Metabolomics Reveals Altered Metabolic Pathways in Experimental Asthma

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Metabolomics refers to the comprehensive analysis of metabolites in biological systems, and has been employed to study patients with asthma based on their urinary metabolite profile. We hypothesize that airway allergic asthma would affect metabolism in the lungs, and could be detected in bronchoalveolar lavage (BAL) fluid (BALF) using a combined liquid chromatography– and gas chromatography–mass spectrometry (MS) platform. The objective of this study was to investigate changes of lung metabolism in allergic asthma by metabolomic analysis of BALF. BALB/c mice were sensitized and challenged with ovalbumin to develop experimental asthma. Dexamethasone was administered to study the effects of corticosteroids on lung metabolism. Metabolites in BALF were measured using liquid chromatography–MS and gas chromatography–MS, and multivariate statistical analysis was performed by orthogonal projections to latent structures discriminant analysis. Metabolomic analysis of BALF from ovalbumin-challenged mice revealed novel changes in metabolic pathways in the lungs as compared with control animals. These metabolite changes suggest alterations of energy metabolism in asthmatic lungs, with increases of lactate, malate, and creatinine and reductions in carbohydrates, such as mannose, galactose, and arabinose. Lipid and sterol metabolism were affected with significant decreases in phosphatidylcholines, diglycerides, triglycerides, cholesterol, cortol, and cholic acid. Dexamethasone treatment effectively reversed many key metabolite changes, but was ineffective in repressing lactate, malate, and creatinine, and induced additional metabolite changes. Metabolomic analysis of BALF offers a promising approach to investigating allergic asthma. Our overall findings revealed considerable pathway changes in lung metabolism in asthmatic lungs, including energy, amino acids, and lipid metabolism.

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CLINICAL RELEVANCE

Metabolomics has been shown to be a promising technique for the identification of patients with asthma based on their metabolite profiles. We hypothesize that there are metabolism changes in asthmatic lungs that can be detected in the bronchoalveolar lavage fluid (BALF) using metabolomics. This study shows that metabolomic analysis of BALF can provide an in-depth understanding of altered lung metabolism in asthma and elucidate unique disease-relevant metabolite profiles. Corticosteroids can help restore certain lung metabolism, but also induce additional metabolite changes.

Asthma is a chronic inflammatory lung disease affecting over 300 million people worldwide, with an estimated additional 100 million persons with asthma by the year 2025 (1). Despite of the fact that the majority of asthma cases can now be well controlled by current medications, asthma still accounts for over 250,000 deaths annually worldwide. As such, there is a constant drive to better understand asthma by identifying new pathogenic molecules, therapeutic targets, and pharmacological agents to ease the rapidly increasing morbidity and mortality associated with the disease. Metabolomics refers to the comprehensive analysis of small molecule metabolites from cells, tissues, organs, or organisms, being influenced by genetic modifications, pathological stimuli, or the environment (2). Metabolomics has been proven to be an acceptable and reproducible platform technology, capable of capturing key molecular signatures and characteristics of diseases at different stages and progression (3, 4).

Metabolomics is a relatively new technique for asthma study, which potentially offers novel and unique insights to widen our understanding of this chronic airway disease. To our knowledge, there have been only four reports employing metabolomics to discover diagnostic biomarkers in urine or exhaled breath condensate from patients with asthma (5–8). These studies were able to discriminate subjects with asthma from healthy populations, and uncovered some potential disease-relevant urinary biomarkers. Nevertheless, these studies focused on identifying biomarkers in the urine or breath, mainly for diagnostic purposes. There is a lack of studies investigating metabolic changes directly in the asthmatic lungs, which potentially generates unique perspectives for understanding disease mechanisms.

Bronchoalveolar lavage (BAL) fluid (BALF), obtained by flushing the lungs with fixed volumes of physiologic solutions, is a physiologically relevant sample for studying respiratory diseases. Metabolomics analysis of BALF collected from patients with cystic fibrosis has been successful in identifying key

disease-related metabolic biomarkers, and has also provided novel insights of the disease (9). Besides, BALF from animals exposed to silica dust (10) or 1-nitronaphthalene (11) has been analyzed using metabolomics to study lung toxicity. To date, there has not been a study using metabolomics technology to analyze BALF from asthma.

