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*EDITORIAL*

# From gastric aspiration to airway inflammation

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**ABSTRACT:** ***From gastric aspiration to airway inflammation. I.A. Brownlee, A. Aseeri, C. Ward, J.P. Pearson.***

**The airways are poorly protected from potentially damaging agents contained within gastric contents. While digestive factors are obvious damaging agents, gastric aspiration may also deliver microbial agents, cytokines or food antigens to airway tissues. Direct damage or the triggering of the inflammatory cascade by gastric aspiration is believed to drive airways disease onset and/or progression.**

**Evidence exists from experimental models demonstrating direct instillation of damaging factors to a range of airways epithelia causes damage and/or an inflammatory response. Clinical longitudinal studies have also noted an association between the presence of biomarkers of reflux in airways samples and disease progression. A shared pathophysiology of many chronic airways diseases is a more negative intrathoracic pressure. Such changes would drive an increased abdominothoracic pressure gradient. These changes in respiratory mechanics mean that chronic lung disease patients may be predisposed to reflux and subsequent aspiration. Therefore, it appears that gastric aspiration and airways disease progression may be linked not solely as cause and effect, but seemingly within a vicious cycle.**

**A range of physiological factors govern both occurrence of gastric reflux into the pharynx/larynx and could also increase the susceptibility of certain individuals to disease progression. A range of long-term surgical and pharmacological intervention studies are necessary to test the benefit of such therapies in reducing disease progression or driving symptom improvement. Such studies may be hampered by the reliability of available therapies in halting gastric aspiration and the difficulty in the clinical or biochemical assessment of gastric aspiration.**

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## Introduction

The term aspiration refers either to the inhalation of food from the oropharynx or gastric contents that have moved up the oesophagus in retrograde fashion [1]. An association between aspiration and inflammatory airways disease was first suggested during the 1960s and 1970s (e.g. [2-5]). Aspiration by either route will lead to the presence of increased amounts of potentially damaging agents into the airways, which is hypothesised to drive the onset and/or progression of a wide spectrum of pathological conditions that affect the lungs and airways [6, 7]. During gastric aspiration, potentially damaging digestive factors from the stomach (via the oesophagus) pass into the relatively unprotected airways. Gastric aspiration is therefore hypothesised as being a prime causative candidate for almost every chronic upper airways disease. This hypothesis has proved difficult to test, due to both the difficulties of assessing the presence of reflux clinically or diagnostically [8-10], alongside the fact that pharmacological and surgical interventions for reflux can only reduce, but not abolish, the occurrence of gastric content reaching the oesophagus/airways.

The symptomology of airways disease is as divergent as the potential aetiologies. One common feature is the presence of an inflammatory response within the affected mucosal surfaces [11-15]. The process of inflammation involves a complex cascade of cellular, molecular and systemic events that are aimed at benefitting the clearance of noxious agents from the mucosal surface. In most pathophysiological cases, the inflammatory response appears to be in excess of the normal state, and is believed to play a role in disease progression. The inflammatory response is not necessarily in proportion to the damaging potential of the initiating agent, and can drive further damage to the surrounding tissues [16].

Previous studies would suggest a high incidence of gastric aspiration in chronic diseases of the lungs and upper airways [17-21]. In some cases, associations have been made between measured occurrence of biomarkers of reflux and disease progression [22] or proxy measures of the inflammatory response [23, 24]. A number of observational studies have also noted an increased co-morbidity between oesophagitis and various airways diseases [25-27]. Perhaps the

most commonly held beliefs are a) aspiration (particularly the reflux of gastric contents) is a potential initial drive for disease progression and b) symptomatic individuals may have more frequent/injurious aspiration events than non-symptomatic individuals. While these hypotheses appear sound, they both neglect key issues within the association of aspiration and airway inflammation, and their impact on disease progression. The remainder of this review will discuss the interplay between gastric aspiration and airways inflammation in greater detail.

## Aspirate content and lung inflammation

While direct aspiration of ingested food can present antigens or bacterial load into the airways, gastric aspiration has further potential modes of antagonism of the airways' mucosa. Firstly, the presence of digestive factors, including enzymes, bile acids and other detergents (e.g. lecitihins) and gastric acid have all previously been shown to have the potential to damage airways mucosa directly [28-31]. The main digestive protagonists from gastric juice that have previously been suggested to drive mucosal damage are gastric acid, pepsin and bile acids.

