



TOF-SIMS analysis of exhaled particles from patients with asthma and healthy controls

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ABSTRACT: Particles in exhaled air (PEx) may reflect the composition of respiratory tract lining fluid (RTLFL); thus, there is a need to assess their potential as sources of biomarkers for respiratory diseases. In the present study, we compared PEx from patients with asthma and controls using time-of-flight–secondary ion mass spectrometry (TOF-SIMS) and multivariate analysis.

Particles were collected using an instrument developed in-house. 15 nonsmoking subjects with physician-diagnosed asthma and 11 nonsmoking healthy controls performed 10 consecutive forced exhalations into the instrument. Particle concentrations were recorded and samples of particles collected on silicon plates were analysed by TOF-SIMS.

Subjects with asthma exhaled significantly lower numbers of particles than controls ($p=0.03$) and the ratio of unsaturated to saturated phospholipids was significantly lower in samples from subjects with asthma (0.25 versus 0.35; $p=0.036$). Orthogonal partial least squares-discriminant analysis models showed good separation between both positive and negative spectra. Molecular ions from phosphatidylcholine and phosphatidylglycerol, and protein fragments were found to discriminate the groups.

We conclude that analysis of PEx is a promising method to examine the composition of RTLFL. In the present explorative study, we could discriminate between subjects with asthma and healthy controls based on TOF-SIMS spectra from PEx.

KEYWORDS: Asthma, breath test, exhaled biomarkers, particles, phospholipids, surfactant

Breath analysis is a noninvasive method of gaining information on the respiratory tract and can be useful for monitoring airway disease. Nonvolatile substances in breath are transported in the form of airborne droplets, *i.e.* particles. Collection of particles in exhaled air (PEx) is a new technique to sample endogenous particles that derive from the respiratory tract. In a previous pilot study, we showed that analysis of PEx using time-of-flight–secondary ion mass spectrometry (TOF-SIMS) provided substantial information on phospholipid composition [1]. Only 10 exhalations are required to detect phospholipids and no sample preparation is needed.

Phospholipids are characteristic components of the surfactant that covers the mucous membranes of the airways [2]. The most abundant lipid is dipalmitoylphosphatidylcholine (PC32:0), which is vital for maintaining a sufficiently low surface

tension in the airways. Phosphatidylglycerol (PG), phosphatidylinositol (PI) and sphingomyelin are also present in lower amounts. PG and PI have recently been shown to play important roles in suppressing inflammatory responses [3, 4]. The phospholipid composition, as measured in bronchoalveolar lavage (BAL), is altered in various respiratory diseases, but the reasons for the changes are uncertain [2]. Thus, analyses of phospholipids in BAL can provide potentially valuable indications of physiological states, but they are far from straightforward, since both the sample preparation and analytical methods involved are laborious and the results may be influenced by large variations in sample recovery and dilution. In addition, BAL is an invasive method associated with certain risks for the patient.

In this study, we investigated and compared TOF-SIMS mass spectra and the composition of

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phospholipids of PEx from subjects with asthma and controls. TOF-SIMS generates comprehensive spectral data and is therefore attracting increasing interest as a tool for classifying biological samples [5–7]. However, since the method is only semiquantitative and visual interpretation of spectra is difficult, multivariate analysis of the acquired data is often very useful [7–9]. Thus, multivariate analysis was applied here, using principal component analysis followed by orthogonal partial least squares (OPLS), to screen TOF-SIMS spectra. We hypothesised that, using this approach, TOF-SIMS could be used to discriminate between subjects with asthma and healthy controls, and that the phospholipid composition would differ between the groups.

METHODS

Study population

15 nonsmoking subjects (eight females) with physician-diagnosed asthma and 11 nonsmoking healthy controls (six females), who were free from symptoms of respiratory infection for ≥ 3 weeks, participated in the study. They all responded to a respiratory questionnaire based on previous validated items and subjects with asthma were additionally evaluated by a clinical allergist to confirm diagnosis.

Asthma diagnosis was based on the presentation of typical symptoms as defined by Global Initiative for Asthma (GINA) guidelines [10]. A significant measure of variable airway obstruction was documented in 14 out of 15 subjects with asthma as indicated by increased peak expiratory flow variability ($\geq 20\%$ variation compared with the mean within 1 week), a positive methacholine challenge test (provocative concentration of methacholine using a 20% fall in forced expiratory volume in 1 s (FEV₁) < 8 mg·mL⁻¹) or a positive reversibility test (12% or 200 mL in FEV₁ after 400 mg of inhaled salbutamol or spontaneous variation in FEV₁ (12%). One of the subjects with asthma was taking high doses of inhaled glucocorticosteroids and long-acting β_2 -agonists, had asthma since childhood and was reluctant to taper medication. 10 out of the 15 subjects with asthma were taking inhaled glucocorticosteroids daily and one subject only during the pollen season.

