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## Variability in Measures of Exhaled Breath Na<sup>+</sup>, Influence of Pulmonary Blood Flow and Salivary Na<sup>+</sup>

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**Abstract:** The assessment of inflammatory markers and ions in exhaled breath condensate (EBC) is being utilized more frequently in diseases such as asthma and cystic fibrosis with marked variability in EBC measures, including those of exhaled Na<sup>+</sup>. We sought to determine if variability in exhaled Na<sup>+</sup> was due to differences in pulmonary blood flow (PBF) or Na<sup>+</sup> in the mouth (salivary Na<sup>+</sup>). We measured exhaled Na<sup>+</sup> three times with coinciding sampling of salivary Na<sup>+</sup> and assessment of PBF (using acetylene rebreathing) in 13 healthy subjects (54% female, age = 27 ± 7 yrs., ht. = 172 ± 10 cm, wt. = 70 ± 21 kg, BMI = 22 ± 7 kg/m<sup>2</sup> mean ± SD). Exhaled Na<sup>+</sup> averaged 2.7 ± 1.2 mmol/l, and salivary Na<sup>+</sup> averaged 5.51 ± 4.58 mmol/l. The coefficients of variation across all three measures in all 13 subjects averaged 30% for exhaled Na<sup>+</sup> and 83% for salivary Na<sup>+</sup>, within subjects the variability across the three measures averaged 30% for exhaled Na<sup>+</sup> and 38% for salivary Na<sup>+</sup>. Across all three measures in all 13 subjects the relationship between PBF and exhaled Na<sup>+</sup> averaged 0.027 ( $P = 0.87$ ), and the relationship between salivary Na<sup>+</sup> and exhaled Na<sup>+</sup> concentrations averaged 0.59 ( $P = 0.001$ ). Also, we sought to determine the relationship between exhaled Na<sup>+</sup> and serum Na<sup>+</sup> in an addition 20 subjects. There was a moderate and significant relationship between serum Na<sup>+</sup> and exhaled Na<sup>+</sup> ( $r = 0.37$ ,  $P = 0.04$ ). These findings suggest there that the variability in exhaled Na<sup>+</sup> is caused, at least in part, by droplet formation from within the mouth as turbulent air passes through and that there is a flux of ions from the pulmonary blood into the airways.

**Keywords:** exhaled breath, lung fluid, airway surface liquid, pulmonary blood

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## Introduction

Epithelial cells of the bronchial tree and alveoli are covered in a surface liquid composed of mucus, ions, inflammatory proteins, and water. Airway surface liquid (ASL) is a fundamental component of the pulmonary defense, and one that has been shown to influence overall pulmonary function.<sup>1,2</sup> Maintenance of an ideal depth of the surface liquid and hydration of the mucus is necessary for proper mucociliary clearance; improper ion transport can result in impaired fluid movement altering ASL depth.<sup>3,4</sup> Even though ASL is crucial for the protection and defense of the lung, it remains one of the few body fluids whose composition is poorly defined as its small volume makes collection of sufficient amounts for quantitative analysis extremely difficult.

Exhaled air, predominantly composed of water vapor, also contains small droplets of fluid that are produced by the shear force of turbulent flow upon exhalation across the airway surface liquid.<sup>5</sup> As such, the composition of one's exhaled breath is believed to be a surrogate, although diluted, marker of the composition of the airway surface liquid (ASL) from any location in the lung, including the alveoli.<sup>6-9</sup> Currently, commercially available condenser systems allow subjects to breathe on a mouthpiece through a cooling system where the exhaled breath and its constituents are collected. The composition of the collected fluid, also known as exhaled breath condensate (EBC), can then be analyzed for solutes, including ions, which could be of particular importance in disease states such as cystic fibrosis.<sup>10</sup>

The usefulness of the EBC method is potentially compromised by the inability to discriminate the exact source of the EBC droplets and the high variability in the determination of ion and inflammatory marker composition between subjects and also within individuals. Previous work has made it clear that extreme variability exists between EBC collections, but as of now the cause or causes of the variability have not been identified.<sup>7,9,11-13</sup> Previous studies have speculated on sources of variability based on collection methods, but have failed to investigate specific physiological sources as potential contributors to this variability. The transport of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  into the intracellular space across the basolateral membrane of epithelial cells is influenced by blood ion levels.<sup>14</sup> Altering the driving force for ion transport by changing the electrochemical

gradient could thereby affect the airway surface liquid ion composition and the reflection of its composition in the EBC collections by influencing apical ion and water movement. Because a large portion of the blood in the pulmonary circulation comes directly from the heart, pulmonary blood flow (PBF) could influence the variability of this measure through the transfer of ions from the pulmonary circulation across the airway epithelia into the alveoli. We hypothesized that increased PBF could increase ASL ion concentrations thereby increasing ion composition of EBC collections.