Gastric acid and pepsin are secreted by the parietal cells and chief cells respectively in the stomach. These gastric secretions are important both in the early stages of protein digestion, but may also act as an important innate barrier to microbes entering the body orally [32-35]. Bile acids are produced by the liver conjugated to glycine or taurine and secreted into the small intestine where they act to emulsify dietary lipids to aid fat digestion and absorption [36, 37]. Small intestinal contents are believed to frequently reflux from the duodenum into the stomach, with well over 50% of gastro-oesophageal reflux disease (GORD) patients reported to experience the movement of mixed gastric and duodenal contents up the oesophagus [38]. A range of small intestinal enzymes could also end up in the stomach by this route. If they are not degraded or denatured during the retrograde passage from duodenum to stomach to the aerodigestive tract. For example trypsin is unaltered by exposure to pepsin at pH 4.0, but inactivated by exposure to pepsin at pH 2.0, they also have the potential to cause mucosal damage.

Aspiration of digestive products may also lead to an indirect drive for mucosal inflammation. Firstly, homogenised and partially hydrolysed foods may act as a more amenable substrate to bacterial species already occurring within the airways. Secondly, the hydrolysis and denaturation of dietary proteins during normal digestion could lead to the appearance of previously sequestered antigen. The potential mechanisms for damage by gastric aspirate are summarised in table 1.

Pharmacological therapies for acid suppression have been shown to greatly reduce oesophageal exposure to low pH [39, 40]. Such therapies will act to reduce the total volume of the gastric juice [41-43], but may act to effectively increase the concentration of pepsin, bile acids and other putatively damaging digestive factors due to the lower volume of gastric secretion. Further from this, recent studies have demonstrated an increased incidence of intestinal infection and communicable diseases following acid suppression [44-48]. This is due to an overgrowth of oral-type bacteria within the stomach [49] as a result of the decreased innate immunity with the removal of the acid barrier. Subsequent aspiration events under such therapies may therefore be of lower total volume and higher pH, but may have a higher concentration of other putatively damaging endogenous and microbial factors.

Direct damage of the airways mucosa by gastric aspiration is the most obvious trigger for an inflammatory response. However, a number of key routes for gastric aspiration-driven inflammation are also hypothesised. The majority of the airways epithelium is lined by a functional mucus barrier that acts to reduce mucosal exposure of damaging inhaled agents, as well as entrapping such agents and facilitating their removal through the process of mucociliary clearance [50, 51]. As such, efficient mucus barrier function plays a vital role in the innate defence of the airways [50]. Many airways diseases are characterised by mucus hypersecretion [52, 53]. This can be a result of increased fluid output, or increased release of mucin granules by the epithelial goblet cells and/or increased gland-based secretion (driven by secretagogues, such as IL-8). If the mucus layer becomes too rheologically thick or thin, mucociliary clearance is greatly reduced, leaving the underlying tissues more susceptible to damage and infection [50].

Inflammatory responses to damaging agents may also be partly driven by the presence of specific receptors within the epithelium. In the case of factors like bacterial lipopolysaccharides, such receptor-mediated pathways are fairly well researched [54, 55] and appear to be mediated by toll-like receptor activation and a subsequent inflammatory cascade The triggering of nociceptors such as the capsaicin receptor in the airways by low pH is also well documented [56, 57]. Evidence would also suggest that recurrent cough could also cause airways damage/act to mediate local inflammatory pathways. In a group of patients with chronic non-productive cough, a subset of individuals who did not have asthma or acidic reflux ("idiopathic" non-productive coughers) had elevated levels of mast cells within their bronchoalveolar lavage fluid in comparison to non-smoking, healthy controls [58]. Recent preliminary data have also suggested that there may be specific receptors that are triggered by the presence of gastric juice factors, such as pepsin and bile [59, 60].

A summary of these pathways is suggested in figure 1 below.

Recent interest in the field has noted that retrograde movement of gas boluses from the stomach may also be an important route of gastric aspi-

ration [61, 62]. While this would not deliver the same volume of gastric contents to the airways, it is believed that aerosolised vapour could act to coat airways mucosa. The aerosolised content would be expected to contain similar damaging agents to those outlined in table 1.