Atopy was defined as a positive skin-prick test to common inhalant allergens.

Control subjects all regarded themselves as healthy and did not report any respiratory symptoms or any medication. All performed a reversibility test with a negative outcome.

The forced vital capacity (FVC) and FEV₁ of all participants was determined using a dry-wedge spirometer (Vitalograph®; Vitalograph, Maids Moreton, UK). They performed at least three technically acceptable trials in accordance with European Respiratory Society (ERS) guidelines [11], and the largest values for FVC and FEV₁ were recorded and compared with predicted values [12]. In addition, the mid-expiratory flow between 25% and 75% of FVC (MEF_{25–75%}) was calculated for all subjects.

The clinical examination started with spirometry. The fraction of exhaled NO (FeNO) was measured using a handheld device, NIOX mini (Aerocrine, Solna, Sweden). A single-breath test at a flow rate of 50 mL·s⁻¹ was performed according to American Thoracic Society (ATS)/ERS recommendations [13]. After sampling PEx, skin-prick and reversibility tests were performed.

Methacholine challenge tests were performed on a separate occasion in those subjects where no other objective measure of variable airway obstruction could be obtained.

The study was approved by the Ethics Committee of Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden. Written informed consent was obtained from all participants.

Collection of PEx and TOF-SIMS analysis

Samples were collected by particle impaction using an instrument developed in-house, as previously described [1]. Participants breathed particle-free air (room air filtered through a HEPA filter; Whatman Inc., NJ, USA) for 3 min before sampling, to avoid contamination from exogenous particles, then performed 10 consecutive forced exhalations, with a target flow of 90% ($\pm 10\%$) of their FEV₁, into the device. The flow rate was displayed on a computer screen so that participants could observe and control their breathing. Particle concentrations were recorded with an optical particle counter (Grimm Model 1.108; Grimm Aerosol Technik GmbH & Co, Ainring, Germany). In addition, PEx were collected on silicon plates that were removed from the instrument immediately after sampling and stored in natural plastic wafer shippers (Fluoroware H22-10; Entegris Inc., Chaska, MN, USA) at -20°C , for no longer than 5 months, until analysis. Sample spots were analysed with a TOF-SIMS IV instrument (IONTOF, Münster, Germany) as previously described [1]. Positive- and negative-ion TOF-SIMS spectra were acquired from two randomly selected spots in each sample.

Statistical data analysis

Univariate analysis based on nonparametric (Mann–Whitney) tests was applied to assess the validity of findings of our pilot study and earlier observations, using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Multivariate regression analysis

For each TOF-SIMS measurement, the spectra from all pixels were summed into a single spectrum, which was binned to a digital resolution of mass (m)/charge (z) 0.2 and converted to Matlab 4 files (Mathworks Inc., Natick, MA, USA) using in-house software in Python (Python 2.5.2 with NumPy 1.2.1; Python Software Foundation, Wolfeboro Falls, NH, USA). Signal intensities of individual ions, m/z 20–1,000, were normalised according to total intensity in the corresponding full spectrum in Matlab 7.6. SIMCA-P+12.0 (Umetrics AB, Umeå, Sweden) was used for multivariate analysis, after mean-centring and Pareto scaling the data [14, 15].

Principal component analysis (PCA) was used to verify the quality of spectra. OPLS-discriminant analysis (DA) was then used to identify differences between patient groups [16, 17].

PCA is a statistical tool that is used to examine relationships between multiple variables simultaneously, by using latent correlations among them to reduce the dimensions of the data. OPLS-DA is a multivariate method that can be used to identify and display differences between patient groups in a single graph (OPLS-DA loading plot) showing all measured variables. The analysis also produces a score plot showing the separation of the groups based on the content of the loading plot. Q₂ is a measure of the quality in multivariate models. For OPLS-DA, Q₂ is based on cross-validation, a procedure in

which fractions of data are systematically excluded. For instance, a model may be calculated using six out of seven of observations (here mass spectra) at a time. A sum of squares is then accumulated for the deviations between the modelled responses (group ratings) and the assigned group (Y). These sum of squares, called the prediction error sum of squares (PRESS), are accumulated for all excluded fractions and compared to the total sum of squares for the variation in Y (SS(Y)) by the formula: $Q^2 = 1 - \text{PRESS} / \text{SS}(Y)$. A value >0.5 is considered large and indicates that the model provides useful systematic information for the present type of biological data.

