Dissection of a Mechanistic Controversy in Cystic Fibrosis

Candace R. Marquette and Douglas B. Luckie*
Department of Physiology, Michigan State University, USA

Abstract

Cystic fibrosis is an autosomal recessive disease caused by mutation of the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. Complications due to chronic lung infections are the leading cause of mortality seen in cystic fibrosis (CF) patients. Two opposing hypotheses for the mechanism of bacterial colonization of cystic fibrosis lungs have co-existed for nearly two decades. The “high salt” hypothesis posits that the bacterial colonization of CF lungs is due to an elevated concentration of sodium and chloride in airway surface liquid (ASL) which inhibits native bactericidal activity. The “low volume” hypothesis, on the other hand, proposes that the volume of the ASL is severely decreased as a result of abnormally increased sodium absorption, which causes cilia to collapse and halt mucus clearance. An analysis of published data for sodium and chloride concentrations measured in the liquid lining of lungs, as well as ASL depth, was conducted. The original published data sets obtained and evaluated in this systematic review provided weak support for either a significant difference in salt concentrations (sodium: \( p = 0.316 \), chloride: \( p = 0.30 \)) or depth (\( p = 0.16 \)). This study found both hypotheses has yet established itself to be more viable and an alternative mechanism for initial bacterial colonization of the lungs in cystic fibrosis is proposed.

INTRODUCTION

Cystic fibrosis (CF) is the most prevalent genetic disease among Caucasians, occurring once in every 3,400 births [1]. Inheritance occurs in an autosomal-recessive pattern and approximately 2-5% of the Caucasian populations are carriers of the disease gene. In total, approximately 30,000 people are currently afflicted with cystic fibrosis in the United States [2]. The underlying etiology of cystic fibrosis can be caused by over 1,500 different mutations in a 1,480 amino acid membrane-bound glycoprotein of the ATP binding cassette super family. A deletion of three base pairs encoding phenylalanine in the 508th amino acid position is the most common mutation within approximately 70% of cystic fibrosis chromosomes [3,4]. Many cystic fibrosis transmembrane conductance regulator (CFTR) protein mutations, particularly the ΔF508 mutation, lead to a misfolding of the quaternary protein structure.

The cystic fibrosis transmembrane conductance regulator (CFTR) protein is present in the membrane of nasal, tracheal, and bronchial epithelial cells. The CFTR protein is bound to a small portion of the C-terminus of CFTR through beta sheet isoform-1 (NHERF1) as shown in Figure (1), is thought to bind to a small portion of the C-terminus of CFTR through beta sheet augmentation [9,10]. The regulatory domain (R domain) of CFTR contains several serine residues that receive protein kinase A (PKA) and are phosphorylated resulting in channel activation [11,12]. As a member of the ATP-binding cassette super family, CFTR has two nucleotide-binding domains (NBD1 and NBD2) and two membrane-spanning domains (MSD1 and MSD2); [13] with six \( \alpha \)-helices each, as seen in Figure (1). The CFTR channel has been shown to transport chloride from the airway lumen to the interstitium through the opening of a pore formed by the membrane spanning domains [13,14]. The NBD1 and NBD2 located on the CFTR channel both bind ATP while perhaps only

Keywords

- ASL
- CFTR
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- Hypothesis
- Lung
- pH

Normal Physiology: Respiratory Systems and CFTR Function

The cystic fibrosis transmembrane conductance regulator (CFTR) protein is present in the membrane of nasal, tracheal, and bronchial epithelial cells. The CFTR protein is bound to the membrane by twelve hydrophobic alpha helical domains. Monomer channels form a multi-protein assembly, likely utilizing two PDZ domains consisting of 80-90 amino acids each [8, 9]. PDZ1, a domain on the Na+/H+ exchanger regulatory factor isoform-1 (NHERF1) as shown in Figure (1), is thought to bind to a small portion of the C-terminus of CFTR through beta sheet augmentation [9,10]. The regulatory domain (R domain) of CFTR contains several serine residues that receive protein kinase A (PKA) and are phosphorylated resulting in channel activation [11,12]. As a member of the ATP-binding cassette super family, CFTR has two nucleotide-binding domains (NBD1 and NBD2) and two membrane-spanning domains (MSD1 and MSD2); [13] with six \( \alpha \)-helices each, as seen in Figure (1). The CFTR channel has been shown to transport chloride from the airway lumen to the interstitium through the opening of a pore formed by the membrane spanning domains [13,14]. The NBD1 and NBD2 located on the CFTR channel both bind ATP while perhaps only
the second domain, NBD2, is hypothesized to hydrolyze ATP to generate energy for the activation of the channel [15].