Table 1. - Potential mediators of airways inflammation and damage within gastric contents

| **Directly damaging agents** | **Agents released by digestion** |
|---|---|
| Digestive factors (i.e. enzymes, bile, acid) | Digested macronutrients |
| Ingested, salivary or gastric microbes | Antigen formed by dietary protein hydrolysis |
| Food particles | Microbe release from gastric bolus |
| Food antigen | |

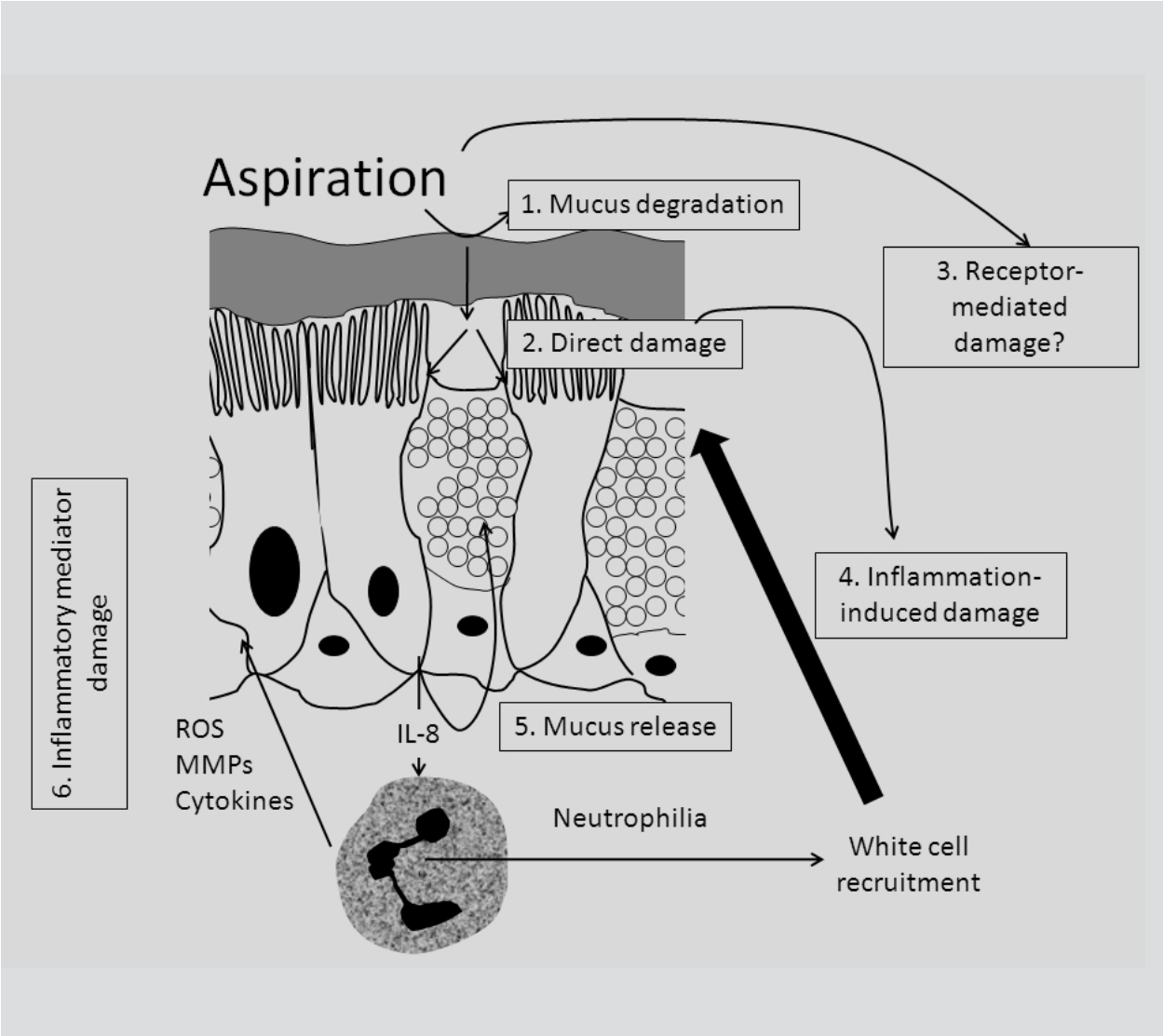


Fig. 1. - A summary of the hypothetical routes through which gastric aspiration could drive mucosal inflammatory responses within the airways. 1. Mucus degradation will potentially expose the underlying mucosa leading either to 2. Direct mucosal injury/insult from gastric aspirate, or indirect mucosal injury/insult from other damaging agents inhaled into the airways. As a result of putative receptor-mediated response, or a consequence of mucosal damage, an increased drive for white cell recruitment occurs, resulting in further 4. Inflammation-induced damage. Further from this, the airways epithelial cells respond to damage by releasing a range of local inflammatory mediators, including IL-8. These mediators drive neutrophil recruitment, as well as 5. Mucus release from goblet cells, and 6. Inflammatory-mediator damage driven by factors released by the recruited neutrophils. Mucus hypersecretion can result in loss of mucociliary clearance, leading to further exposure of the mucosa to damaging agents. Reactive oxygen species (ROS) and matrix metalloproteinase (MMP) release will lead to further mucosal and epithelial damage from within.

## Reflux pathophysiology

Reflux of gastric contents is an episodic event in both the physiological and pathophysiological state [63]. 24-h ambulatory impedance monitoring of 72 healthy adults [64] demonstrated that the median number of gastro-oesophageal reflux events to occur in a day was 44, with a great deal of inter-individual variation (the 25th and 75th percentiles were 25 and 58 events respectively). These episodes are characterised by increased gastric motility, a transient reduction in lower oesophageal sphincter tone and increased intragastric pressure. Recent literature has focussed on a central role for transient lower oesophageal sphincter relaxations (tLOSRs) [65-69]. While evidence would suggest that tLOSRs are no more frequent in GORD patients than asymptomatic