In this study, we employed an integrated approach of combining liquid chromatography (LC) and gas chromatography (GC) mass spectrometry (MS) metabolomics methods to analyze BALF from experimental asthma. We further investigated the effects of dexamethasone on metabolic profiles of BALF in the murine model of asthma. Our findings revealed, for the first time, substantial alterations in energy, lipid, and sterol metabolism in BALF from an experimental murine model of asthma, including potentially important metabolites for phenotyping asthma. We further demonstrated that dexamethasone was able to reverse many of these changes in BALF metabolome in experimental asthma, but also initiated changes in some other metabolites.

MATERIALS AND METHODS

Details of sample preparation and GC/MS and LC/MS methods are available in the online supplement.

Animals

Female BALB/c mice, 6 to 8 weeks old (Centre for Animal Resource [CARE], Canning Vale, Western Australia, Australia), were sensitized and challenged with ovalbumin (OVA) to develop allergic airway inflammation (OO), as previously described (12). Dexamethasone (Dex) was dissolved in saline, given intraperitoneally to mice at 1 mg/kg 1 hour before each aerosol challenge (OO/Dex). Saline aerosol was used as a negative control (OS), while naive mice were used as baseline controls (N). Animal experiments were performed according to the institutional guidelines for the Animal Care and Use Committee of the National University of Singapore.

Chemicals

Dex, N-(9-fluorenylmethoxycarbonyl)-glycine, and N-methyl-N-trimethylsilyl-trifluoroacetamide were purchased from Sigma-Aldrich (St. Louis, MO). Formic acid, pyridine, high-performance LC-grade methanol, and ethanol were purchased from Merck (Darmstadt, Germany).

BALF Collection and Analysis

Mice were anesthetized 24 hours after the last aerosol challenge, and BAL was performed. In essence, tracheotomy was performed for each animal using a cannula inserted into the trachea. Ice-cold PBS ($3 \times 500 \mu\text{l}$) was instilled into the lungs, and a consistent volume of BALF, ranging between 1,400 to 1,450 μl , was recovered from each animal. Total and differential cell counts were determined from BALF collected as described previously (12, 13). BALF supernatants were frozen at -80°C before further analysis.

GC/MS Analysis

GC/MS derivatization was based on our earlier method (14). Briefly, 1.0 μl derivatized supernatant was injected splitlessly with an Agilent 7,683 Series autosampler into an Agilent 6890 GC system equipped with a HP-5MSI column (Agilent Technologies, Santa Clara, CA). Compounds were identified by comparison of mass spectra and retention time with those of reference standards, and those available in libraries (NIST 0.5; Agilent Technologies).

LC/MS Analysis

LC/MS analysis was performed on an Agilent 1200 high-performance LC system equipped with a 6410 QQQ mass detector. Compounds showing significant differences between groups were searched in the Human Metabolome Database (www.hmdb.ca) using mass-to-charge ratio (m/z) and identified by either MS/MS fragmentation patterns or reference standards.

Data Preprocessing

Each chromatogram obtained from GC/MS and LC/MS analysis was processed for baseline correction and area calculation using the MZmine 2.0 software package (15). Data were combined into a single matrix by aligning peaks with the same mass and retention time for GC/MS and LC/MS data, respectively. Area of each peak was normalized to that of internal standard (N-(9-fluorenylmethoxycarbonyl)-glycine) in each data set. The missing values were replaced with a half of the minimum value found in the data set (4).

Statistical Analysis

Cell count data are presented as means ($\pm$ SEM). One-way ANOVA followed by Dunnett's test was used to determine significant differences between groups, with significant levels at P less than 0.05. Correlations between metabolites and inflammatory cells were calculated using Pearson's correlation analysis. Multivariate statistical analysis was performed using SIMCA-P software version 11.0 (Umetrics AB, Umea, Sweden). Orthogonal projections to latent structures discriminant analysis (OPLS-DA) was used to model the discrimination between treatment and control subjects by visualization of score plots. Before OPLS-DA, data were mean centered and unit variance scaled. Metabolites heatmap and non-parametric test (Wilcoxon, Mann-Whitney test) were conducted using MultiExperiment View V4.6.1 (www.tm4.org).