Alongside the CFTR channel on the apical membrane of airway epithelial cells and the serous cells of the submucosal gland is an epithelial sodium channel (ENaC). Consisting of α, β, and γ subunits, ENaC is believed to be inhibited by the CFTR channel, thus slowing the movement of sodium into the interstitium [16-19]. The inhibition of ENaC is thought to be controlled by a complex relationship between the PDZ binding domains of the Na+/H+ exchanger regulatory factor isoform-1 (NHERF1), which bind both the C-termini of the CFTR and YES-associated protein-65 (YAP65) to PDZ1 and PDZ2, respectively [10]. The non-receptor tyrosine kinase, c-YES, interacts with YAP65. The c-YES kinase is a member of the c-src family, which is known as strong inhibitors of ENaC and may inhibit ENaC channel activity through said interaction with YAP65 and the PDZ domains [10].

The apical surface of the airways is protected by two layers; a periciliary liquid layer or as it will be called in this paper, the airway surface liquid (ASL), and a mucus layer of mostly mucin glycoproteins produced by mucosal cells. These two layers work as an innate immune system that is the first line of defense against bacterial pathogens [20-21]. The airway surface liquid is thought to be approximately 10 µm in depth [22]. Cilia of the airway epithelial cells are about 7 µm, and facilitate mucociliary clearance towards the mouth as a component of the innate immune system [23-24]. Due to the low viscosity of ASL, the mucus layer, floating on top, is pushed by the cilia at a rate of 3 mm/min for bacterial clearance [22].

Abnormal Physiology: Effects of Cystic Fibrosis

Cystic fibrosis disrupts multiple organ systems throughout the body. One of the most common symptoms of digestive disease in CF is pancreatic exocrine insufficiency [25]. A decrease in sodium bicarbonate secretion, which lowers pH, negatively affects the efficacy of pancreatic enzymes and precipitation of bile salts in the duodenum thereby preventing the proper breakdown and absorption of food [26-29]. Over the course of a lifetime, damage to the pancreas can decrease insulin secretion and result in CF-related diabetes [30].

CFTR plays a major role in fluid secretion. Hence, proper function of the sweat glands is also affected by CF. Children with cystic fibrosis has characteristically abnormal salty skin during perspiration [31]. The secretory coil of sweat glands consists of three types of cells; myoepithelial (ME), beta-adrenergic-insensitive (β-I), and beta-adrenergic-sensitive (β-S). However, only the β-S cells in the secretory tissue of the sweat glands are affected in CF. Skin chloride concentrations greater than 60 mEq/l are indicative of CF in children; due to the lack of reabsorption of chloride ions by sweat duct cells prior to secretion onto the skin. In sweating, primary fluid begins as an isotonic solution, and under normal physiologic conditions would be secreted as a hypotonic solution. In CF, the lack of chloride absorption results in a final fluid that remains isotonic [32-35].

Decreased mucus clearance allows for a build up within the airways of mucus with increased viscoelasticity. The mucus layer is the first line of defense against pathogens but abnormalities caused by a mutation of the CFTR protein results in chronic pulmonary infections and bronchiectasis. The main pathogen seen in these infections is bacteria [7,36]. At birth the lungs of CF patients are mostly normal and not infected. From 1 to 2 years old, until 10, children are commonly infected with Staphylococcus aureus and Haemophilus influenza. After 10 years of age, leading cause of mortality in cystic fibrosis patients [37]. Currently, the main pathogen is Pseudomonas aeruginosa [38-39].