RESULTS

Characteristics of subjects and the median amounts of PEx collected from them are presented in table 1. Subjects with asthma exhaled significantly lower numbers of PEx (of all size classes) than controls ($p=0.03$). The mass median aerodynamic diameter was the same in both groups, *i.e.* 0.73 μm .

TOF-SIMS analysis

The overall patterns of peaks in spectra from subjects with asthma and controls were similar. Typical positive- and negative-ion spectra for a healthy subject are shown in figure 1. In positive-ion spectra, both fragments and molecular ions ($[\text{M}+\text{H}]^+$, $[\text{M}+\text{Na}]^+$ and $[\text{M}+\text{K}]^+$) of PC were observed. The major signals were from sodium, potassium and PC fragment ions in the lower mass region, and from various species of PC in the higher mass range. In negative-ion spectra, lower mass regions were dominated by signals from CN^- , CNO^- , silicon oxides (*e.g.* SiO_2^- and SiO_3H^-), PO_3^- , and the free fatty acids C16:1, C16:0, C18:1 and C18:0. In higher mass ranges, signals from phosphatidic acid (PA), PG and PI were detected (table 2). Overall, there was high variability in signal intensity among spectra from different individuals. In addition, as observed in our previous study [1], signals from molecular ions were generally stronger in the rim of the spots, probably due to matrix effects.

Multivariate analysis

The PCA analysis of both positive and negative spectra showed that the final dataset does not have any outliers or groupings depending on uncontrolled factors, *e.g.* sample handling or instrument instability.

For the OPLS-DA analysis, data from samples from subjects with asthma and controls were placed in two different groups to see if the mass spectra supported this division. The resulting models showed good separation for both positive- and negative-ion spectra (see score plots in fig. 2). The corresponding OPLS-DA loading plots (figs 3 and 4) show the variation in m/z information that explains the division in the score plot. In this context, an asthma patient sample with a positive value on the score x -axis corresponds to positive values on the y -axis of the loading plot. Peaks contributing to the separation between groups in positive- and negative-ion spectra are visible in the loading plots. In summary, in the low mass range, signals from silicon are stronger among samples from subjects with asthma, while sodium and potassium are stronger among controls. Peaks that are commonly observed in TOF-SIMS spectra, such as phthalates (common contaminants from surrounding air) and fragments from organic compounds, appear strong for samples from both subjects with asthma and controls. In the high mass range, molecular ions from phospholipids (PC30:0+H, PC32:0+H and PC32:0+Na) are strong among samples from subjects with asthma. In negative-ion spectra, silicon signals and typical fragments from protein and phosphate are stronger among samples from subjects with asthma. In the high mass range, molecular ions from phospholipids, such as PG28:0, PG32:0, PG34:1 and PG36:1, are higher among samples from subjects with asthma.

In addition, an OPLS-DA analysis was performed solely on positive spectra of samples from subjects with asthma, which were divided into two groups based on the subjects' intake of inhaled glucocorticosteroids. The resulting model showed a complete separation without overlap between subjects taking glucocorticosteroids and subjects not taking glucocorticosteroids (the corresponding score plot is shown in supplementary information). In the higher mass region, signals from molecular ions PC32:0 were stronger in samples from subjects taking glucocorticosteroids.

The within-sample (between-spot) repeatability in the score plot was estimated by calculating the coefficient of variation using the standard deviation of scores in the predictive component divided by the mean distance between subjects with asthma and controls. To test the within-individual variability in the OPLS score plot, double or triple samples

TABLE 1 Basic data on subjects with asthma and controls

	Controls [#]	Subjects with asthma [†]
Males/females n	5/6	7/8
Age yrs	37 (21–55)	30 (19–55)
Atopy %	36	79 [‡]
FVC % pred	115.3 (93–129)	102.6 (77–126)
FEV1 % pred	108 (80–126)	97 (75–116)
MEF25–75%	3.8 (1.6–6.0)	3.6 (1.5–6.2)
FeNO ppb	20 (11–33)	55 (13–223)
Number of collected particles 0.50–2.0 μm median (range)	44000 (4500–2100000)	23000 (8100–97000)

Data are presented as mean (range), unless otherwise stated. FVC: forced vital capacity; % pred: % predicted; FEV1: forced expiratory volume in 1 s; MEF25–75%: mid-expiratory flow at 25–75% of FVC; FeNO: exhaled nitric oxide fraction. [#]: n=11; [†]: n=15; [‡]: number based on 14 subjects; data are missing for one subject.