RESULTS

Development of OVA-Induced Airway Inflammation and Reversal with Dex

BALF was collected 24 hours after the last OVA or saline aerosol challenge, and total and differential cell counts were performed. OVA sensitization, followed by saline inhalation, did not promote significant increases in inflammatory cell recruitment as compared with naive mice (Figure 1). OVA inhalation markedly increased total cell and eosinophil counts, with moderate increases in macrophage, lymphocyte, and neutrophil counts, as compared with saline aerosol control ($P < 0.05$). Dex treatment (1 mg/kg) suppressed eosinophil, macrophage, and lymphocyte recruitment ($P < 0.05$), but was ineffective in repressing neutrophil counts.

Multivariate Analysis of Metabolic Fingerprint by GC/MS and LC/MS

We investigated BALF samples from four groups of mice (naive [N], $n = 12$; sensitized [OS], $n = 12$; asthma model [OO], $n = 12$; asthma treated with Dex [OO/Dex], $n = 8$) using GC/MS and LC/MS. Over 80 peaks in GC/MS were consistently detected in at least 90% of the BALF samples. Similarly, more than 300 peaks were collected in both positive and negative modes of LC/MS after 90% filter threshold. In OPLS-DA analysis, the value of R^2Y describes how well the data in the training set are mathematically reproduced, ranging between 0 and 1, where 1 indicates a model with a perfect fit. Models with a Q^2 value greater than or equal to 0.5 are generally considered to have good predictive capability. Statistical analysis of lung metabolite profiles showed clear separation of OO from OS and N groups (Figures 2A and 2B), with strong modeling fit R^2Y values of 0.866 (GC/MS) and 0.863 (LC/MS), as well as good prediction Q^2 values of 0.495 (GC/MS) and 0.694 (LC/MS). Experimental asthma lung metabolite profiles with Dex treatment, OO/Dex, also showed distinct separation from OO (Figures 2C and 2D), with robust modeling fit R^2Y values of 0.881 (GC/MS) and 0.941 (LC/MS), and high-prediction Q^2 values of 0.725 (GC/MS) and 0.859 (LC/MS).

Altered Lung Metabolism in Experimental Asthma and Effects with Dex Treatment

BALF metabolites were significantly altered in OO as compared with OS. Fold changes are depicted in the heatmap for each

Figure 1. Ovalbumin (OVA)-induced inflammatory cell pulmonary recruitment and treatment using dexamethasone (Dex). Inflammatory cell counts were performed in bronchoalveolar lavage fluid (BALF) obtained from naive untreated mice (N), sensitized mice 24 hours after the last saline aerosol (OS), OVA aerosol (OO), or 1 mg/kg Dex (OO/Dex). Differential cell counts were performed on a minimum of 500 cells to identify macrophages (Mac), eosinophils (Eos), lymphocytes (Lym), and neutrophils (Neu). Values are expressed as means ($\pm$ SEM) for 8–12 mice per group. #Significant difference from OS, $P < 0.05$; *significant difference from OO, $P < 0.05$.

treatment group (Figure 3A). Altered energy metabolism was noted in allergic airway inflammation, with significant increases in pulmonary levels of lactate, malate, and creatinine (Figure 3B). On the other hand, concentrations of carbohydrates, including mannose, galactose, and arabinose, were significantly reduced in experimental asthma as compared with saline-challenged controls (Figure 3B). Alterations in lipid and sterol metabolism were also observed, with substantial decreases in phosphatidylcholines (PCs), diglycerides (DGs), triglycerides (TGs), cholesterol, cholic acid, and cortol, and increases in choline and hexadecenoylcholine levels only (Figure 3C). Detailed data on fold changes in various PCs, DGs, TGs, as well as other

metabolites, are available in the online supplement (see Figure E1 in the online supplement). Dex showed reversed changes of most altered metabolites, except for some metabolites, such as lactate, malate, and creatinine.

Correlations between Specific Metabolites and Various Inflammatory Cells

Among the list of metabolites found to be significantly altered in the BALF of experimental asthma (Figure 3A), 13 specific metabolites were noted to possess significant linear correlations to inflammatory cell counts (Table 1) and to various inflammatory cell percentages (Table 2). Energy metabolites (lactate and malate) and lipid metabolites (choline and hexadecenoylcholine) showed moderate-to-strong correlations to recruited inflammatory cells, especially to percentage of macrophage (negative correlations) and eosinophils (positive correlations) (Pearson coefficient, $r > 0.5$, $P < 0.01$). On the other hand, carbohydrates (galactose and mannose), sterols (cholesterol, cortol, and cholic acid), and other lipid metabolites (PCs, DGs, and TGs) had substantial negative relationships to total cell, eosinophil, and neutrophil percentages (Pearson coefficient, $r < -0.5$, $P < 0.01$), and were also positively correlated to macrophage percentages (Pearson coefficient, $r > 0.5$, $P < 0.01$). In most cases, lymphocyte counts (Table 1), but not percentages (Table 2), were shown to be moderately associated with most of these metabolites (r in the range of ± 0.3 to ± 0.5 ; $P < 0.05$).