The Cystic Fibrosis Controversy: High Salt or Low Volume Airway Surface Liquid

There are two conflicting hypotheses concerning the origin of

Figure 1 Basic components of cystic fibrosis transmembrane conductance regulator (CFTR) channel and hypothesized interactions with epithelial sodium channel (ENaC). Located on the apical side of airway epithelial cells, the CFTR channel passively transports chloride across the membrane. Shown above are the regulatory and nucleotide binding domains, along with the site of the ΔF508 mutation. The passive chloride channel, CFTR, consists of 2 membrane spanning domains, MSD1 and MSD2, along with 2 nucleotide-binding domains, NBD1 and NBD2. Amino and carboxyl groups at either end with a regulatory domain, the R domain, in the middle. The PDZ domains of the Na+/H+ exchanger regulatory factor isoform-1 (NHERF1) between CFTR and YES-associated protein-65 (YAP65) connecting to ENaC where c-YES, a non-receptor tyrosine kinase, may inhibit activity(adapted from Rowe et al. [13]).
the abnormal bacterial colonization seen in CF airways; the “high salt” hypothesis and the “low volume” hypothesis. In 1996, Smith et al. showed that ASL collected from primary lung epithelial cultures of non-CF patients exhibited bactericidal effects on *P. aeruginosa* and other strains of bacteria [40]. However, when introduced to ASL collected from cultures generated from CF patients, bacteria thrived. Smith et al. suggested that normal ASL may contain small defensin-like molecules of less than 10 kDa with broad-spectrum bactericidal activity, whose activity was absent in the ASL of CF patients. Ion concentrations were measured in the ASL of both non-CF and CF cell cultures, showing that CF ASL had an increased concentration of chloride (Cl-) ions, and that this most likely was inhibiting the ability of the salt-sensitive bactericidal molecule [40]. This study by the Welsh group from the University of Iowa, in conjunction with similar previous studies laid the foundation for the high salt hypothesis: namely, elevated Cl- concentrations, as a result of defective Cl- transport by CFTR, inhibits bactericidal activity and allows for bacterial colonization within the airways [41-42]. The high salt hypothesis is modeled in Figure (2).

However, in 1997, Knowles et al., argued, that it is unlikely that airway Cl- concentrations are higher than plasma levels, as this is not compatible with the normal physiologic processes involved [43]. In their study, ion composition of the airway surface liquid was compared between normal participants and CF patients in nasal passages and lower airways. No significant differences in Na+ or Cl- concentrations were found. In nasal passages the concentrations of Na+ observed were lower than that of plasma (~109 mM), whereas Cl- concentrations were comparable to plasma levels (~125 mM). Looking at ion composition in the lower airways, Na+ and Cl- were found to be lower than plasma levels and hypo-osmotic in comparison to the results of Smith et al. As a result, a low volume hypothesis, proposed by Boucher came to be an alternative to the results of Smith airways at Chapel Hill, and over time gained credence. The low volume hypothesis postulates that Cl- concentrations are similar to plasma levels in CF and non-CF airway surface liquid, but that increased Na+ absorption leads to an intracellular flux of Cl- (via a non-CFTR pathway) and a net movement of water to maintain isotonicity, thereby collapsing the cilia and halting mucus clearance modeled in Figure (2).

For nearly 20 years, these two groups have supported their respective hypotheses with experimental data with no clear resolutions concerning the mechanism underlying CF. Hence a critical examination of the data is needed to determine whether the high salt or low volume hypothesis is most likely to be correct.

![Figure 2](image.png)
Developing more effective treatments for patients with cystic fibrosis depends heavily on gaining a better understanding of the disease mechanism in affected organ systems. However, currently, the field of cystic fibrosis research is somewhat stalled in a debate over the fundamental mechanism of pulmonary disease, the leading cause of mortality in cystic fibrosis. On one hand, Dr. Michael Welsh’s research group at the University of Iowa has put forward the “high salt” hypothesis; where CF is linked to increases in Cl- (salt) in the ASL that inactivate antimicrobial defenses (Figure 2). In contrast, Dr. Richard Boucher’s group from the University of North Carolina supports the “low volume” hypothesis; where increased activity of Na channels (ENaC) removes water from the ASL, draining it of its volume (Figure 2). These two competing hypotheses invoke very different mechanisms leading to a milieu permissive for bacterial colonization of the lungs and the resulting respiratory complications associated with infection. To evaluate these opposing models, this meta-analysis of published data focused upon whether: (i) the salt concentration of ASL, and/or (ii) the height of ASL, differed between normal and CF conditions.