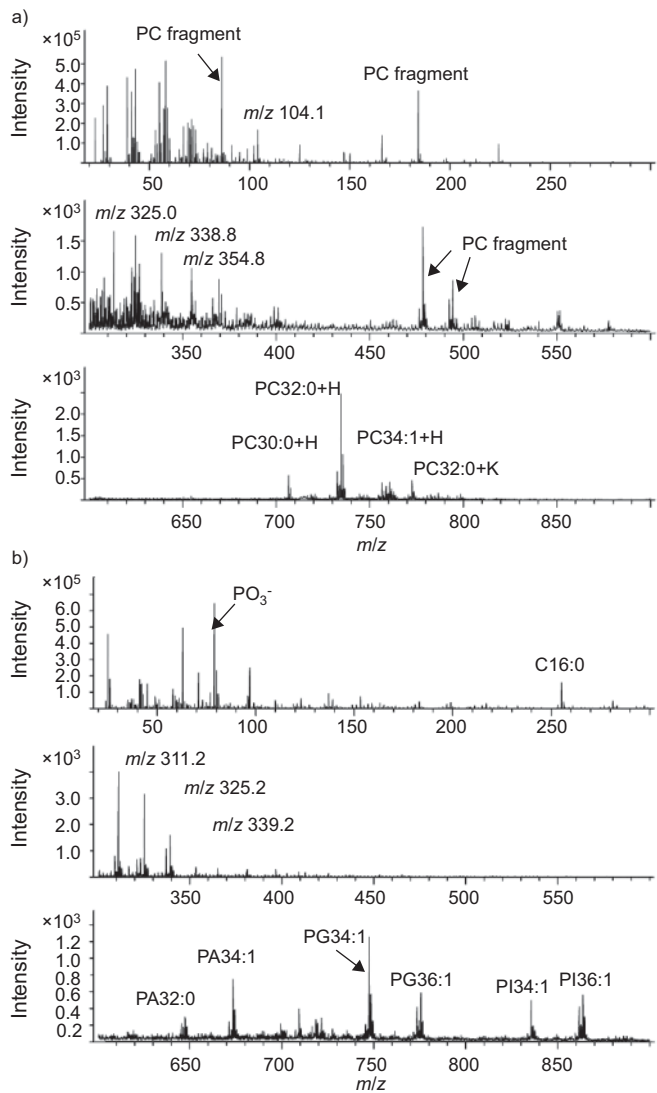


FIGURE 1. a) A typical positive-ion time-of-flight–secondary ion mass spectro-metry (TOF-SIMS) mass spectrum from a sample spot of exhaled particles from a healthy control. b) A typical negative-ion TOF-SIMS mass spectrum from a sample spot of exhaled particles from a healthy control. PC: phosphatidylcholine; PA: phosphatidic acid; PG: phosphatidylglycerol; PI: phosphatidylinositol; *m*: mass; *z*: charge.

from three controls and one subject with asthma were included in the original model which then was re-fitted. The coefficient of variation within-sample was 14% and within-individual 17%. For ratios of unsaturated to saturated phospholipids, the coefficient of variation within-samples was 4% and within-individual was 26%.

Study of possible matrix effects

As the controls exhaled significantly higher amounts of PEx than subjects with asthma, the possible effects of variations in sample amounts on the spectra were assessed by a separate OPLS analysis, using solely spectra from control samples, in which particle concentrations were the Y variables and the TOF-SIMS spectra the X variables. A strong correlation was found between particle concentration and variation in molecular ions of phospholipids ($Q^2=0.97$). PC32:0+H, PC32:0+Na

TABLE 2 Summary of peak assignments of time-of-flight–secondary ion mass spectrometry spectra obtained from exhaled particles