Gross contamination of EBC by liquid saliva has been reported as unlikely through measurements of undetectable salivary amylase activity, which is a good indicator of salivary contamination.<sup>6,7,9,15</sup> However, droplet formation from salivary liquid within the mouth during exhalation could be a potential contributor to EBC ion composition that has not been yet been studied or ruled out. Measuring saliva falling into the EBC sample during collection using amylase as a marker, does not exclude salivary liquid droplet formation from turbulent airflow passing through the mouth, which can be even greater than what is present in the airways. As such, in addition to the droplets generated from the airway surface liquid, the mouth could produce its own droplets becoming a potential contributor to the variability in EBC ion concentrations as differences in salivary liquid ion concentrations could alter the ion concentrations in EBC independent of the ion composition of the ASL.

The potential to utilize EBC as a method of determining the composition of the still ambiguous ASL is promising, but in order for this technique to be used to diagnose, treat or monitor patients with various lung diseases the cause of the large intrasubject variability needs to be better understood.<sup>1,7,10</sup> Hence, the purpose of this study was to examine whether the source of the previously-described variability in EBC was due to physiological parameters. An increase in PBF could increase the ion pool of the interstitial space which could then influence ion flux between the ASL and the airway epithelium. Due to the potential for salivary droplet formation from within the mouth, an increase in  $\text{Na}^+$  concentration in the salivary liquid could result in an increase exhaled  $\text{Na}^+$  concentration. As such, both PBF and the amount of  $\text{Na}^+$  in the mouth could contribute to variability in EBC collection



Na<sup>+</sup> concentrations both within an individual during multiple collections and between individuals. We hypothesized that there would be a relationship between PBF and salivary Na<sup>+</sup> with exhaled Na<sup>+</sup> over three EBC collection sessions within an individual.

In addition, in order to demonstrate that the composition of exhaled Na<sup>+</sup> may also be influenced by ion regulation within the lung and the flux of ions from the pulmonary capillaries to the interstitial space finally the airway lumen, we recruited 20 additional subjects and measured exhaled Na<sup>+</sup> and serum Na<sup>+</sup> levels. In this second study we hypothesized that the ion composition of the blood would influence the flux of ions from the airway epithelium into the ASL and would be reflected in exhaled Na<sup>+</sup>.

## Methods

### Subjects

The protocol was reviewed and approved by the University of Arizona Institutional Review Board, all subjects agreed to participate in the study, and all aspects of the study conformed to the Declaration of Helsinki. The subjects provided written informed consent.

### Protocol

Exhaled breath condensate (EBC) was collected three times within two hours on the same day for all subjects with a minimum of 20 minutes between each collection. EBC was collected for 20 minutes on the Jaeger EcoScreen (Cardinal Health, Yorba Linda, CA). All subjects were seated and asked to breathe quietly for ten minutes prior to collection to ensure that data was collected while the subject was in a resting state. During EBC collection, subjects remained seated with feet flat on the floor in an upright position and wearing a nosepiece. At the halfway point of each collection, subjects briefly came off the Ecoscreen to provide a saliva sample. Following each EBC collection, pulmonary blood flow (PBF) was assessed in triplicate.

We also sought to determine the relationship between exhaled Na<sup>+</sup> and serum Na<sup>+</sup> in an additional 20 subjects to determine if exhaled Na<sup>+</sup> is representative of ion values within the blood. We performed this additional study to demonstrate that the Na<sup>+</sup> flux in the lung is dynamic, moving from the blood to the interstitial space then to the alveolar space. Exhaled breath condensate was collected for 25 minutes followed by

the assessment of PBF. Serum Na<sup>+</sup> was drawn at the midway point of EBC collection.

