing deglutitive inhibition is often presented as diffuse esophageal spasm or spastic achalasia (79), and it is suggested that esophageal motility abnormalities in patients on chronic opiate medication mimic these clinical conditions (51). The effects of remifentanil on esophageal motility could be either centrally or peripherally mediated, given that both central inhibitory vagal pathways and regional inhibitory myenteric nerves are involved in esophageal deglutitive inhibition.

Naloxone, a competitive opioid receptor antagonist, did not counteract the effects of remifentanil; however, because of the limited sample size, it is unclear whether the effects of remifentanil on esophageal motility are mediated through opioid receptors or whether other receptors are also involved. The dopamine antagonist metoclopramide did not attenuate the effects of remifentanil. On the contrary, there was a tendency toward amplification of these effects which suggests that metoclopramide cannot be used to prevent remifentanil-induced esophageal dysmotility.

Effects of remifentanil on pharyngeal swallowing

In study IV, we evaluated the effects of remifentanil on pharyngeal swallowing using HRIM combined with AIM analysis to derive pressure flow and UES relaxation metrics. Remifentanil in particular influenced most pressure flow variables towards the direction that suggests greater swallow dysfunction: vigor of the pharyngeal contraction was weakened, pharyngeal bolus propulsion was diminished, and UES flow resistance increased. In this study, the effects of remifentanil on pharyngeal swallowing were compared with the effects of morphine. Even morphine resulted in weaker pharyngeal contraction, and SRI and UES resistance were increased; however, the remifentanil’s effects were greater overall. Furthermore, there was a significant difference between drugs regarding one variable in particular. TZn-PeakP was shortened with remifentanil, while no differences were observed with morphine. TZn-PeakP measures the time from bolus passage into the pharynx from the oral cavity to pharyngeal contraction and is thought to reflect the strength of lingual bolus propulsion and/or whether the pharyngeal response (also called the pharyngeal swallow trigger) is coordinated and well timed with respect to the bolus transfer. Many dysphagia patients demonstrate weak lingual bolus propulsion, poor oral bolus containment, and/or delayed pharyngeal trigger, and TZn-
PeakP is shorter in relation to these particular modes of swallow defect (80). As a corollary to these objective findings, we found in study I that subjects who reported swallowing problems following remifentanil exposure reported the inability to initiate a swallow. Hence, we conclude that remifentanil may have a greater effect on lingual bolus propulsion and/or pharyngeal swallow trigger than morphine.

Oropharyngeal swallowing is a complex, stereotyped sequence of the inhibition and activation of several pairs of muscles driven by several motor neuron pools located in various cranial motor nuclei in the brainstem and the upper part of the cervical spinal cord (26), which in turn are triggered by both central and peripheral inputs. Consequently, several sites of action for opioids, both peripheral and central, or muscle tone/rigidity, are possible.

Both young and old volunteers were enrolled in study IV. Generally, the older volunteers exhibited differences in pressure flow variables that suggest greater swallow dysfunction compared with young volunteers. These findings are consistent with a previous report evaluating older adults with AIM analysis (81). In addition, when stratified by age, pressures during UES relaxation (UES nadir pressure and UES intrabolus pressure) exhibited a heterogeneous treatment effect, and UES resistance showed a similar trend: these variables increased in young volunteers exposed to remifentanil in particular, while the old volunteers started from almost twofold higher baseline values and were less influenced by the treatments. One explanation may be that old volunteers already exhibit values near the upper limit for a specific variable with no capacity for additional increase. Another explanation may be that the lower drug dosages administered to older subjects were insufficient to induce an effect in these variables. Other variables in study IV did not exhibit differing effects caused by drugs when volunteers were stratified by age, although this might be a power issue: although twelve old volunteers were planned to participate in accordance with power analysis, we managed to include only seven.