volunteers, the likelihood that GORD patients will reflux during the period of sphincter relaxation is almost twice as high [69]. While significantly higher occurrences of reduced lower oesophageal pressure are noted in GORD patients complicated with severe hiatal hernia [70, 71], it must be noted that this particular patient group is not indicative of GORD patients *per se* [63]. Functionally, reflux from the stomach to the oesophagus has the potential to occur whenever the intragastric pressure exceeds lower oesophageal sphincter pressure. Manometry studies have noted a higher pressure gradient across the lower oesophageal sphincter in GORD patients than non-symptomatic controls, with the difference in gradient owing to a higher gastric pressure [72]. Recent assessments of gastrointestinal motility have also suggested that the position of the "acid pocket" (i.e. secreted gastric juice that sits above the meal bolus) in relation to the diaphragm [73] may drive reflux. Further functional studies have suggested that the occurrence of retrograde waves of peristalsis up the oesophagus appears to be propagated by the occurrence of tLOSRs [65], which could act to further increase aspiration events. A previous study in non-symptomatic volunteers noted that barostat distension of the stomach resulted in increased number of tLOSRs than distension to the same degree by an ingested meal [68]. This would suggest that the drive for gastric motility given by postprandial luminal content may reduce the frequencies of tLOSRs.

Intragastric pressure is controlled by a complex array of neurohumoral pathways that govern lower oesophageal and pyloric sphincter tone, gastric compliance, gastric secretion volume and gastric motility. Alongside vagal innervation, three main hormonal drives may govern all of these factors [74]. Cholecystokinin (CCK) release from the duodenal I cells is a major drive for reduced gastric emptying, while gastrin release from gastric G cells increases gastric mixing. Both of these factors could drive an increased intragastric pressure. At the same time, motilin release from intestinal enteroendocrine cells acts to increase the rates of gastric emptying, and thus would be expected to decrease intragastric pressure. Previous reviews have suggested the potential of these agents and their receptors as targets for reflux therapy [75, 76].

