Original article

Urea in exhaled breath condensate of uraemics and patients with chronic airway diseases

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Abstract. Exhaled breath condensate (EBC) is composed mainly by water and also contains non-volatile mediators, which are expired in small droplets of airway fluid. Urea has been proposed as a normalization factor for EBC non-volatile biomarkers. Aim of this study was to assess volatility and diffusivity of urea *ex vivo* and to measure its EBC concentrations in different clinical conditions. Volatility was assessed quantifying EBC concentrations collected at 4 different temperatures, whereas diffusivity was tested by measuring urea concentrations in both plasma and EBC from uraemic patients on intermittent haemodialysis. Urea was also measured in EBC from patients with chronic airway diseases, *i.e.*, chronic obstructive pulmonary disease, asthma, and cystic fibrosis. The concentration of urea but not its absolute amount in EBC increased with condensation temperature. Haemodialysis influenced EBC and plasma urea concentrations in a similar way. The concentrations of urea in chronic airway diseases did not significantly differ from those of controls. Urea is a non-volatile molecule *ex vivo* and EBC urea depends on its concentrations in plasma. Urea concentrations in EBC are unaffected by three chronic airway diseases. We suggest that there is no need to normalize non-volatile biomarkers in EBC for urea concentrations to account for inter-individual variability. However, in repeated measurements within the same individual, the use of urea either as a normalizing factor or as covariate variable could be proposed to control intra-individual variability. (www.actabiomedica.it)

Key words: Exhaled breath condensate, urea, normalization factor, asthma, COPD, cystic fibrosis

Introduction

Exhaled breath condensate (EBC) is an aqueous biological fluid obtained by cooling exhaled air during normal breathing. Due to its total non-invasiveness, EBC has attracted attention for the study of different pulmonary diseases (1-4) and to assess early biochemical lung changes in subjects exposed to pneumotoxic substances (5-6). The use of EBC in the clinical practice is not widely accepted due to discrepancies in collection procedures and sample analysis among different laboratories and, most of all, in data interpretation (7). Recent studies on EBC formation have demonstrated that it contains traces of non-volatile mediators soluble in condensed water vapor (8). Non-volatile mediators are thought to be expired as a result of convective processes in small droplets of airway fluid generated from an uncertain airway location (9-11); however, 99% of EBC is represented by water, which is collected after condensation of water vapor outside the lung on a cool surface (12). These observations raised the question on how much condensed water dilutes expired droplets (12-14).

Some normalizing factors for EBC biomarkers have been proposed, including total ion concentra-

tions (as the sum of ions: Na^+, Cl^-, K^+), conductivity, and urea after EBC lyophilization (15). Whereas conductivity after EBC lyophilization has the disadvantage of a large inter- and intra-subject variability (16) and there is not sufficient evidence that salts are equally concentrated in the airway lining fluid and blood of healthy and diseased subjects (17), urea might be the ideal candidate normalization factor for its physical and chemical properties. In fact, urea is a small molecule, uncharged at physiologic pH, not metabolized by lung cells, and it is thought to be diffusible (18). For all these reasons, urea has been adopted as a possible indicator of normalization in broncoalveolar lavage fluid (BALF) (19-21). Recently, the ratio of plasma to condensate urea concentrations has been proposed as a simple method to estimate the dilution of non-volatile compounds in EBC as compared to blood (8).

Whereas the dilution factor (*i.e.*, the ratio between the same analyte in two different biological matrices) of urea in EBC as compared to plasma has been already calculated for controls and COPD subjects (12), the use of urea in EBC as normalization factor of other EBC biomarkers is still poorly studied (a normalization factor is defined as the ratio between two analytes in the same matrix). Before proposing urea EBC concentrations as a normalization factor for non-volatile biomarkers, some issues need to be addressed.

Therefore, the aims of this study were:

i) to establish whether urea could be considered as a non-volatile compound *ex vivo*, by comparing urea EBC concentrations in samples collected from healthy volunteers at four different temperatures (-10, -5, 0, and +5°C);

ii) to assess the diffusivity of urea between blood and airways, when a forced change of blood urea concentration occurs, *i.e.*, by collecting EBC and blood samples from patients with renal disease on intermittent haemodialysis (HD) before and after the treatment;

iii) to measure EBC urea concentrations in patients with different lung diseases, *i.e.*, chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis.