### Assessment of pulmonary blood flow

Pulmonary blood flow was assessed with a previously-validated 8–10-breath acetylene wash-in technique using a 5-liter rebreath bag containing 0.7% C<sub>2</sub>H<sub>2</sub> and 9% He which has been compared to the direct Fick equation.<sup>16–18</sup> Briefly, a pneumotachograph was connected to a non-rebreathing Y valve (Hans Rudolph, KC, MO) with the inspiratory port connected to a pneumatic switching valve (Hans Rudolph, KC, MO) which allowed for rapid switching from room air to the test gas mixture. Gases were sampled using a mass spectrometer (Perkin-Elmer 1100, Yorba Linda, CA). The volume of gas used to fill the rebreath bag for was 1575 ml. At the end of a normal expiration (end-expiratory lung volume, EELV) the subjects were switched into the rebreath bag and instructed to nearly empty the bag with each breath for 8–10 consecutive breaths. Consistent bag volumes were assured using a timed switching circuit which, given a consistent flow rate from the tank, resulted in the desired volume.<sup>19</sup> The switching circuit and tank were calibrated prior to each test to ensure accurate volumes. Following each maneuver, the rebreath bag was emptied with a suction device and refilled immediately prior to the next maneuver. At the start of each maneuver, there was no residual gas in the dead space of the apparatus, nor from the exhaled air from the subjects, as determined through gas sampling with the mass spectrometer.

The rate of diffusion of acetylene was used to assess pulmonary blood flow. Acetylene upon diffusion across the alveolar-capillary membrane cannot bind to hemoglobin so diffusion occurs till equilibrium between the partial pressures in the alveoli and capillary have been reached, this means that the rate of diffusion is limited by the rate at which this partial pressure gradient can be re-established or in other words the rate at which a new volume of blood is transported through the lungs.<sup>20</sup> The rate of disappearance of acetylene with each breath was then calculated from the “slope of the exponential disappearance of acetylene” with respect to the inert helium using custom software.<sup>21</sup>

### Assessment of serum Na<sup>+</sup>

Serum Na<sup>+</sup> concentrations were determined from a venous blood sample collected at the midway point



of the exhaled breath collection using ion-selective electrodes at the University of Arizona Medical Center Pathology Laboratory.

### Collection of exhaled breath condensate

The condensing system used in the present study was the Jaeger Ecoscreen cooling unit which is an electrically cooled Teflon-lined double lumen system. The condenser consists of two tubes: an inner 10 cm long tube (1.3 cm diameter) and a second outer 8 cm long tube (2.3 cm in diameter; condensing surface area = 63 cm<sup>2</sup>) (3). Sample collection cups are screwed onto the bottom of the condenser and the condenser is snapped tightly into the inhalation/exhalation valve. This entire unit is then placed on the condensing system with the condenser and cup inserted into the lumen of the cooling system which was cooled to  $-20^{\circ}\text{C}$  to facilitate condensation of exhaled breath. For the study, a mouthpiece was attached to the elbow joint of the inhalation/exhalation valve. A new condenser was used for each collection point within a subject. During each collection, subjects were instructed to wear a nose clip while performing calm tidal breathing on the mouthpiece. One should note that the mouthpiece on the Ecoscreen is equipped with a saliva trap and the device angles upward away from the mouth so that saliva travels back into the mouth rather than into the condenser to avoid salivary contamination; subjects were also instructed to swallow salivary accumulations as necessary to make certain there was no gross contamination of the sample. An individual's exhaled breath flows through the condenser where it is precipitated and the droplets are collected in the sample collection cup.

### Quantification of ion concentrations

Exhaled breath condensate and salivary samples were stored at  $-80^{\circ}\text{C}$  until the time of final batch analysis. Previous testing by our lab and others found no difference in ion concentrations of the same samples tested directly after collection and again after being frozen.<sup>9</sup> Sodium concentrations were measured using an atomic absorption spectrophotometer (Analyst 100; Perkin-Elmer, Norwalk, CT) with a detection limit of 0.01  $\mu\text{mol/L}$ . emission wavelength of 566.5 nm Na<sup>+</sup> as previously described.<sup>22</sup> Briefly, the Na<sup>+</sup> cation lamp provided absorbance measurements of samples. Samples were diluted as needed in order for absorbance to be

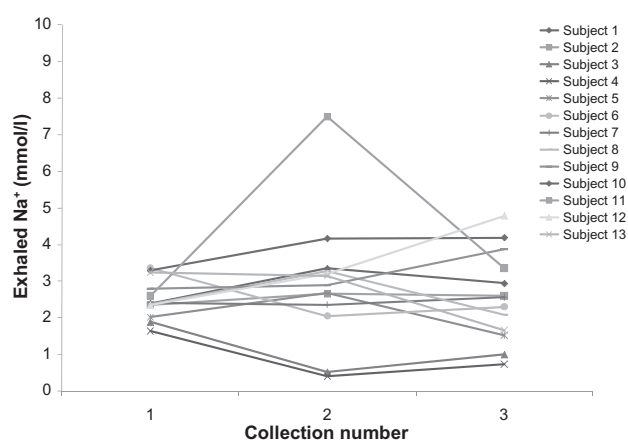
measured within the prepared standard solution curve with known concentrations of sodium diluted with Milli-Q water (Millipore, Burlington, MA) to concentrations of 0.01, 0.05, 0.10, 0.15, 0.20 mmol/L.