**Subjective swallowing difficulties**

In study I, we showed that remifentanil induced subjective swallowing difficulties. Seven of ten volunteers reported more difficulty swallowing during remifentanil infusion than either before infusion or 15 min after ending remifentanil infusion. Most volunteers went from experiencing no difficulty to severe swallowing difficulty immediately after the remifentanil target concentration was achieved. A significant increase in subjective
swallowing difficulties during exposure to remifentanil was also observed in study II, although we observed no association between pulmonary aspiration detected with lung scans and swallowing difficulties. However, these findings could not be reproduced in studies III and IV. The manometric results in these studies suggest that remifentanil affects pharyngeal and esophageal swallowing towards the direction of greater dysfunction while the mean values of swallow metrics remained within the sub-clinical normal range, and therefore did not lead to symptoms in the tested subjects. The volunteers performed dry swallows in studies I and II and swallowed liquid boluses in studies III and IV (study II: 2 mL water; study III: 10 mL saline). Wet swallows are easier to perform than dry swallows, and swallowing liquid is probably sufficient to overcome the sensation of swallowing difficulty induced by remifentanil. It might be claimed that the volunteers experienced swallowing difficulty when performing dry swallows because of a lack of saliva in studies I and II; however, when the remifentanil infusion was stopped, the subjective swallowing difficulties were reversed.

**Limitations**

The blood concentration of remifentanil was not measured in these studies. This data would have strengthened the studies the actual blood concentrations reached with TCI methodology vary considerably among individuals. However, most volunteers were relatively young and of normal weight, which minimizes this variability.

The level of sedation was not monitored in these studies, and whether eventual sedation induced by remifentanil contributed to the increased incidence of aspiration and to the effects on pharyngeal swallowing and esophageal motility cannot be addressed. A previous study using the modified observer’s assessment of the alertness-sedation scale showed that remifentanil infusion with a target concentration of 3 ng/ml, which was used in all of the four studies presented here, does not induce deep sedation (82). Furthermore, remifentanil alone in concentrations in the clinical range is insufficient to induce loss of consciousness (83, 84). All volunteers responded promptly to instructions during the experiments (e.g., when liquid boluses were administered for swallow assessment), indicating that volunteers were not deeply sedated.

In study IV, dosages of remifentanil and morphine aimed to represent clinically relevant dosages. Owing to different pharmacokinetic profiles, no actual equipotency was searched.
No power analysis was performed in studies I and III. Study I was the first evaluation of whether methylnaltrexone was able to reverse remifentanil-induced effects on EGJ and swallowing. Twelve volunteers were recruited based on the results of a previous study that investigated methylnaltrexone’s influence on the effects of morphine on gastric emptying, in which 11 volunteers were enough to ensure statistical significance (85). In study III, we recruited 15 volunteers based on the results of two previous studies that investigated the effects of morphine on esophageal motor response, in which eight and ten volunteers, respectively, were sufficient to ensure statistical significance (49, 86). Furthermore, in study IV, in accordance with the power analysis, we planned to recruit 12 young volunteers and 12 old volunteers. However, only seven old volunteers were included owing to poor interest, and the small number of old individuals weakened the study’s statistical power to demonstrate differences between age groups.

A manometric catheter can influence the experience of eventual swallowing difficulties. However, exposure to remifentanil was always compared with baseline, after the catheter had been placed and before remifentanil had been administered. Furthermore, volunteers were instructed to ignore the sensation of the catheter in the pharynx.

**Clinical implications**

In this thesis, we have explored deficiencies in protection against pulmonary aspiration owing to drugs used in anaesthesia and remifentanil in particular. These findings are relevant to perioperative anaesthesia in clinical practice, particularly in the context of monitored anaesthesia care and during intensive care.

Remifentanil has gained wide acceptance in clinical settings and is used not only as an adjuvant in general anesthesia, but also in monitored anesthesia care as a single infusion or in combination with hypnotics. Remifentanil is particularly suitable for use in conscious sedation during painful but minor surgical procedures because of its rapid onset (1 – 2 min) and short duration of action (5 – 7 min) (63). Moreover, remifentanil has recently become an alternative in labor analgesia when neuraxial analgesia is contraindicated (61, 62). Furthermore, remifentanil use is not unusual in the intensive care unit, and pharyngeal dysphagia (87, 88) and silent aspirations (89) are common after prolonged endotracheal intubation. Our studies indicate that remifentanil impairs several levels of airway protec-
tion. And although effects on pharyngeal and esophageal swallowing remained within the sub-clinical range, we believe that a compound effect induced by remifentanil alters airway protection towards greater dysfunction. These findings may improve clinical practice by encouraging that the drug is used with caution. In circumstances in which the patient is spontaneously breathing, swallowing keeps the pharynx free of material that might otherwise threaten the airway. Therefore, swallowing dysfunction induced by remifentanil is an essential problem to take into account. Additionally, we found that even morphine influences pharyngeal swallowing, although not to the same extent as remifentanil, and based on our results we think morphine should be chosen as analgesia in susceptible patients. We even confirmed the previous findings (31, 32, 81) that the functional swallowing reserve declines in healthy older individuals, and drugs that further deteriorate swallowing function should be used with great attention in the elderly. Furthermore, with regard to our findings, we believe the fasting guidelines for women in labor receiving remifentanil should be reviewed.