Dex Alters Lung Metabolite Profile in Experimental Asthma

Apart from reversing metabolite alterations in inflamed lungs, we observed that Dex could also induce additional metabolite changes in experimental asthma. Noticeably, Dex caused substantial induction of fatty acids, including steridonic acid, eicosapentaenoic acid, and petroselinic acid, as well as various monoglycerides, DGs, and TGs (Figure 4). Other lipid-based

Figure 2. Statistical discrimination of OVA-induced lung inflammation and treatment with Dex. (A) Orthogonal projections to latent structures discriminant analysis (OPLS-DA) score plot obtained from gas chromatography (GC)/mass spectrometry (MS) data comparing N, OS, and OO mice. (B) OPLS-DA score plot obtained from liquid chromatography (LC)/MS comparing N, OS, and OO mice. (C) OPLS-DA score plot obtained from GC/MS data comparing OO mice and OO/Dex mice. (D) OPLS-DA score plot obtained from LC/MS data comparing OO and OO/Dex mice. Black unfilled circles, N mice ($n = 12$); blue unfilled diamonds, OS mice ($n = 12$); red filled circles, OO mice ($n = 12$); green filled diamonds, OO/Dex mice ($n = 8$). The x axis, $t[1]$, and y axis, $t[2]$, indicate the first and second principle components, respectively.

Figure 3. Key metabolite changes induced by airway inflammation and reversal with Dex treatment. (A) Significant fold level changes in BALF metabolites are expressed as a heatmap showing metabolite changes in all treatment groups, detected by either LC/MS or GC/MS. Detailed fold changes in various phosphatidylcholines, diglycerides, triglycerides, and other metabolites can be found in Figure E1. Fold changes are derived from the fold difference over normalized means for each metabolite. Green squares indicate a reduction of up to two-

fold; black squares indicate no significant fold changes; red square indicates an increase of up to twofold. ⁺No significant change in OO/Dex as compared with OO, $P > 0.05$. (B) Mass abundance of key metabolites involved in the alteration of energy metabolism in asthma, detected by GC/MS or LC/MS. [#]Significant change in OO compared with OS or N, $P < 0.05$; *significant change in OO/Dex compared with OO, $P < 0.05$. (C) Mass abundance of key metabolites involved in lipid metabolism in asthmatic lungs, detected by GC/MS or LC/MS. [#]Significant change in OO compared with OS or N, $P < 0.05$; *significant change in OO/Dex compared with OO, $P < 0.05$.

metabolites, such as monostearin, 2-palmitoylglycerol, sphingomyelin, and lyso PCs, were also found to be up-regulated with Dex. Besides, amino acids, such as N,N-dimethyl-tyramine and N-acetyl-tyrosine, were also found to be increased with corticosteroid treatment. Interestingly, 2-oxoarginine, a metabolite of arginine catabolism, suggestive of impaired arginase activity, was noted to be reduced by Dex.

DISCUSSION

Metabolomics, especially MS-based metabolomics, is an emerging analytical tool in human disease research, due to its high sensitivity and capability to simultaneously measure many metabolic biomarkers in a single biological sample (16). Recent studies have used metabolomics to analyze the urine or breath condensate of patients with asthma, and demonstrated that this approach could offer opportunities for disease-related biomarker discovery (5–8). To date, there are still no metabolomics studies that have investigated these metabolic changes directly in asthmatic lungs, which would provide novel insights in to changes in lung metabolism under asthmatic conditions. In this study, BALF derived from OVA-induced asthma was analyzed using an integrated LC/MS- and GC/MS-based metabolomics approach. We hypothesized that asthmatic conditions would lead to alterations in lung metabolism, which can be reflected in the BALF. Dex, a potent corticosteroid, was administered to investigate the effects of corticosteroids in lung metabolism resulting from asthma.