### Statistical analysis

All statistical comparisons were performed using the SPSS statistical software package (v. 17.0, Chicago, IL). Descriptive statistics were performed to calculate mean, minimum, maximum, and standard deviation for all collections. Coefficients of variation were calculated for exhaled Na<sup>+</sup>, salivary Na<sup>+</sup> and PBF using the average of the standard deviations to mean concentration values for the three collections within each subject. Pearson correlation coefficients were calculated to determine the relationship between PBF and exhaled breath condensate Na<sup>+</sup> concentrations, salivary Na<sup>+</sup> and exhaled Na<sup>+</sup> concentrations. Also serum Na<sup>+</sup> and exhaled Na<sup>+</sup> concentrations as well as PBF and exhaled Na<sup>+</sup> were examined with a Pearson correlation in 20 subjects in the additional study. An alpha level of 0.05 was used to determine statistical significance. All data are presented as mean  $\pm$  SD.

### Results

Thirteen healthy subjects were recruited for the repeatability portion of this study (54% female, age =  $26 \pm 7$  years, ht. =  $173 \pm 9$  cm, wt. =  $71 \pm 18$  kg, BSA (body surface area) =  $1.8 \pm 0.2$  m<sup>2</sup>, BMI =  $24 \pm 4$  kg/m<sup>2</sup>). The variability between collected EBC samples within an individual showed gross variability for Na<sup>+</sup> across three consecutive collections (Fig. 1). All EBC



**Figure 1.** Individual variability of exhaled ion concentrations in three simultaneous exhaled breath condensate collections. Ion concentrations are in mmol/l for all 13 subjects of initial at each of the three exhaled breath condensate (EBC) sample collections.



samples contained detectable levels of Na<sup>+</sup>. Exhaled Na<sup>+</sup> averaged  $2.7 \pm 1.2$  mmol/l and salivary Na<sup>+</sup> averaged  $5.51 \pm 4.58$  mmol/l. The coefficients of variation across all three measures in all 13 subjects averaged 30% for exhaled Na<sup>+</sup> and 83% for salivary Na<sup>+</sup>, within subjects the variability across the three measures averaged 30% for exhaled Na<sup>+</sup> and 38% for salivary Na<sup>+</sup> (Table 1). Pulmonary blood flow averaged  $3.8 \pm 1.5$  l/min with the coefficient of variation for triplicate measures within a subject being 8%. Cardiac index for these subjects was  $2.1 \pm 0.6$  l/min/m<sup>2</sup>. Across all three measures in all 13 subjects the relationship between PBF and exhaled Na<sup>+</sup> averaged 0.027 ( $P = 0.87$ ) (Fig. 2), and the relationship between salivary Na<sup>+</sup> and exhaled Na<sup>+</sup> concentrations averaged 0.59 ( $P = 0.001$ ) (Fig. 3).

To determine if exhaled Na<sup>+</sup> is reflective of the amount of Na<sup>+</sup> in the blood, we explored the relationship between serum Na<sup>+</sup> and exhaled Na<sup>+</sup> in an additional study. For this study 20 subjects were recruited (40% female, age =  $29 \pm 9$  yrs., ht. =  $174 \pm 11$  cm., wt. =  $72 \pm 13$  kg., BSA =  $1.9 \pm 0.2$ , BMI =  $24 \pm 4$  kg/m<sup>2</sup>). Again all EBC samples contained detectable levels of Na<sup>+</sup>. In this group of subjects, exhaled Na<sup>+</sup> averaged  $2.8 \pm 1.7$  mmol/l and serum Na<sup>+</sup> averaged  $139 \pm 2$  mmol/l. Pulmonary blood flow averaged  $3.7 \pm 1.2$  l/min with the coefficient of variation for triplicate measures within a subject being 5%. Cardiac index for these 20 subjects was  $2.0 \pm 0.6$  l/min/m<sup>2</sup>. Among these 20 subjects, again there was not a significant relationship between PBF and exhaled Na<sup>+</sup>  $r = 0.29$ ,  $P = 0.12$ . There was however, a moderate and significant relationship between serum Na<sup>+</sup> and exhaled Na<sup>+</sup> ( $r = 0.37$ ,  $P = 0.04$ ,  $r^2 = 0.20$ , Fig. 4). When the subject who was determined to be an outlier was removed the correlation between serum Na<sup>+</sup> and exhaled Na<sup>+</sup> improves to  $r = 0.52$ ,  $P = 0.03$ ,  $r^2 = 0.27$ .