Methods

Subjects

i) 20 Healthy non-smokers [10 males, median age 33, range 29-47 years] were enrolled to check urea volatility *ex vivo*. Fifteen control subjects repeated their collection after 24 h at -5°C.

ii) 16 Dialyzed out-patients [12 males, median age 76, range 43-88 years] were enrolled to check urea diffusivity. The dialysis (HD) treatment lasted 4 h for 12 subjects, 3 h for 2 subjects, and 3.5 h for the remaining ones. EBC and plasma samples were collected before (PRE-HD) and after dialysis (POST-HD).

In 8 dialyzed in-patients [5 males, median age 66, range 38-81 years], the time-dependence of EBC urea concentration during two cycles of HD was assessed. EBC was collected at different times: PRE-HD, POST-HD, and 1, 2, 3, 24 and 48 h POST-HD.

iii) This part of the study included 11 mild-to-moderate clinically stable COPD patients [11 males, median age 75, range 68-85 years], 24 mild asthmatics [13 males, median age 16, range 6-58 years] and 25 patients with cystic fibrosis [14 males, median age 26, range 6-41 years]. Lung diseased patients have been diagnosed according to international guidelines.

iv) Reference values for urea in plasma and EBC were obtained from 50 healthy subjects [20 males, median age 30, range 6-47 years].

All the subjects gave informed consent for participation in the study. The study was conducted in conformity with the declaration of Helsinki and was approved by the Ethical Committee of the University of Parma.

Sample collection

EBC collection: EBC was collected with TURBO-DECCS (Italchill, Parma, Italy) as previously reported (6, 16). To test the volatility of urea *ex vivo*, subjects belonging to group 1 were asked to breathe tidally through the mouthpiece without nose clip for 10 min at different chilling temperatures (-10, -5, 0 and +5°C). Subjects were divided in 4 groups (codes 1-4) with a different sequence of the 4 temperatures according to a Latin-squares scheme.

For all subsequent studies, the temperature of collection was set at −5°C and subjects breathed tidally for 15 min. EBC samples were stored in microcentrifuge tubes at −20°C before the analysis.

Alpha amylase activity was assessed with EnzChek amylase assay kit (E-11954) from Molecular Probes (Eugene, OR), whose limit of detection is 0.2 mU/mL and was negative in all samples.

Plasma collection: For the measurement of urea, 10 ml of plasma were collected in tubes and stored at -20 °C until analyses.

Sample Analysis

Urea in EBC: For the determination of urea in EBC, a high performance liquid chromatography (HPLC) method for the determination of aqueous samples was adapted by Mace et al. (22) and improved in terms of sensitivity. Analytical determinations were performed using an Agilent 1100 quaternary gradient pump equipped with a on line degasser, a variable wavelength detector, and a ChemStation for data acquisition (Agilent, Palo Alto, CA, USA). Isocratic elution of urea was achieved on a Dionex CS12 column (250 x 4.0 mm i.d., 4 μm) equipped with a CG12 guard column (50 x 4.0 mm i.d., 4 μm, Dionex Corporation, Sunnyvale, CA, USA*)* using a mobile phase composed by 20 mM methanesulfonic acid at a flow rate of 0.5 ml/min. The UV detector was set at 190 nm. The use of methanesulfonic acid provided lower background absorbance thus improving the sensitivity of UV detection. The autosampler was upgraded with extensive loop for 100-500 μl injection and a sample volume of 150 μl was injected onto the column without further dilution. The calibration curve of urea was linear in the range 0.25-10 μM and the limit of detection (LOD), calculated as 3 SD of the blank, was approximately 37.5 pmol injected. The inter-day and intra-day precision of the method, expressed as coefficient of variation (CV), was <15% at the concentration of 1 μM and <10% at 5 μM. Urea was purchased from Sigma (Sigma-Aldrich, Milan, Italy) as a aqueous standard solution (40% w/v). Stock solutions were prepared by dissolving appropriate amounts of the standard solution in ultra-purified water.

In 12 randomly selected subjects urea EBC concentration was confirmed by means of a recently published method (23) to detect urea in EBC by Liquid Chromatography - tandem mass spectrometry (LC-MS/MS) (Fig. 1).

EBC volume: The collected EBC volume was measured using a calibrated 200 μl micropipette (Gilson International, Den Haag, The Netherlands) with an experimental error of ± 10 μl.