Positive ions		Negative ions	
Assignment	<i>m/z</i>	Assignment	<i>m/z</i>
PC fragment	58.1	C16:1	253.2
PC fragment	86.1	C16:0	255.2
PC fragment	184.1	C18:1	281.2
PC fragment	476.4	C18:0	283.3
PC fragment	478.4	PA32:1	645.4
Lyso-PC/PC	494.3	PA32:0	647.4
fragment			
Lyso-PC/PC	522.3	PG28:1	663.4
fragment			
Lyso-PC/PC	524.4	PG28:0	665.4
fragment			
PC28:0+H	678.5	PG32:0	671.5
PC30:0+H	706.6	PA34:1	673.5
PC30:0+Na	728.5	PG32:0	721.5
PC32:1+H	732.6	PG34:1	747.5
PC32:0+H	734.5	PG36:2	773.5
PC30:0+K	744.5	PG36:1	775.5
PC32:1+Na	754.5	PI34:2	833.6
PC32:0+Na	756.5	PI34:1	835.6
PC34:1+H	760.6	PI36:2	861.6
PC34:0+H	762.6	PI36:1	863.6
PC32:1+K	770.6		
PC32:0+K	772.5		
PC34:1+Na	782.5		
PC34:0+Na	784.5		
PC34:1+K	798.6		
PC34:0+K	800.6		

All mass (*m*)/charge (*z*) values correspond to singly charged monoisotopic ions. Molecular species of phospholipids are named *x:a*, where *x* is the number of carbons and *a* is the number of double bonds. Secondary ions are detected as protonated ions [M+H]⁺, sodiated ions [M+Na]⁺ and potassiated ions [M+K]⁺ in positive ionisation mode and deprotonated [M-H][−] in negative ionisation mode. PC: phosphatidylcholine; C: free fatty acid; PA: phosphatidic acid; PG: phosphatidylglycerol; PI: phosphatidylinositol.

and PC32:0+K (and in the low mass range Si, K, SiOH and phthalate fragments) were stronger in positive-ion spectra of samples with low particle amounts. PG34:1 and PG36:1 were the identified phospholipids that dominated in negative-ion spectra of samples with low particle amounts.

Univariate analysis

We also tested the validity of findings from our previous pilot study by applying univariate analysis to data acquired in the present study. The results indicated, contrary to previous findings, that ratios of levels of PO₃[−] to CN[−], CNO[−] and the sum of CN[−] and CNO[−] in PEx did not significantly differ between the groups.

The ratio of unsaturated to saturated phospholipids was significantly lower in samples from subjects with asthma than in samples from controls (0.25 *versus* 0.35; *p*=0.036), despite

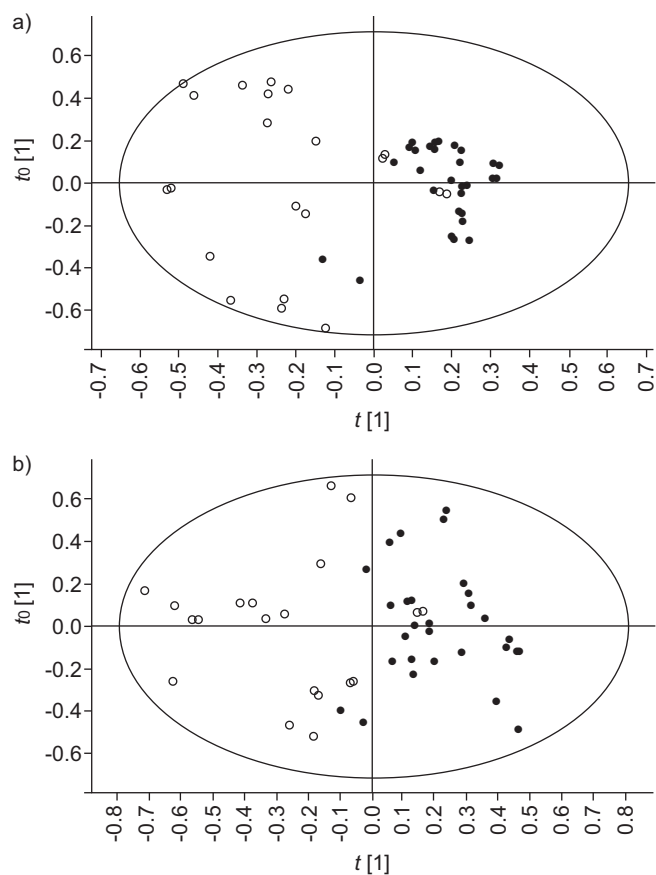


FIGURE 2. Orthogonal partial least squares-discriminant analysis score plot of a) positive-ion spectra ($R^2X(\text{cum})=0.606$, $R^2Y(\text{cum})=0.655$, $Q^2(\text{cum})=0.517$, where $R^2X(\text{cum})$ is the cumulative sum of squares (SS) of all the X values explained by all extracted components, and $Q^2(\text{cum})$ is the cumulative SS of the Y values explained by all extracted components), and b) negative-ion spectra ($R^2X(\text{cum})=0.694$, $R^2Y(\text{cum})=0.602$, $Q^2(\text{cum})=0.568$). Duplicate samples were analysed. $t[1]$: the predictive component; $t_0[1]$: first orthogonal component. \circ : subjects with asthma; \bullet : control.