## Discussion

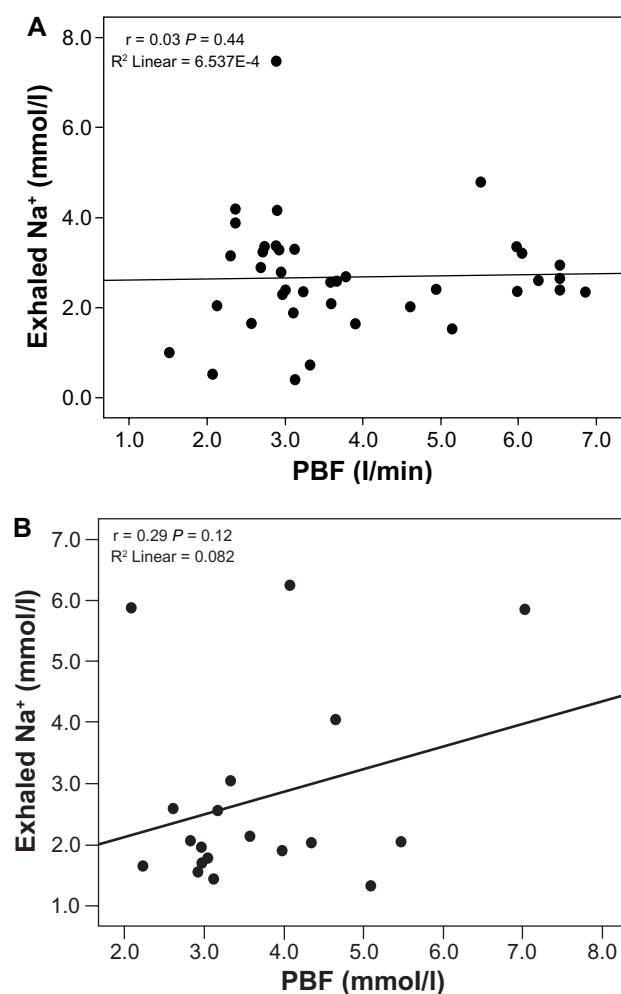
Our findings demonstrate marked variability for Na<sup>+</sup> across three consecutive collections with exhaled Na<sup>+</sup> averaging  $2.7 \pm 1.2$  mmol/l. The coefficients of variation for exhaled Na<sup>+</sup> across three measures averaged 30% which is as good or better than has been previously documented.<sup>9,23</sup> In our study we show that no pattern in the variability exists between collection points within a subject (Fig. 1). Our study investigated PBF and saliva as potential ion reservoirs and, therefore, two possible sources for the variability observed in our work and the work of others both within an individual and between individuals. Our findings that salivary Na<sup>+</sup> concentrations showed a positive correlation to exhaled Na<sup>+</sup> concentrations appear to support our initial hypothesis of droplet formation from within the mouth contributing to EBC ion concentrations. Analysis of saliva composition in earlier work has demonstrated high concentrations of Na<sup>+</sup> (3–125 mmol), Cl<sup>-</sup> (16–54 mmol) and K<sup>+</sup> (4–30 mmol).<sup>24–26</sup> As such, generation of salivary liquid droplets in addition to droplets formed from the airway surface liquid due to turbulent airflow through the mouth and bronchial tree appear to collectively dictate EBC composition.

The concentrations of Na<sup>+</sup> ( $2.7 \pm 1.2$  mmol/l) in EBC collected on the Ecoscreen obtained in this study are in agreement with those obtained by Zacharasiewicz et al ( $1.6 \pm 2.1$  mmol/l) where collections were conducted using the Ecoscreen for 10 minutes.<sup>9</sup> Our observed Na<sup>+</sup> concentration was lower than that of Gessner et al ( $8.5 \pm 0.9$  mmol/l) who also performed the collection using the Ecoscreen system for 30 minutes.<sup>15</sup> The concentrations of Na<sup>+</sup> we observed are higher than those reported by Svensson et al, Effros et al ( $0.24 \pm 0.14$  mmol/l), and Griese et al ( $0.089 \pm 0.04$  mmol/l).<sup>6,9,11,27</sup> These disparities in Na<sup>+</sup>