Urea in plasma: Urea in plasma were measured with AutoAnalyzer IL-LAB300 plus (Instrumentation Laboratory, Lexington, MA, USA).

Statistical Analysis

Data distribution for urea concentrations in healthy volunteers during collection of EBC at different four temperatures was assessed using the Shapiro-Wilk test. Geometric means and geometric standard deviation [GSD] were used for data with a log-normal distribution. Between-group differences were calculated using one-way ANOVA for repeated measures, followed by the Tukey's *post-hoc* test using log-transformed variables. Correlations between variables were tested with the Pearson test. Urea EBC levels in patients undergoing HD *vs* healthy

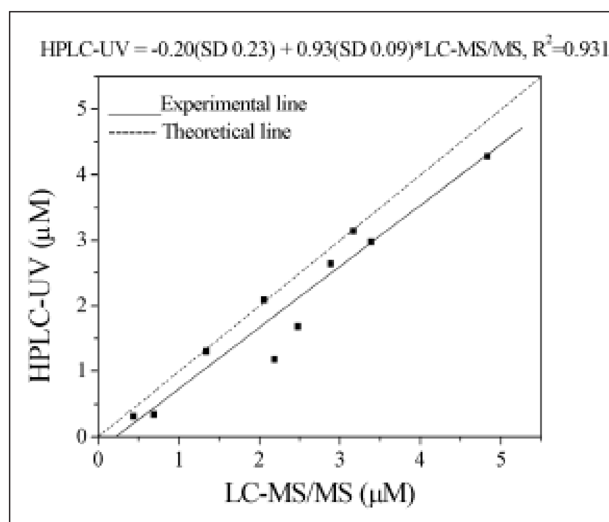

Figure 1. Correlation diagram between the results obtained by applying HPC-UV (x axis) and LC-MS/MS (y axis) to the measurement of the EBC urea concentration. The regression line and the degree of correlation are reported

subjects were expressed as median and interquartile range (IQR). PRE-HD and POST-HD data were compared with controls using the Kruskal-Wallis test followed by the Dunn *post hoc* test (the equivalent form of the parametric Dunnett test). Comparison between PRE-HD and POST-HD data were performed using the Wilcoxon test. A regression model was used on the log-transformed data to study the relationship between urea concentrations in EBC and plasma, the distribution of standardized residuals being normal. The dummy variable method (16, 24) was used to test the weight of intra-individual effect on the relationship. Data were analyzed using SPSS 15.0 (SPSS inc., Chicago, IL, USA) and PRISM 4.0 (Graphpad Software, San Diego, CA, USA). Statistical significance was assumed for p values of less than 0.05.

Results

The trend of EBC urea concentration and EBC urea total amount (obtained by multiplying urea concentration by EBC volume) with increasing condensation temperature is shown in Figures 2A and 2B, respectively. There were significant differences between urea concentrations in EBC samples collected at +5°C (geometric mean [GSD], 1.55 [1.86] μM) and those of EBC samples collected at the other tested temperatures (0.86 [2.47], at -10°C; 0.81 [2.14], at -5°C; 0.82 [2.49], at 0°C; p<0.05 for all comparisons). No differences were observed in the absolute amount of urea in EBC samples collected at different temperatures (1.28 [2.22], at -10°C; 1.06 [1.94], at –5°C; 0.98 [2.15], at 0°C; 1.50 [2.05] μM, at +5°C, respectively).

Looking at the intra-subject variability, for the subgroup of 15 subjects who repeated their collection after 24 h at -5°C, EBC urea concentrations were 0.89 [2.02] μM and 0.79 [1.84] μM at the first and the second collection, respectively, without any significant difference and with very similar variability (25). The average inter-day coefficient of variation (CV) was 27.3%.