high variability within each group (0.13–0.42 and 0.14–0.68, respectively).

DISCUSSION

Sampling and analysing PEx, which have phospholipid profiles indicating an origin in RTLF [1], offer a novel, noninvasive way of examining the physiological status of subjects' airways. In the present study, we discriminated between subjects with asthma and healthy controls using multivariate analysis of TOF-SIMS spectra of PEx. Loading plots from the OPLS analysis show that fragment ions and inorganic ions (present in the low mass range) are important differentiators between the groups. It is difficult to interpret the variation in individual fragment peaks, although their pattern is significant for the division of the groups. Hence, we focus on the molecular ions to address possible reasons for the observed differences in the mass spectra. Major differences in this respect were that spectra of samples from subjects with asthma yielded stronger signals of PC32:0 and several PG species compared with controls.

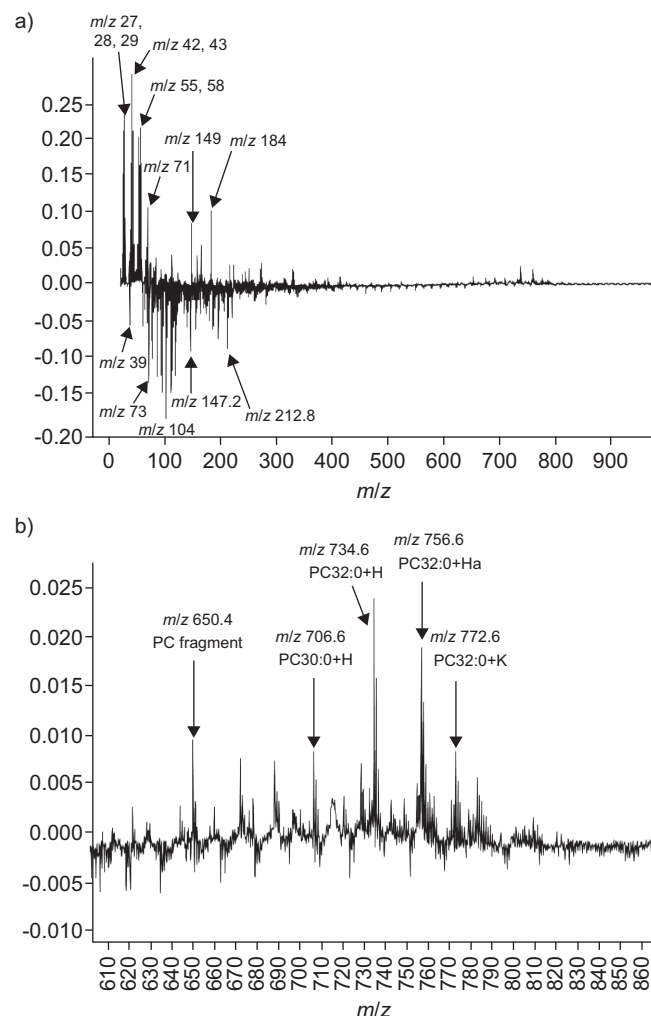


FIGURE 3. a) Orthogonal partial least squares-discriminant analysis (OPLS-DA) loading plot of positive-ion time-of-flight–secondary ion mass spectrometry (TOF-SIMS) spectra. Arrows indicate the m/z peaks with strong impacts on the separation between groups. A positive value on the y-axis corresponds to m/z peaks that are relatively stronger in spectra of samples from subjects with asthma than in control spectra. b) Detailed view of the high mass range of the OPLS-DA loading plot (shown in entirety in part a) of positive-ion TOF-SIMS spectra. Identified peaks are indicated. PC: phosphatidylcholine; m : mass; z : charge.