Variations in Cell Culture Limit Their Impact on Resolving the Debate

The use of cell culture in CF research has many benefits. Cell cultures are easily maintained and manipulated for analysis of isolated conditions impacting the disease. For example, the mechanism of regulation of ENaC by the CFTR channel has been studied in detail using cultured cells. And in 1998, Matsui et al from the Boucher group, and Zabner et al from Welsh’s group, both published Na+ and Cl- measurements in cultured human airway epithelial cells. Matsui et al., reported the ASL concentrations of 140mM Na+ and 130mM Cl- were unchanged by disease, yet Zabner et al observed altered values that increased from 50 to 100 mM Na+ and 37 and 90mM Cl- for normal and CF cells, respectively.

A closer look into each study’s methods reveals this discrepancy may be a result of variation in the cell culture techniques. For example, for a time the Welsh group may have been studying cells that were not ciliated or well differentiated. Those cells had a low amiloride sensitive short circuit current “Isc (amil)”, which is in flux across the epithelia without ENaC activity [44,45]. Later, cell cultures in the 1998 Zabner paper demonstrated a high Isc (amil) and found an increased liquid absorption in normal compared to CF epithelia with transepithelial electrical resistance (TER) as depicted in Figure (3). In addition, Zabner reported TER for all epithelial as $\geq 800 \ \Omega \times \text{cm}^2$ which is uncharacteristically high compared with other studies. For example, Calu–3 cells in culture traditionally have achieved a transepithelial resistance of approximately $400 \ \Omega \times \text{cm}^2$, and the resistance in Matsui et al was reported as $> 300 \ \Omega \times \text{cm}^2$ [46,47].

A difference in the transepithelial resistance across studies is important in this debate, as TER is indicative of the level of resistance to passive ion flow through tight junctions. A higher

![Figure 3](image-url)
Data from CF Murine Models May Undermine the Low Volume Position

Comparatively, the in vivo murine model has innumerable applications in research across the sciences. The murine model’s popularity stems from the ease with which transgenic mice strains can be generated through crossbreeding well-established strains. However, the limitations of mice, exhibiting a specific mutation to study a disease, such as cystic fibrosis, must not be ignored. By the year 2007, eleven mouse models of cystic fibrosis had been generated and studied [50-63]. The first cystic fibrosis mouse was a knockout generated by the Boucher group in 1992 [54,55]. It had a very low survival rate with less than 5% of the animals surviving until adulthood [55]. This appeared to be a result of severe gastrointestinal impairment, yet the airways showed no symptoms of CF. Additional genetic manipulations then led to the generation of a ΔF508 CFTR mouse [62]. Ultimately, various recognizable CF symptoms were found in most of the various mouse models generated, and these were seen across the affected organ systems in CF mice. The gastrointestinal system exhibited the most cystic fibrosis complications across the different murine models. Several CF mice were also reported to demonstrate a failure to thrive along with mucus accumulation and intestinal obstruction [54]. Obstruction of the mouse intestines were similar to that presented by meconium ileus, which is typically experienced by CF patients. Models also presented with familiar pancreatic symptoms. Typically the blockage of small pancreatic ducts was seen, but less impact overall on pancreatic function [59].

Unfortunately, since 1993, all studies have shown that CFTR-null murine models are an ineffective form of examining pulmonary effects of cystic fibrosis. Mice do not develop any of the typical characteristics of lung disease such as peptides secreted by airway epithelial cells and inflammation from neutrophil and macrophage activity [51-53]. In order for inflammation and decreased bacterial clearance to be seen, mice had to be repeatedly exposed to unusually high numbers of bacteria [64]. A range of Na+ and Cl- concentrations, as well as ASL volumetric depths, in murine ASL measurements have been reported, yet no significant differences have been found. This lack of difference between normal and CFTR-null mice were accounted for by the novel finding that mice express an alternate pathway in the airway: Ca-activated Cl- channels that hence can replace the CAMP-dependent CFTR chloride movement lost in CF [65,66]; (Figure 3). Yet, perhaps this unusual situation sheds some light on our debate.

Conclusions from murine model studies: Increased activity of Ca2+-activated chloride channels in CFTR-null mice have been demonstrated [65,66]. Hence we can assume that in this animal model a dysfunctional CFTR is expressed alongside a viable alternative Cl- path (do not need a paracellular one) and yet it does not express key features of pulmonary disease. This could be evidence cited to undermine or even refute the low volume hypothesis model as presented by the Boucher group (Figure 2b). If their mechanism is accurate, it would lead to a loss of ASL volume and thus infections in the mouse airway. However, this is not the case. One might respond to this position by arguing both hypotheses fail to manifest in the CF mouse, thus neither
is undermined. Perhaps, yet given low volume is a physical mechanism, suppressed cilia cannot move mucus, it seems that it should manifest in the mice, while a chemical mechanism like that of high salt is less predictable in different species. Yet to be conservative, following a careful analysis of ASL Na+ and Cl- concentrations reported in both murine models and human airway epithelial cell cultures, important downfalls of each model for cystic fibrosis research have been identified and our comparative analysis ruled out significance in findings obtained by these models partly due to the limitations described.