Reflux symptoms are prevalent in the majority of airways diseases. For instance, previous reports would suggest that around 80% of adult asthmatics have symptoms of reflux [77, 78]. It must be noted that the association between gastric aspiration and airways pathophysiology is often hypothesised to be a result of aspiration driving the disease process. However, the association between the two processes in not fully defined, and a number of researchers have suggested that the negative intrathoracic pressure caused by a result of airways obstruction and/or respiratory distress may act to drive reflux by increasing the likelihood of gastric contents refluxing into the oesophagus [78-80]. A preliminary temporal association study was carried out between episodes of coughing/wheezing in asthmatics and reflux occurrence, as assessed by pH-metry in 2001 [81]. Within this population group, reflux events preceded cough events by less than 2 minutes 40% of total cough events per patient, with only 6% of reflux events being preceded by cough events over the same time-course. These figures were elevated to 50% and 12% respectively when a five minutes inter-event time cut-off was applied. In more recent studies where impedance monitoring has been used to assess reflux in paediatric asthma [82], cystic fibrosis [83] and chronic cough [84] patients. In all cases within these studies, it must be noted that an appreciable number of cough events were associated with reflux events within two of these studies, 26.6% total of events within a 5-minute window [82] in paediatric asthmatics and 30.6% within a two-minute window in chronic cough patients [84]. Such temporal studies may not best represent the interplay between intrathoracic pressure and reflux/aspiration. In terms of intrathoracic pressure mediating reflux, cough represents a short-term negative intrathoracic pressure change, as opposed to a more uniform, long-term change towards lower pressure seen in disease processes where there is chronic lung obstruction. In terms of aspiration driving airways symptoms, cough is an immediate response, believed to be triggered by a range of cough receptors [85]. As there are previous reports of refluxers having reduced laryngeal sensitivity [86-89], it is perhaps unsurprising that the cough reflex is not always elicited in response to gastric aspiration. Over a short time scale, gastric aspirate may not be damaging enough to the airways mucosa to drive a relevant clearance response such as cough. As previously discussed, aspiration of aerosolised gastric contents may also lead to damaging material entering the airways, that would be considerably less likely to elicit an immediate cough reflex than a large volume reflux event. One previous study used a canine model to assess the impact of balloon-catheter-induced upper airways obstruction on reflux occurrence (assessed by pH-metry). Within this study, there was a strong and significant positive correlation ($R = 0.928$, $P =$

0.023) between the change towards a more negative intrathoracic pressure and percentage of time proximal pH was below 4 in five dogs [90]. While this study is low in numbers, it may model how long-term pressure changes affect gastric aspiration.

From the above, there is evidence that the development of an abdominothoracic pressure gradient may drive further reflux and gastric aspiration, with consequent worsening of disease symptoms. While there is no conclusive proof as to whether airways symptoms drive reflux or vice versa, it is perhaps more likely to consider that these events could conspire to worsen chronic disease progression through a vicious cycle [91]. Preliminary longitudinal data from our own group would suggest that reflux is a common occurrence post lung transplantation, even at timepoints when lung mechanics should be close to normal [92].

Our group has consistently shown that pepsin, as a marker of gastric aspiration, is elevated in lung transplant recipients [21, 22] and has been associated with neutrophilic airway inflammation and pathologist graded acute rejection [22]. Acute airway rejection is known to be associated with airway inflammation and to constitute a risk factor for chronic allograft dysfunction, recognised physiologically by fixed airflow limitation (Bronchiolitis Obliterans Syndrome: (BOS) [93]. The pathology underlying BOS involves inflammation and airway remodelling and fibrosis [93-95]. This pattern of airway damage, as in the case of reflux and aspiration, is implicated in the progression of a number of airways diseases including asthma and COPD [96]. In lung allografts, this can be very aggressive and is the main reason for the chronic loss of lung allografts [93]. In other more common lung diseases such as COPD the pattern of airway injury develops over a longer time frame.

One potential link between aspiration and airways remodelling is perturbation of TGFβ homeostasis. TGFβ is a pleiotropic growth factor (reviewed elsewhere [16, 97]) that is implicated in both airway repair and pathophysiology, and which is elevated in airway disease including BOS post transplantation [98].