The concentrations of urea in EBC from patients undergoing HD and controls are reported in Table 1. In PRE-HD subjects, urea concentrations in EBC were significantly higher than those detected in the EBC of POST-HD subjects and healthy controls (p<0.005 and p<0.001, respectively). There was also a significant difference in urea EBC concentrations be-

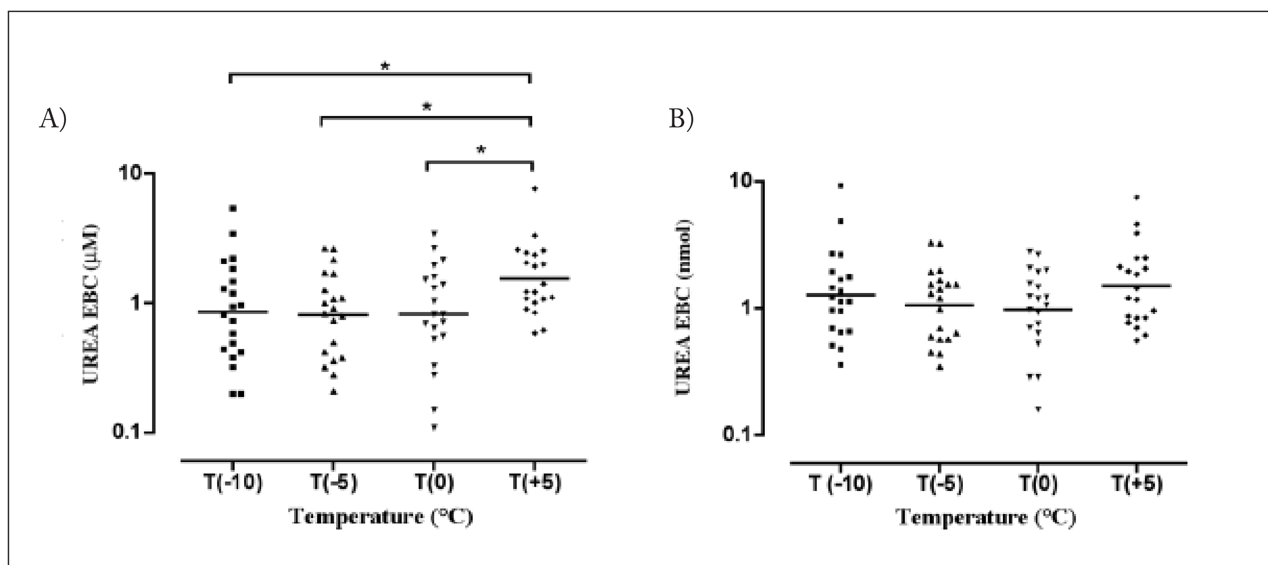

Figure 2. A) Concentrations of EBC urea (μM) at different cooling temperatures in 20 healthy subjects. Data are reported in log scale as scattergram. Horizontal bars represent geometric means. *p<0.05.
B) Absolute amounts of EBC urea (nmol) at different cooling temperatures in 20 healthy subjects. Data are reported in log scale as scattergram. Horizontal bars represent geometric means

Table 1. EBC and plasma concentrations of urea. Data are expressed as median and interquartile range

	Urea EBC (μM)	Urea plasma (mM)
PRE-HD	3.0 (1.5-4.0)[a]	22.2 (19.9-23.3)[d]
POST-HD	1.8 (1.2-2.8)[b, c]	6.7 (5.3-8.8)[e, f]
Controls	1.0 (0.7-1.3)	4.5 (4.2-5.3)

[a] p< 0.001 *vs* controls, [b] p<0.01 *vs* controls, [c] p<0.005 *vs* PRE-HD, [d] p< 0.001 *vs* controls, [e] p<0.05 *vs* controls, [f] p<0.0005 *vs* PRE-HD

tween POST-HD subjects and healthy controls (p<0.01).

Considering PRE-HD and POST-HD samples together, the correlation between plasma and EBC urea concentrations was modest (r=0.31, p=0.09, slope 0.32±0.18). However, looking at the intra-subject contribution to correlation with the dummy variable method (16, 24), the correlation was highly significant (p=0.01, slope 0.32±0.11).

In EBC samples the time course of decrease of urea is illustrated in Figure 3. After a rapid decrease during the dialysis time, urea concentrations returned to PRE-HD levels within 48 h.

The concentrations of urea in EBC of COPD, cystic fibrosis and asthma patients were 0.92 [2.22], 0.95 [1.84] and 1.01 [1.89] μM, respectively, and did not significantly differ from those of controls, as shown in Figure 4. Urea concentrations in EBC did not show any relationship with age.