**Table 1.** Coefficients of variation for in exhaled and salivary sodium.

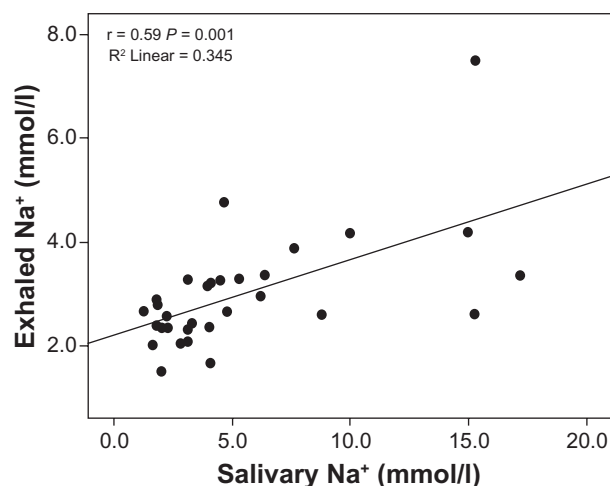
	Average	Standard deviation	Minimum	Maximum	Coefficient of variation
Across all collections					
Exhaled Na <sup>+</sup> (mmol/l)	2.70	1.20	0.40	7.50	0.30
Salivary Na <sup>+</sup> (mmol/l)	5.51	4.58	1.29	17.17	0.83
Within subjects					
Exhaled Na <sup>+</sup> (mmol/l)	2.68	0.76	0.90	7.50	0.30
Salivary Na <sup>+</sup> (mmol/l)	5.58	2.47	1.29	17.17	0.38

Variability in exhaled and salivary Na<sup>+</sup> across all collections (3 collection for 13 subjects, N = 39) and the average within a subject across the three collections in each of the 13 subjects from the initial study.



**Figure 2.** Relationship between pulmonary blood flow and exhaled sodium concentration. **A)** Data presented include all three samples collected within each of the 13 subjects in the initial study. **B)** Data from single collection in additional 20 subjects. Results from the Pearson correlation analysis are shown on the graph.

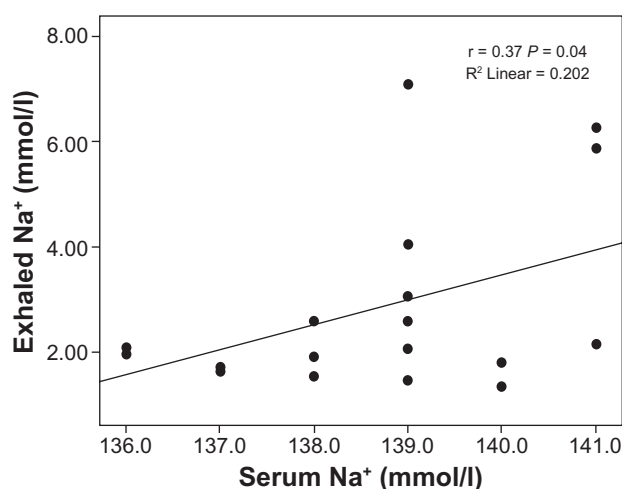
concentrations between our study and those of others, like the variability associated with the EBC collection itself, could be due to a variety of potential sources including equipment utilized, sampling procedures, methods for quantification, and physiological factors. First, differences in concentrations may be attributed to the use of different collection systems; the studies conducted by Effros et al and Griese et al both utilized custom made systems, whereas collections in our study and those made by Zacharasiewicz et al were completed on the Ecoscreen. Previous research has found differences in the volume collected (repeatability and variability, with lower variability found with the Ecoscreen), and the yield of specific constituents between the commercially available EBC systems such



**Figure 3.** Relationship between salivary sodium concentration and exhaled sodium concentration. Salivary sodium concentration (mmol/l) versus exhaled sodium concentration (mmol/l) for all three samples collected for each of the 13 subjects. Results from the Pearson correlation analysis are shown on the graph.

as the Jaeger Ecoscreen and Respiratory Research ‘R tube’ that have been primarily used and evaluated.<sup>7,9,13</sup> Custom made systems can potentially introduce even greater variability for collections made on different systems due to non-standardized methods of operation.<sup>7</sup> With the intention of potentially using EBC clinically, the use of a commercially available system is advantageous.

Second, the differences in concentrations may be due to differences in collection time between our study and previous studies as the collections made



**Figure 4.** Relationship between serum sodium concentration and exhaled sodium concentration from single collection in the additional 20 subjects. Results from the Pearson correlation analysis are shown on the graph.



by Effros et al and Griese et al which were one hour and 2–4 hours respectively. The lengthening of the collection time could cause lower concentrations due to the increased dilution of ASL droplets with water vapor during the collection period on the custom made systems. Although it may have been better to use one of the collection times used in previous work using the Ecoscreen instead of 20 minutes. Our collection time of 20 minutes was a median between the two collection times in previous studies using the Ecoscreen. As collection time increased from 10, 20 to 30 minutes the concentration of Na<sup>+</sup> increased from  $1.6 \pm 2.1$  mmol/l,  $2.7 \pm 1.2$  mmol/l to  $8.5 \pm 0.9$  mmol/l. Therefore, it appears that there is a positive relationship between collection time and concentration when using the EcoScreen for EBC collection which needs to be further evaluated in order for a standardized method for EBC collection to be established for clinical application of this technique to be feasible.