**Published Data on Na+ and Cl- in ASL of Human Subjects**

In vivo measurements of Na+ and Cl- concentrations in the ASL of normal and CF human subjects appear to be the most reliable source of data from which to draw a conclusion of the accuracy of the high salt hypothesis.

With regard to Na+ concentration, values obtained from 4 different studies indicated a reasonably steady "normal" range between 82-103 mM. Corresponding ASL Na+ concentrations were reported in patients with cystic fibrosis by Joris et al. in 1993, Knowles et al. in 1997, and Hull et al., in 1998. The described range of ASL Na+ concentrations in CF patients was similar to the range of sodium in normal subjects; 82 mM to 121 mM of sodium. An independent samples t-test determined that there was no significant difference between human in vivo ASL Na+ concentrations in normal and CF subjects.

Subsequently, the concentration of chloride ions in the airway surface liquid of both normal and cystic fibrosis patients measured in vivo underwent a separate meta-analysis. Overall, there are five studies that report normal Cl- ASL concentrations: Gilljam et al., 1989; Joris et al., 1993; Knowles et al., 1997; Hull et al., 1998; Jayaraman et al., 2001. The range of Cl- ASL in normal subjects was between 78 mM to 108 mM, with no significant difference between the five data points shown through a Grubbs’ outlier test. Thus, chloride concentrations in normal subjects range from approximately 70 mM to 110 mM, depending on the individual. Four of the aforementioned papers report corresponding Cl- ASL measurements in CF subjects: Gilljam et al., 1989; Joris et al., 1993; Knowles et al., 1997; Hull et al., 1998. Chloride measurements in CF subjects were between 75 mM and 170 mM. To determine if normal concentrations of chloride are significantly different from those of cystic fibrosis patients, independent samples t-test was performed, which indicated no significant difference.

Thus, these data, when pooled, do not provide statistical support that either the ASL Na+ or Cl- concentration is increased in cystic fibrosis.

With the exception of Gilljam et al., in 1989, all other studies measured human in vivo ASL salt concentrations using varying forms of filter paper to collect ASL through a bronchoscope from the distal trachea or bronchi, which has been argued to have its drawbacks as well. Protocols utilizing the placement of a filter paper to the airway can wick fluid from areas outside the paper’s diameter and likely from inside the cell, since the removal of fluid will cause a shift in fluid to the apical surface. However, all three studies used this same methodology, which limits the concern that differences in reported findings between studies are caused by this technique. It should be noted that, although it is unlikely to explain cross-study variations, filter paper collection may artificially increase or decrease salt concentrations measured in ASL.

The technique used by Gilljam et al., in 1989 to collect ASL, and the manner in which error was reported, explains the larger margin of error relative to those of the other 3 human in vivo studies. Gilljam et al. aspirated ASL from the bronchial surface through a bronchoscope into a collection vial, while the other studies used filter paper collection techniques similar to each other. Aspiration of the very thin layer of liquid lining the lungs may permit significant evaporation of fluid as it ascends the bronchoscope, resulting in a higher salt concentration by default. In addition, error measures were reported as one standard deviation instead of as a standard error measure (SEM) as done in Joris et al in 1993, Knowles et al in 1997, and Hull et al in 1998. For comparison, the error of ASL chloride concentrations was reduced from 79 mM for CF patients and 54 mM for normal subjects to 26.3 mM and 18 mM respectively, by calculating the SEM using the reported sample size and standard deviation.