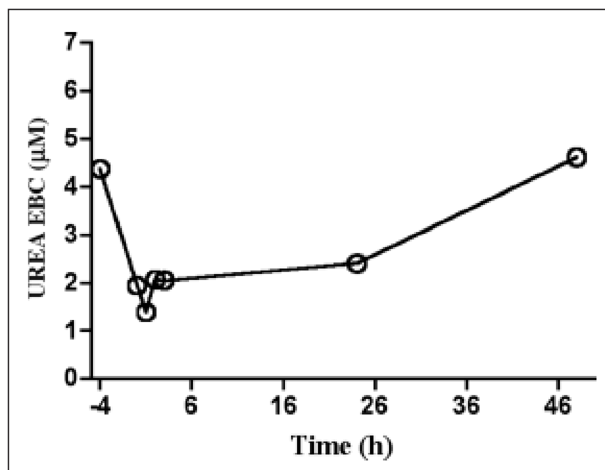

Figure 3. Time-course of urea concentrations in the EBC of 8 patients underwent HD. Points are median values at the following sampling times: PRE-HD, POST-HD, and after 1, 2, 3, 24 and 48 h POST-HD. The interquartile range is: Pre-HD, 2.8-5.7 μM; Post-HD, 1.4-2.4 μM; after 1 h, 1.3-1.6 μM; after 2 h, 1.0-2.8 μM; after 3 h, 1.5-2.8 μM; after 24 h, 2.1-2.8 μM; after 48 h, 3.1-6.4 μM

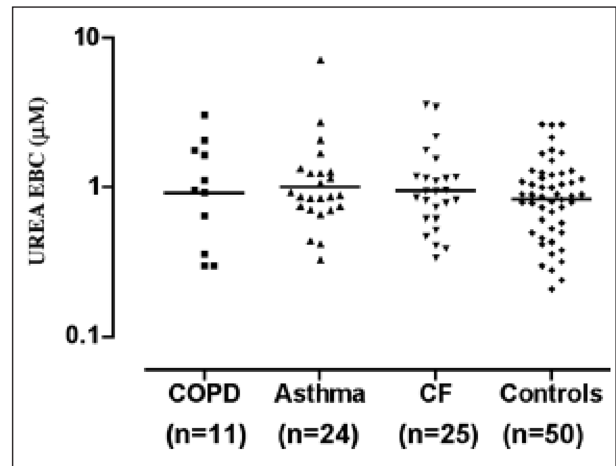

Figure 4. Concentrations of EBC urea (μM) for patients with different clinical conditions. Data are reported in log scale as scattergram. Horizontal bars represent geometric means. COPD=chronic obstructive pulmonary disease; CF= cystic fibrosis

Discussion

The present paper shows that urea is a diffusible compound with a very limited volatility, if any. Urea concentrations in EBC from patients with COPD, cystic fibrosis and asthma patients do not differ from those of controls.

In this study, we applied a method that allowed the determination of urea in EBC samples without any previous lyophilization procedure (22). EBC urea concentrations found in healthy controls were of the same order of magnitude (median [IQR], 1.0 [0.7-1.3] μM) as compared to those previously reported by other authors, *i.e.*, 0.73±0.12 μM (17). However, small differences could be accounted for by pre-analytical procedures (*e.g.*, sample lyophilization) and methods of EBC collection, which may cause a slight loss of urea influencing its concentrations during lyophilization. The inter- and intra- variability of urea found in this study was clearly lower than that measured for

conductivity and was similar to those observed for H_2O_2 (16).

In this study, the concentration of urea in EBC was determined by HPLC-UV. Due to the low concentration of urea in EBC and the scare selectivity of spectrophotometric detection, a subset of EBC samples was analyzed also by LC-MS/MS. Shown in Figure 1, the two methods gave comparable results, thus confirming the applicability of the HPLC-UV method to the determination of urea in EBC samples.

The assessment of the effect of condensation temperature on urea EBC concentration has been a step forward to characterize physical and chemical properties of urea volatility *ex vivo*. Our results showed that urea EBC concentration decreased with the reduction of collection temperatures. Such differences were no longer significant when the absolute amount of urea recovered was considered. Taken together, these data demonstrated that while the number of exhaled urea molecules appeared to be constant at the studied temperatures, its concentration in EBC decreased as the number of condensed water molecules increased at low temperatures, with a consequent dilution of non-volatile molecules. This trend was similar to what observed for conductivity in our previous study (16). The absolute amount of urea is not temperature dependent, whereas the observed decrease in urea concentrations at lower temperature should depend on number of condensed water molecule with dilution of non-volatile mediators. Thus, urea can be considered as a prevalently non-volatile compound *ex vivo*. However, a very low contribution of evaporation could not be definitely ruled out. A consequence of this result is that only non-volatile molecules could eventually be normalized to EBC urea concentrations (12).