Third, differences in concentrations may also be attributed to differing quantification methods, which included ion chromatography by Svensson et al and ion electrode by Zacharasiewicz et al and Effros et al with detection limits of 1 mmol/l and 0.017 mmol/l respectively. The analytical sensitivity of atomic absorption spectrophotometry used in the present study provided detection limit of 0.01 mmol/l allowing for ion concentration to be measured in all of the samples.

Finally, the differences in concentrations could be attributed to salivary contamination. However, gross contamination by liquid saliva is not likely as it has been ruled out in previous research studies using the Ecoscreen demonstrating undetectable salivary amylase activity with detection limits of 0.05  $\mu\text{mol/l}$ <sup>15</sup> and 5 U/l,<sup>9</sup> as well as a study using a custom collection system only detecting low concentrations 4.67 U/l in 75% of the samples which  $2 \times 10^{-5}$  fold less than saliva concentrations.<sup>11</sup> Because amylase has not been found in previous studies using the Ecoscreen collection system, and because our aim was not to determine if there was gross salivary contamination of the EBC samples, rather we were interested in the potential for generation of droplets from within the mouth from the salivary liquid contributing to measured Na<sup>+</sup>, we did not measure salivary amylase activity. Subjects were

instructed to swallow accumulated saliva as necessary during the collection and, as described, the Ecoscreen has a saliva trap and upward angling mouthpiece which would require the subject to forcibly spit into the system in order to contaminate the sample. If the higher concentrations found in the present study were due to considerable saliva contamination of the EBC samples then one would expect much larger discrepancies that we noticed because ion concentrations present in saliva are drastically larger (Na<sup>+</sup> = 3–125 mmol/l).<sup>24–26</sup>

We hypothesized that with transport of K<sup>+</sup>, Cl<sup>-</sup> and Na<sup>+</sup> through the basolateral membrane from the extracellular fluid which has an electrolyte composition similar to that of the plasma, and into the intracellular space of the airway epithelia cells may potentially influence apical ion and water movement by altering the electrochemical gradients thereby affecting the ASL composition and the reflection of its composition in the EBC collections.

Our values for PBF seem low, but the system setup was the same for all subjects in both studies so all values should be low by the same factor. Our finding that PBF did not show a significant correlation to exhaled Na<sup>+</sup> concentrations did not support our original hypothesis that a greater PBF would result in a greater concentration of Na<sup>+</sup> in the alveolar lumen due to increased exposure of the extracellular fluid to blood ion levels. Since blood in the pulmonary circulation includes both pulmonary blood flow from the right ventricle which is in contact with the alveoli as well as oxygenated blood from the left ventricle to supply the lung tissue, our measure of pulmonary blood flow may not have been specific enough as it does not include blood supplied by bronchial arteries. As the large airways will be a primary source of exhaled breathe droplet formation due to greater turbulent airflow in the larger airways than compared to the droplet formation from the alveoli and lower airways, a possible explanation for why PBF did not show a strong correlation with EBC sodium is that the specific bronchial blood supply to the trachea and upper airways has the greater influence on exhaled ions and our assessment of PBF was too broad of a measure of blood flow to allow for a relationship to be found.

In contrast to PBF, serum Na<sup>+</sup> levels in the blood are consistent between all regions of the lung regardless of



where the droplet formation is occurring. We found a relationship (albeit a moderate relationship) between serum  $\text{Na}^+$  and exhaled  $\text{Na}^+$  which suggests that the ion composition of the blood still has the potential to be a contributing factor to variability where serum  $\text{Na}^+$  concentrations appear to dictate airway surface liquid sodium concentrations. Due to the body's tight regulation of  $\text{Na}^+$ , the narrow range for serum  $\text{Na}^+$  values does increase the likelihood that a significant correlation between serum and EBC  $\text{Na}^+$  would be found. Our findings highlight the importance of future work being conducted where serum  $\text{Na}^+$  concentrations are artificially raised to confirm the relationship between serum and exhaled  $\text{Na}^+$ . As well, measurement of bronchial blood flow with measurement of exhaled  $\text{Na}^+$  and serum  $\text{Na}^+$  would allow for determination if there is a relationship to blood flow when the flow is compartmentalized to the upper airways and again confirm the relationship between serum and exhaled  $\text{Na}^+$ .