The finding of no difference between CF and normal human Na+ and Cl- ASL concentrations disagrees with the findings of Joris et al., in 1993 and Gilljam et al., in 1989. CF subjects from the 1993 Joris paper were found to have approximately 45% more Na+ in ASL than their normal counterparts [42]. However, the sample size for normal subjects was seventeen compared to only three CF subjects. Additionally, the three CF subjects included a 6-month-old undergoing surgery to remedy a collapsed lung, and a 16 year old and 81 year old hospitalized for elective surgical procedures. By the age of 5, 60% of people with CF have symptoms of lung disease [7]. Therefore, it is likely that due to the age of the older CF subjects, at least two-thirds of the CF patient sample in the 1993 Joris paper had lung infections or inflammation. Between studies, there were not only differences in CF subjects measured but also in normal subjects. Gilljam et al. measured subjects who did not have CF, but were diagnosed with chronic bronchitis. The significant difference found in Na+ and Cl- concentrations by Joris et al. and Gilljam et al., is likely due to the limited and skewed range between CF subjects’ ASL measurements and the wider range of unhealthy normal subjects.

**Conclusions from human subject’s studies:** Through analysis of available data from both the Welsh group of the University of Iowa who support the high salt hypothesis and Boucher group of the University of North Carolina at Chapel Hill who support the low volume hypothesis, available evidence suggests that neither Na+ nor Cl- concentrations are statistically higher in the ASL of cystic fibrosis vs. normal patients. The high salt hypothesis model described in Figure (2b) is therefore unlikely to be an accurate depiction of CF on the airways cellular level.

Airway surface liquid depth is the widely measured and defining variable of the low volume model for CF lung disease proposed by the Boucher group (Figure 4). For years, the low volume hypothesis has claimed that the characteristic lung infections of cystic fibrosis initiate from a decrease in airway
surface liquid depth. Water is hypothesized to move into the cell via osmosis following an increased absorption of Na+ through an uninhibited ENaC channel, thus reducing the thickness of the ASL. Eventually, the loss of water is thought to lower ASL below the height of the cilia (7 μm), resulting in ciliary collapse, loss of mucus transport, and disruption of normal bacterial clearance.

The standing mucus then becomes a breeding ground for bacteria such as Pseudomonas aeruginosa.

In order to determine the soundness of this hypothesis, all published ASL depths obtained from normal and CF models were gathered and compared. Normal ASL depths measured by Matsui et al. in 1998, Tarran et al., in 2001, Song et al., in 2009, Chen et al. in 2010, and Harvey et al. in 2011 varied from 4μl to 20μl. In comparison, ASL depths of CF models ranged from 4.5μl to 10μl. The highest values for both normal and cystic fibrosis ASL depth were found to be significant outliers (Grubbs’ test). Both outliers were observed by Matsui et al., 1998. Despite the significant outliers, an independent samples t-test resulted in a p-value of 0.01. An independent samples t-test resulted in a p-value of 0.16. (Right) Normal ASL Depth compared across models. Normal ASL depth in μl for in vivo and ex vivo human tissue (labeled “Human Tissue”), human lung epithelial cell cultures (labeled “Human Cell Culture”), ex vivo bovine tissue, bovine lung epithelial cell cultures (labeled “Bovine Cell Culture”), ex vivo porcine tissue (labeled “Porcine Ex Vivo”), and samples from murine models to show differences in ASL height across models and sampling procedures. ("Low Volume" refers to research affiliated with the Boucher group, “High Salt” for work from the Welsh group, while the term “Independent” refers to research groups not affiliated with Boucher or Welsh; values are mean ± SEM unless absent in original publication).

Under CF-like conditions, ASL fluid was not increased in the presence of secretory stimuli, as it was in tissue with unhindered CFTR activity. These findings by Song et al. in 2009, suggested a transient change in ASL depth in response to extracellular conditions was lost in cystic fibrosis. Consequently, a second look into how the ASL depth of subjects with cystic fibrosis responds under different agonists and inhibitors compared to normal subjects should be considered. Transient increases in the depth

Figure 4 Non-CF vs. CF: ASL depth. (Left) Comparison of the depth of normal ASL and CF ASL. Matsui et al., 1998 depths of normal ASL and CF were tested by a Grubbs’ test for outliers resulting in a p-value of 0.01. An independent samples t-test resulted in a p-value of 0.16. (Right) Normal ASL Depth compared across models. Normal ASL depth in μl for in vivo and ex vivo human tissue (labeled “Human Tissue”), human lung epithelial cell cultures (labeled “Human Cell Culture”), ex vivo bovine tissue, bovine lung epithelial cell cultures (labeled “Bovine Cell Culture”), ex vivo porcine tissue (labeled “Porcine Ex Vivo”), and samples from murine models to show differences in ASL height across models and sampling procedures. ("Low Volume" refers to research affiliated with the Boucher group, “High Salt” for work from the Welsh group, while the term “Independent” refers to research groups not affiliated with Boucher or Welsh; values are mean ± SEM unless absent in original publication).
of ASL were observed when exposed to CFTR agonist, forskolin, or ENaC inhibitor, amiloride [69].