The study on patients undergoing HD demonstrated that urea is a diffusible molecule. HD induced a significant decrease of urea in plasma associated with a decrease in EBC urea concentrations. The kinetic of EBC urea was similar to what reported in literature for plasma (26). This result confirms the idea that free diffusion phenomena tend to normalize urea levels in blood and airways and that EBC urea concentration reflects the trend of urea in pulmonary lining fluid. The apparently reduced excursion in EBC (Table 1) and the obvious time lag between airways and blood are accounted for by inter-compartmental differences in terms of diffusion. Considering that urea is not produced at the pulmonary level (18, 27-30), these results can be explained only by supposing free diffusion of urea between blood and airways.

Despite the observed poor correlation between EBC and plasma urea concentrations, the intra-subject contribution to correlation was highly significant, indicating that individual variation of urea in plasma are reflected by parallel variation of urea in EBC. The correlation could improve if kinetic factors (time lag between central and peripheral compartments) associated with HD were considered.

Moreover, urea in EBC is only a little fraction of that present in the airways and its final concentration in EBC depends on the physical phenomena of exhaled breath formation and collection.

Normalization factor is currently used to normalize the concentration of biomarkers influenced by specific biological phenomena in the same biological fluid (e.g. creatinine in urine), while dilution factor is calculated with the same analyte between two different matrices and is dependent by volume of both them (31).

Finally, our data show that the concentrations of urea in EBC are not significantly affected by the three pulmonary diseases studied (COPD, asthma, and cystic fibrosis). This result is consistent with previous findings by Effros et al., who also showed that urea concentrations in plasma are similar for both COPD patients and normal subjects (17). As there is no evidence that these pulmonary pathologies modify the urea concentration, we suggest that there is no need to normalize non-volatile biomarkers in EBC for urea concentrations to account for inter-individual variability.

Conclusion

This study demonstrates that urea is a non-volatile diffusible molecule *ex vivo* mainly and that urea in EBC behaves like its concentrations in plasma. Urea concentrations in EBC are unaffected by three

chronic airway diseases (COPD, cystic fibrosis, and asthma). In repeated measurements within the same individual, the use of urea either as a normalizing factor or as covariate variable could be proposed to control intra-individual variability.