We have shown that TGFβ can initiate epithelial mesenchymal transition (EMT) in epithelial cells from lung allografts [99]. In EMT, epithelial cells lose epithelial characteristics adopting a mesenchymal, fibroblast phenotype which may cause airway fibrosis through the production of collagen. The process of EMT has been documented in lung development, metastatic disease and a range of other settings involving organ fibrosis [100]. Consistently TGFβ is recognised to be a prototypical drive for EMT and it is therefore of interest that bile acid challenge of human air-

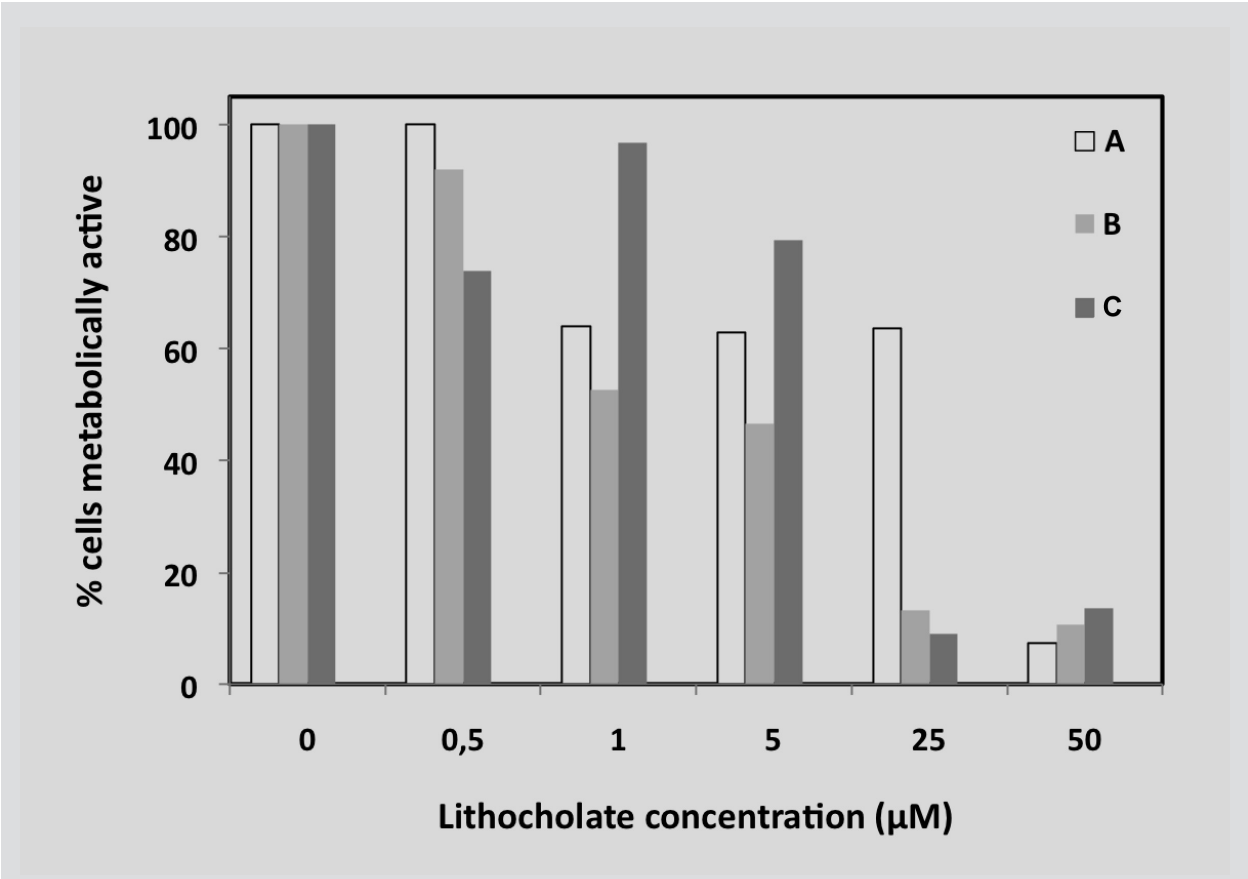


Fig. 2. - The effect of varying levels of lithocholic acid on cell viability in primary lung epithelial cultures from 4 separate lung transplant recipients (here labelled A - C), 6 - 15 months post-transplantation. Cell viability (i.e. % cells metabolically active) was assessed by CellTiter-Blue cell viability assay (Promega. Madison, WI) [115, 116].

way epithelial cells has been shown to lead to the release both of inflammatory cytokines and TGFβ [101]. Most TGFβ is held in an in-active latent form. This may be activated through protease release from inflammatory cells, as well as directly by stomach acid. Type IV collagenases such as MMP-9 are also released in the inflammation with which aspiration is associated. Damage of airway epithelial basement membranes, which contain type IV collagen, is also known to promote EMT [100].