Alterations in the phospholipid composition in RTLF associated with asthma have been rarely studied; previous studies of associated changes in the composition of airways surfactant have focused on induced sputum or BAL. It has been speculated that in asthma, surfactant dysfunction (due to decreased surface activity) is involved in airway obstruction and inflammation [18–20]. WRIGHT *et al.* [21] observed that the proportion of PC32:0 was decreased in sputum, but not in BAL in patients with asthma, while CHANG *et al.* [22] found that PC32:0 correlated with eosinophilic cationic protein in sputum of children with asthma. It has also been observed that children with asthma have higher levels of PC32:0 in sputum compared with control subjects [23]. In atopic asthma, experimental antigen exposure has been shown to induce a reduction in PG in large surfactant aggregates collected from BAL [24]. Leakage of plasma proteins seems to be important for inactivation of

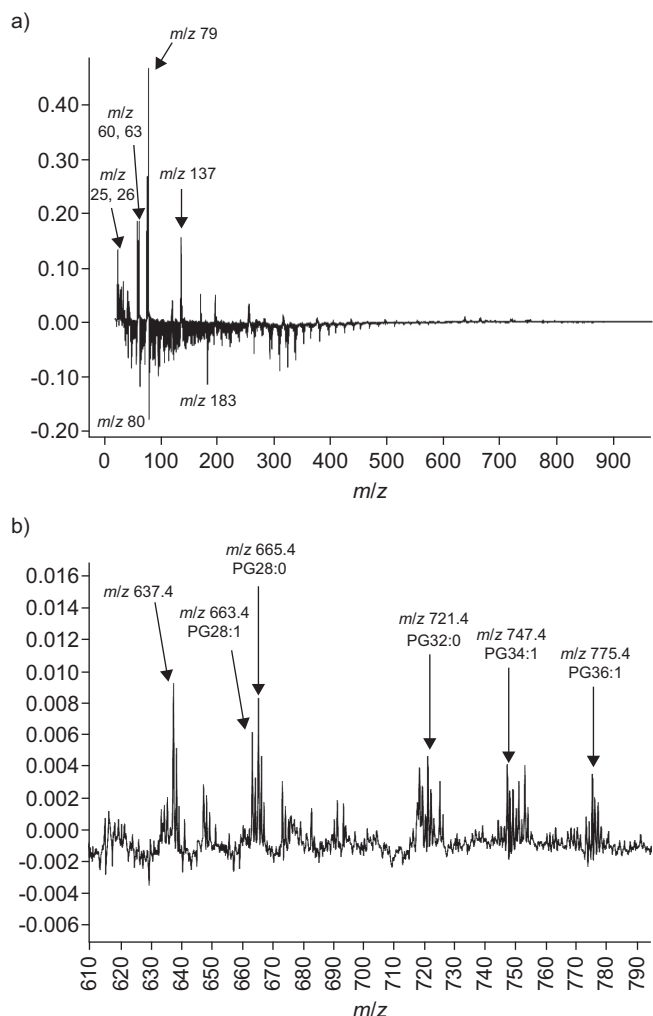


FIGURE 4. a) Orthogonal partial least squares-discriminant analysis (OPLS-DA) loading plot of time-of-flight-secondary ion mass spectrometry (TOF-SIMS) negative-ion spectra. Arrows indicate the mass (*m*)/charge (*z*) peaks with strong impacts on the separation between groups. A positive value on the y-axis corresponds to *m/z* peaks that are relatively stronger in spectra of samples from subjects with asthma than in control spectra. b) Detailed view of the high mass range of the OPLS-DA loading plot (shown in entirety in part a) of TOF-SIMS negative-ion spectra. Identified peaks are indicated. PG: phosphatidylglycerol.

surfactant [19, 21, 25]. It is not possible to identify specific proteins by TOF-SIMS, but CN^- and CNO^- are considered unspecific fragments from proteins and peptides, and according to the loading plots, both CN^- and CNO^- were elevated among subjects with asthma. The ratio of proteins to phospholipids can be estimated by comparing signals from CN^- and CNO^- , and the signal from PO_3^- , which is a strong phospholipid fragment. In the present study, we observed no significant difference in this ratio between groups. We also investigated the difference in the ratio of saturated to unsaturated phospholipids between the groups and found that the ratio was significantly elevated among asthmatics. Unsaturated fatty acids are more rapidly oxidised than saturated species, suggesting that an increased ratio of saturated to unsaturated species may reflect the increased oxidative stress known to occur in asthma [26].