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References

1. Montuschi P. Exhaled breath condensate analysis in patients with COPD. *Clin Chim Acta* 2005; 356: 22-34.
2. Hunt J. Exhaled breath condensate: an evolving tool for non-invasive evaluation of lung disease. *J Allergy Clin Immunol* 2002; 110: 28-34.
3. Zanconato S, Carraro S, Corradi M, et al. Leukotrienes and 8-isoprostane in exhaled breath condensate of children with stable and unstable asthma. *J Allergy Clin Immunol* 2004; 113: 257-63.
4. Carpagnano GE, Foschino Barbaro MP, et al. Exhaled markers in the monitoring of airways inflammation and its response to steroid's treatment in mild persistent asthma. *Eur J Pharmacol* 2005; 519: 175-81.
5. Goldoni M, Catalani S, De Palma G, et al. Exhaled breath condensate as a suitable matrix to assess lung dose and effects in workers exposed to cobalt and tungsten. *Environ Health Perspect* 2004; 112: 1293-8.
6. Caglieri A, Goldoni M, Acampa O, et al. The effect of inhaled chromium on different exhaled breath condensate biomarkers among chrome-platin workers. *Environ Health Perspect* 2006; 114: 542-6.
7. Horvath I, Hunt J, Barnes PJ. ATS/ERS Task Force on exhaled breath condensate. Exhaled Breath Condensate: methodological reccomandations and unresolved questions. *Eur Respir J* 2005; 26: 523-48.
8. Dwyer TM: Sampling airway surface liquid: non-volatiles in the exhaled breath condensate. *Lung* 2004; 182: 241-50.
9. Briant JK, Lippmann M. Particle transport through a hollow canine airway cast by high-frequency oscillatory ventilation. *Exp Lung Res* 1992; 18: 385-407.
10. Brown JS, Gerrity TR, Bennett WD, Kim CS, House DE. Dispersion of aerosol boluses in the human lung: dependence on lung volume, bolus volume and gender. *J Appl Physiol* 1995; 79: 1787-95.
11. Lindsley WG, Collicott SH, Franz GN, Stolarik B, McKinney W, Frazer DG. Asymmetric and axisymmetric constant curvature liquid-gas interfaces in pulmonary airways. *Ann Biomed Eng* 2005; 33: 365-75.
12. Effros RM, Hoagland KW, Bosbous M, et al. Dilution of respiratory solutes in exhaled condensates. *Am J Respir Crit Care Med* 2002, 165: 663-9.
13. Effros RM, Dunning MB, Biller J, Shaker R. The promise and perils of exhaled breath condensates. *Am J Physiol Lung Cell Mol Physiol* 2004; 287: 1073-80.
14. Effros RM, Su J, Casaburi R, Shaker R, Biller J, Dunning M. Utility of exhaled breath condensates in chronic obstructive pulmonary disease: a critical review. *Curr Opin Pulm Med* 2005; 11: 135-9.
15. Effros RM, Biller J, Foss B, et al. A simple method for estimating respiratory solute dilution in exhaled breath condensates. *Am J Respir Crit Care Med* 2003; 168: 1500-5.
16. Goldoni M, Caglieri A, Andreoli R, et al. Influence of condensation temperature on selected exhaled breath parameters [online]. *BMC Pulmonary Medicine* 2005; 5: 10. http://www.biomedcentral.com/1471-2466/5/10 [1 Sept 2005].
17. Effros RM, Peterson B, Casaburi R, et al. Epithelial lining fluid concentrations in chronic obstructive lung disease patients and normal subjects. *J Appl Physiol* 2005; 99: 1286-92.
18. Ward C, Duddridge M, Fenwick J, et al. The origin of water and urea sampled at bronchoalveolar lavage in asthmatic and control subjects. *Am Rev Respir Dis* 1992; 146: 444-7.
19. Rennard SI, Basset G, Lecossier D, et al. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. *J Appl Physiol* 1986; 60: 532-8.
20. Mills PC, Chen Y, Hills YC, Hills BA. Comparison of surfactant lipids between pleural and pulmonary lining fluids. *Pulm Pharmacol Ther* 2006; 19: 292-6.
21. Marcy TW, Merril WW, Rankin JA, Reynolds HY: Limitations of using urea to quantify epithelial lining fluid recovered by bronchoalveolar lavage. *Am Rev Respir Dis* 1987; 35: 1276-80.
22. Mace KA, Duce RA. Determination of urea in atmospheric aerosols and natural waters - a cation exchange method. *Intern J Environ Anal Chem* 2002; 82: 341-52.
23. Esther CR Jr, Jasin HM, Collins LB, Swenberg JA, Boysen G. A mass spectrometric method to simultaneously measure a biomarker and dilution marker in exhaled breath condensate. *Rapid Commun Mass Spectrom* 2008; 22: 701-5.
24. Glantz SA, Slinker BK. Primer of Applied Regression and Analysis of Variance. 2nd edition. Columbus: McGraw-Hill Education, 2000.
25. Vass G, Huszar E, Barat E, et al. Comparison of nasal and oral inhalation during exhaled breath condensate collection. *Am J Respir Crit Care Med* 2003; 167: 850-5.
26. Misra M, Nolph K. A simplified approach to understanding urea kinetics in peritoneal dialysis and hemodialysis. *Contrib Nephrol* 2006; 150: 20-7.
27. Feng NH, Hacker A, Effros RM. Solute exchange between the plasma and epithelial lining fluid of rat lungs. *J Appl Physiol* 1992; 72: 1081-9.
28. Harris TR, Roselli RJ. A theoretical model of protein, fluid, and small molecule transport in the lung. *J Appl Physiol* 1981; 50: 1-14.

29. Chinard FP. Quantitative assessment of epithelial lining fluid in the lung. *Am J Physiol* 1992; 263: L617. “Abstract”
30. Dargaville PA, South M, Vervaart P, McDougall PN. Validity of markers of dilution in small volume lung lavage. *Am J Respir Crit Care Med* 1999; 160: 778-84.
31. Barr DB, Wilder LC, Caudill SP. Urinary Creatinine Concentrations in the U.S. population. Implications for urinary biologic monitoring measurements. *Environmental Health Perspectives* 2005; 113: 192-200.

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