In addition to *ex vivo* studies on primary airway epithelial cells suggesting the potential for EMT in lung allografts, we have also shown that airway biopsies from stable lung transplant recipients express markers of the EMT proteome [99, 102]. Overall aspiration may be linked both to airways inflammation and remodelling/fibrosis. This potential linkage requires further appropriate translational research [103].

## Increased susceptibility to aspiration-induced airways damage

Previous studies in non-symptomatic individuals would suggest that reflux into the oesophagus and even the pharynx is a frequent occurrence within normal life [104-107]. It might therefore be expected that gastric aspiration is also an occurrence that most individuals will have experienced. Assessment of bronchoalveolar lavage fluid in individuals asymptomatic of upper airways disease suggests that biomarkers of gastric aspiration can occur at low but detectable levels in 2 of 4 healthy individuals [22]. Some background levels of pepsin may be present in the broncho-alveolar lavage fluid of healthy individuals, as pepsinogen C (a precursor of human pepsin 5 and 6) has previously been reported to occur in type 2 alveolar cells [108]. While this data would suggest that gastric aspirate does not occur as frequently or to the same degree in a healthy population, it is important to consider that there may be other factors that predispose some individuals to airways injury as a result of gastric aspiration, either as a result of increased volume of aspirate reaching the airways, or due to a lack of defence mechanisms against aspirate-induced inflammation or injury. Data from the authors' laboratories would suggest that primary cell cultures of lung epithelia from different individuals react differently to insults from putatively damaging agents in gastric aspirate, such as the secondary bile acid lithocholic acid. While all cultures appear to be close to completely unviable at the highest concentration of lithocholate used (i.e. 50 μM), the drop-off of viable cells numbers follows a different gradient between individuals.

The data above are suggestive of genetic variability in the inflammatory response caused by such mediators of damage. Previous studies have also suggested that certain patients groups who are more or less susceptible to reflux-mediated disease tend to show specific genotype [17], or differential expression of a specific genes involved in airway protection [28, 109, 110]. While such studies would give credence to genetic predisposition as an important factor in aspiration-driven disease progression, it is important to consider that "susceptibility" may also come about as a result of temporal, anatomical or physiological changes in airways functionality.

In general, increased airways damage or inflammation as a result of aspiration is likely to be as a result of decreased defence against aspiration, or reduced clearance rates of damaging agents that are delivered to the mucosa. As described above, previous studies have suggested an elevated threshold for laryngeal sensitivity in refluxers [86-89], which is higher than in asymptomatic individuals [111]. A less sensitive larynx would not perform its respiratory defence mechanisms [112, 113] as well as normal upon exposure to reflux. As a result, if the larynx were insensitive to contact with gastric contents, gastric aspiration would be more likely to occur. Factors such as vagal damage (e.g. following lung transplant surgery [114]) may also drive an increased incidence of gastric aspiration by affecting aerodigestive tract motility, and may also reduce cough and mucociliary clearance. Previous events resulting in damage or an inflammatory response in the airways could lead to an increased likelihood of subsequent damage as a result of gastric aspiration insult. This could be as a result of a loss of the innate barrier function of airways mucus, or because the underlying mucosa has become easier to penetrate. In a similar fashion, loss of effective mucociliary clearance will result in increased mucosal exposure to the damaging agents in gastric aspirate.

## Future work

Current evidence is indicative of an association between airways disease progression, inflammatory processes and gastric aspiration. The next step from a clinical perspective may be the development and implementation of pharmacological or surgical interventions in the relevant patient groups, targeted at reducing gastric aspiration rates with objective measures of airways disease as the primary outcomes in adequately powered, controlled studies. In conditions where improvement of disease symptoms is unlikely (e.g. idiopathic pulmonary fibrosis), a reduction in disease progression rates may be the most relevant outcomes to measure. Such studies will be demanding and necessitate the collaboration of both gastroenterology and pulmonary specialists, and may be hampered by the difficulties in assessing occurrence of gastric aspiration/reflux clinically. Biochemical analysis of biomarkers of gastric aspiration may be a useful predictive surrogate of gastric aspiration, but further work is necessary to characterise how long such biomarkers occur within the airways following an aspiration event. Methodological standardisation is an important requirement.

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