With this study there are some limitations that deserve discussion. The collection time was 20 minutes for the initial study and lengthened to 25 minutes in the additional study to increase the size of the sample collected so the two studies collection method was not completely uniform. However, it is important to point out that the addition of 5 minutes to the collection time did not demonstrate a significant difference in exhaled  $\text{Na}^+$  concentration as the mean for the 20 minute collection was  $2.7 \pm 1.2$  mmol/l compared to the mean of  $2.8 \pm 1.7$  mmol/l for collections of 25 minutes. There was greater variability in exhaled  $\text{Na}^+$  concentration measured within a subject during the same day than there was for the means between the studies with different collection times. Although the collection of the data having to be split between two studies may not be ideal the importance of presenting these findings is meaningful in order for this technique to have clinical usefulness in the future.

From this study and the work of others it appears that for exhaled breath condensate to have clinical application, future work should continue to determine the primary source of EBC and the source(s) of the variability in this measure. To aid in making EBC collection a clinically useful technique results from previous studies and our study suggest that one, there is utilization of the same device for collection of exhaled breathe condensate, where commercial

devices would probably be more ideal for consistency in methods of operation. Two, there is uniformity in the collection time used by different groups, controlling for humidification, and measurement of a stable ion, for example urea, in order to remove differences in concentrations measured that can be attributed to the dilution of ASL droplets by water vapor. Three, subjects fast and rinse the mouth with distilled water before collection to minimize salivary differences in ion composition and try to minimize salivary contribution to exhaled ion measurement. Four, a salivary sample is also collected along with EBC collection where eventually a correction equation can be determined to factor salivary contribution out of the exhaled breathe condensate ion concentration. Finally, there is the development of a standard rate and depth of breathing for EBC collection as it has been shown that minute ventilation is the major factor determining the volume collected, but solute levels have not been shown to be impacted by alterations in breathing pattern in other constituents.<sup>28,29</sup> As well other research supports the potential for anatomical differences the ionic composition of exhaled breathe<sup>8,30</sup> and that breathing pattern and recruitment may alter the anatomical site of origin of the ASL droplets collected in the exhaled breathe.<sup>13</sup>

## Implications

These findings have important implications in the assessment of ion and fluid regulation in the lung. As water follows the movement of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  in the lungs, the determination of the ion composition of the EBC could potentially serve as an assessment of lung fluid regulation and homeostasis of ionic composition. To date, the diagnosis, assessment of severity, and progression of respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis through the quantification of airway biochemistry and inflammation are obtained through the use of invasive procedures such as bronchoalveolar lavage, induced sputum or biopsy. However, due to its simplicity, minimal risk, and potential for frequent collections, the analysis of exhaled breath to study inflammatory markers and ionic composition as a non-invasive method for elucidating physiologic and pathologic processes in the lung has become a promising and potentially preferable alternative. Exhaled breath





condensate improves upon the limitations due to age, complications relating to disease severity, and potential pro-inflammatory effects associated with the more invasive methods.<sup>7,10,11,13</sup> If the variability within the measurement of exhaled ions from exhaled breath condensate within a subject can be determined, then the collection of exhaled breathe condensate can have clinical application.

## Conclusion

The results of this study confirm previous research demonstrating significant within-subject variability of measures of exhaled Na<sup>+</sup>. Our findings suggest that the variability in exhaled Na<sup>+</sup> is caused, at least in part, by droplet formation from within the mouth as turbulent air passes through. Although one cannot discriminate the exact source of the EBC, our findings would suggest that EBC droplets are produced from both the bronchial tree and the mouth with salivary Na<sup>+</sup> concentration appearing to contribute to the Na<sup>+</sup> levels measured in exhaled breath condensate (EBC). Our findings highlight the importance of concurrent salivary sampling with EBC collections to allow for factoring out of salivary droplet ionic contributions from the ASL droplets if one hopes to utilize EBC as a means of determining ASL ionic composition in health and disease. Furthermore, we found that the variability in exhaled Na<sup>+</sup> concentration is not related to differences in pulmonary blood flow. However, we noted that exhaled Na<sup>+</sup> is influenced by the amount sodium in the blood in the finding of a positive relationship between serum Na<sup>+</sup> and exhaled Na<sup>+</sup> suggesting exhaled Na<sup>+</sup> may be an index of pulmonary ion regulation of the airway surface liquid.

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## Disclosures

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