**Future perspectives**

This thesis presents insights into the effects of remifentanil on pharyngeal and esophageal defence mechanisms against pulmonary aspiration. Remifentanil-induced aspiration was directly demonstrated using a scintigraphic method. However, there are still many questions to be answered. For example, it would be of clinical value to visualise the precise route of the aspirated material in order to differentiate between pharyngeal-to-pulmonary and gastric-to-pulmonary aspiration; it should be feasible, although logistically and economically difficult, to implement the dynamic collection of a series of lung scans by having the study subject in the gamma camera during the entire study session.

How different drugs used for sedation in monitored anaesthesia care influence defences against pulmonary aspiration has not been completely explored. More volunteer studies to evaluate the effects of for example, propofol and dexmedetomidine are needed. Perioperative studies to assess pharyngeal swallowing with focus on the immediate postoperative phase would also be interesting; this period should be vulnerable considering postoperative pulmonary complications such as pneumonia, as the patient is spontaneously breathing, is exposed to residual effects of general anaesthesia, and the requirements of analgesia are usually high.
Outcome studies to determine to what extent pulmonary complications emerge after monitored anaesthesia care in high-risk patients are also needed. Another interesting area is that regarding patients in the intensive care unit with tracheostomy or after prolonged endotracheal intubation. These patients are often encouraged to undertake oral feeding soon after extubation in order to fulfil the nutritional and psychological needs, and studies that assess swallowing function and the impact of different sedative drugs on swallowing in these patients would be valuable.
Conclusions

Based on this thesis it is concluded that:

- Remifentanil may increase risk for gastro-esophageal reflux by decreasing the inspiratory EGJ augmentation, and thereby interfering with the function of the crural diaaphragm.

- Remifentanil induces dysfunction of esophageal motility by impairing the deglutitive inhibition of the distal esophageal body. This may also contribute to elevated risk of regurgitation.

- Remifentanil infusion at concentrations used in monitored anesthesia care increases the incidence of aspiration.

- Remifentanil in particular influences pharyngeal swallowing towards greater dysfunction, as does morphine in relevant doses.

- Remifentanil affects several levels of defence against pulmonary aspiration. The risk of gastric-pulmonary aspiration is increased by impaired protection against gastro-esophageal reflux and retrograde regurgitation of gastric contents. Pharyngeal-to-pulmonary aspiration can occur owing to dysfunction of pharyngeal swallowing.

- Remifentanil induces subjective swallowing difficulties when subjects perform dry swallows, but not when liquid is swallowed.
Summary in Swedish


I mina studier har vi dels använt manometriska mätningar för att undersöka effekten av remifentanil på esofagussfinktrarna, samt på esophagalea och pharyngeala sväljningsfasen med fokus på skydd mot aspiration. Vi har även testat förekomsten av pulmonell aspiration i samband med remifentanil administration med en röntgenologisk metod. Eftersom försökspersoner i våra tidigare studier spontant rapporterade sväljningssvårigheter vid remifentanil infusion har vi även studerat huruvida remifentanil inducerar sväljningssvårigheter.

I första arbetet visades att remifentanil försämrar barriärfunktionen av den nedre esofagussfinktern genom att sänka tryckaugmentationen i denna högtryckszon under inspiration. I det andra arbetet påvisades förekomst av pulmonell aspiration hos friska frivilliga försökspersoner efter 60 minuters remifentanil infusion. I tredje och fjärde studien studerades effekten av remifentanil på den esophageala respektive pharyngeala sväljningen och man såg att båda sväljningsfaserna påverkades negativt. Remifentanil inducerade subjektiva sväljningssvårigheter i de första två arbetena då försökspersoner utförde torra sväljningar, dessa fynd kunde dock inte upprepas i de sista två arbetena, då försökspersoner svalde vätska.

Sammanfattningsvis, remifentanil försämrar skyddet mot pulmonell aspiration genom en påverkan på flertal fysiologiska försvarsmechanismer av luftvägen i såväl farynx som esophagus. Detta, tillsammans med det faktum att vi kunde påvisa förekomst av aspiration i samband med remifentanil infusion bör leda till att större försiktighet iakttas vid remifentanil infusion på vakna patienter.
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