In both negative- and positive-ion spectra, molecular ions from phospholipids were stronger in spectra from subjects with asthma. Indeed, no phospholipid molecular ions were stronger in spectra of samples from controls. This distinction may be due to samples from asthmatic subjects containing smaller amounts of particles. The possible matrix effects caused by variations in sample amounts were therefore investigated further. Some of the phospholipid molecular peaks were found to correlate to the particle amount. In positive spectra, low particle amounts were correlated with stronger signals from PC32:0, while in negative-ion spectra, low particle amounts correlated with stronger signals from PG34:1 and PG36:1. However, PG28:0 did not correlate with particle amounts, but is important for distinguishing between controls and subjects with asthma. Samples from two control subjects had extremely high particle concentrations (400,000 and 2,100,000 particles, respectively; see table 1 for median values), and, since particle concentration was shown to affect the chemical analysis, the investigated relationships were also modelled without data from these subjects. The Q2 values for the resulting models of both negative- and positive-ion spectra were improved (0.52 versus 0.91 for positive spectra and 0.57 versus 0.64 for negative spectra) and the OPLS loadings for the models for asthma and the model for particle amount were not correlated. The division between groups in the new models was stronger than in the original models (see online supplementary material for score plots). Furthermore, PC32:0, PG32:0, PG34:1 and PG36:1 were stronger differentiators of the groups in the new models, indicating that the method could be further improved by standardising sampled particle numbers for TOF-SIMS analysis.

The effect of inhaled glucocorticosteroids on spectra from subjects with asthma was also examined using OPLS-DA. The results indicate that inhaled glucocorticosteroids may alter the surfactant composition and, hence, the spectra of PEx. The results agree with studies showing that glucocorticosteroids stimulate the synthesis of PC [27, 28]. An alternative explanation is that those taking inhaled glucocorticosteroids have a more severe disease and that this is reflected in the mass spectra. The groups were, however, too small to draw any further conclusions.

It is not known if the number of PEx is affected in airway inflammation. In the present study, controls exhaled significantly higher numbers of PEx than subjects with asthma. Subjects performed forced exhalations, since this manoeuvre produces much higher particle numbers than tidal exhalations. The subjects were instructed to exhale at a maximal flow corresponding to 90% of their FEV₁ ($\pm 10\%$). The reasoning behind this was that the intra-thoracic flow may be highly important for the formation of particles during forced exhalations, especially during dynamic compression of the airways, which may be an important mechanism for endogenous particle production. In this small group, inhaled glucocorticosteroids did not affect particle number. We have recently shown that airway re-opening is an important mechanism for PEx formation [29]. We have not controlled for this in the present study. However, all subjects were given similar instructions: to inhale as deeply as possible and then exhale maximally. Subjects with asthma may, however, have been unable to exhale as deeply as control subjects because of premature airway closure. The site of airway closure may have taken place at earlier generations of the bronchial tree for subjects with asthma compared with controls, which could affect the

particle number and possibly also the composition of particles. Furthermore, the location of the flow-limiting segment may differ between subjects with asthma and controls, which in turn may also affect the location of particle production if particles are formed due to shear forces produced by the high flows.

Differences in the composition of the RTLTF may also contribute to the observed difference in PEx concentrations between controls and subjects with asthma. Nebulised isotonic saline has been shown to influence the number of PEx during tidal breathing [30], possibly by influencing the viscosity of the respiratory tract lining fluid. Whether PEx concentrations are really lowered in asthma is a matter for future studies.

Asthma is a very heterogeneous disease; ideally more subjects would have been included in our study, and possibly steroid-naïve subjects with a more uniform disease. The subjects with asthma in the present study were recruited by advertisements in the hospital or the university and presented with varying disease severity (from mild-intermittent to moderate) and medication varied. Hence, the present work should be considered an explorative study of both a new sampling method and an analytical method that has not previously been used in respiratory research. We were nevertheless able to show that it is possible to distinguish between subjects with asthma and controls based on TOF-SIMS spectra of PEx and that differences in the phospholipid composition were important for the separation between groups.

Conclusions

Collection and analysis of PEx seem to be a promising new method for examining the RTLTF and monitoring changes in the composition of surfactant in respiratory disease. The signals from PC32:0 and several species of PG were higher in samples from asthmatic subjects, and these subjects also had higher ratios of saturated to unsaturated phospholipids compared with controls.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

Statements of interest for A-C. Almstrand, M. Josefson, J. Lausmaa and A-C. Olin can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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