The Cause of Cystic Fibrosis Lung Disease: High Salt or Low Volume Hypotheses

After critical analyses of the existing data from the Boucher group of the University of North Carolina who support the low volume hypothesis and the Welsh group of the University of Iowa who have proposed the high salt hypothesis, as well as data from various independent groups, the most likely conclusion is that neither hypothesis is correct, as suggested by in vivo human or porcine studies. These studies strongly suggest that airway surface liquid does not have an increased salt concentration in cystic fibrosis, nor is the depth of the airway surface liquid lowered or depleted in the disease.

As neither of the major hypotheses of cystic fibrosis lung disease appears to be supported, it begs the question: what is an alternative for the underlying cause of pulmonary infection in cystic fibrosis?

A handful of groups researching the underlying cause of cystic fibrosis lung disease have now refocused on differences in extracellular pH [70-72]. This shift from the concentrations of Cl- and Na+ in ASL, instead rather to pH, is a direct consequence of the original finding that CFTR not only transports Cl- ions but bicarbonate as well [73]. In fact, a study done utilizing a virtual gland apparatus has shown that approximately 50% of the anion secretion in the airway is bicarbonate, making this a now very significant area of research [74]. Increased levels of bicarbonate in the ASL of CF subjects could alter pH, which would have broad-sweeping effects across organ systems that have previously not been able to be tied to a simple defect in Cl- conductance [75]. Over several studies, the Verkman group initially reported finding no significant change in pH between genotypes, but in 2004 when submucosal gland fluid from human and porcine airways was examined; a significant difference between the pH of ASL from CF and normal models was found [69].

Differences in the pH of ASL from normal and cystic fibrosis models have been reported in several studies over the years [69, 71,76-79]. The most reproducible and compelling differences, due to similarity in disease progression, are found in the porcine model and in vivo human studies. In Figure (5), in vivo ASL pH measurements for normal human subjects and CF subjects are shown with the mean value for each group, indicated by the large corresponding marker. Mean measurements for human subjects with CF and those of normal individuals were reported in Jayaraman et al., 2001 while Song et al., 2006 and Cho et al., 2011, reported individual pH measurements. The mean pH for normal subjects was 7.22, with a standard deviation of 0.22, while the mean pH for subjects with CF was 6.92, with a standard deviation of 0.35. An independent sample t-test showed a significant difference between the ASL pH of normal and CF patients. In 2014, AbouAlaiwa et al., measured ASL pH in normal and CF carriers to compare to CF neonates who were homozygous for CFTR mutations. In neonates, the difference between normal and CF subjects is more pronounced, as shown in Figure (5). Although there is a larger sample size of “normal” subjects compared to CF neonates, a significant difference was found in an independent samples student’s t-test. Thus, a difference in pH most is likely present at birth, before inflammation and exposure to bacterial pathogens that could result in infections and impact milieu.

Thus, a lower ASL pH in those with cystic fibrosis at birth and throughout later stages of life is a potential mechanism as cause of lung infections and pulmonary complications associated with cystic fibrosis.

As a part of Marquette’s graduate work, during the year 2013 original data was meticulously obtained from the original publishing authors and head to head independent samples two-tailed t-tests performed to look for significance between data sets from different studies. No statistical tests found significance supporting reliable differences. Hence after an in depth analysis of the two conflicting hypotheses concerning the difference in the airway surface liquid and cystic fibrosis lung disease, this...
study finds that either the high salt hypothesis or the low volume hypothesis is accurate. According to recent research, the data more likely support a significant decrease, and thus acidity, in the pH of the airway surface liquid of CF patients. In fact, while this systematic review was performed in early 2014, since then follow-up studies have further bolstered that pH is altered in the ASL of CF pigs [80]. Forthcoming experiments will further the study of airway surface liquid and etiology of CF lung disease by evaluating changes in pH as well as ASL depth. The lack of passive transport of bicarbonate ions is predictably a significant change in cystic fibrosis airway milieu and should be an important future direction of research.

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