Our results indicate that BALF, obtained from animals with OVA-induced asthma, encompass unique disease-relevant metabolic signatures, detected by a combination of GC/MS and LC/MS analysis. The measurements of multiple metabolites, known as a metabolic signature, versus using a single metabolite, as demonstrated here, enabled discrimination of diseased animals from healthy control animals. These metabolic signatures, coupled with good predictive values of the mathematical modeling, revealed major alterations in lung metabolism in an experimental murine model of asthma that were substantially reversed by Dex, suggesting that they are disease-relevant metabolic changes. These alterations of the BALF metabolome in experimental asthma and reversal effects by a corticosteroid are illustrated in Figure 5, adapted from a recent study (17).

In this study, a prominent metabolic feature observed in animals with asthma was an alteration to cellular energy metabolism in the lungs, possibly related to an increased respiratory burden in supplying energy to recruited inflammatory cells. Increases in lactate, malate, and creatinine levels (Figure 3B) suggest a stronger demand for energy in allergic airway inflammation. Lactate and malate are products of altered energy metabolism produced during abnormal lung respiration, especially under hypoxic or inflamed conditions (9). Correlation analysis performed in this study has revealed strong positive correlations between lactate and malate versus macrophages and eosinophils, suggesting that these energy metabolites may be contributed more significantly from these two cell types (Tables 1 and E1). Elevated levels of lactate have been reported in BALF from patients with cystic fibrosis (9), urine of children with asthma (7), and inflamed lungs exposed to silica dust (10) or 1-nitronaphthalene (11), indicating consistent impairment of energy metabolism in these respiratory diseases. Malate is a metabolic product from carbohydrate breakdown via the tri-carboxylic (TCA) cycle to provide additional energy in the form of adenosine triphosphate (ATP). Although increased levels of malate have not been previously reported in asthma, it has been shown that fumarate and succinate, two upstream metabolites of malate in the TCA cycle, were correspondingly depleted in experimental asthma (6), supporting the involvement of the TCA cycle with increased formation of malate in experimental asthma. Increases in pulmonary levels of creatinine, a downstream metabolite of creatine, suggest promotion of energy metabolism via the urea cycle. Creatine is involved in muscular protein turnover and energy supply to muscles, such as the airway smooth muscles (18). Substantial increases in creatine have been observed in inflamed lungs due to sepsis (19) or silica exposure (10). Metabolomic analysis of urine from children with asthma, especially those having an asthma exacerbation, has shown similar increases in creatine, which were attributable to the increased respiratory burden (7).

Carbohydrates, such as glucose, are sources of energy in pulmonary cells, and have been observed to be reduced in urine of experimental asthma (6). In our study, the levels of carbohydrate-related metabolites, such as mannose, galactose, and arabinose, were substantially lower in the experimental model of asthma as compared with healthy controls (Figure 3B), as these sugars might

Figure 3. (Continued).

have been broken down into lactate and malate to provide additional energy. Significant inverse correlations have been observed between galactose and mannose with eosinophils and neutrophil levels, suggesting that these inflammatory cells may be associated with the reduction of galactose and mannose (Table 1). Mannose has been shown to exert anti-inflammatory and protective effects in the lungs against LPS-induced injury (20). Arabinogalactan,

a downstream product formed from arabinose and galactose, has also been shown to have protective effects in allergic asthma (21). Depletion of these saccharides in asthmatic lungs of mice may contribute to the development of airway inflammation and increase in severity of the disease. To our knowledge, this is the first time that reduced mannose, arabinose, and galactose levels have been reported in experimental asthmatic lungs.

TABLE 1. PEARSON CORRELATION COEFFICIENTS OF BRONCHOALVEOLAR LAVAGE FLUID METABOLITE LEVELS VERSUS VARIOUS INFLAMMATORY CELLS RECRUITMENT

	Total	Mac	Eos	Lym	Neu
Energy metabolites					
Lactate	0.662*	0.691*	0.632*	0.366†	0.371†
Malate	0.485*	0.441*	0.482*	N.S.	N.S.
Carbohydrates					
Galactose	-0.562*	-0.450*	-0.573*	-0.350†	-0.569*
Mannose	-0.549*	-0.444*	-0.559*	-0.339†	-0.548*
Sterols metabolites					
Cholesterol	-0.498*	-0.346†	-0.509*	-0.487*	-0.532*
Cortol	-0.619*	-0.505*	-0.618*	-0.486*	-0.587*
Cholic acid	-0.648*	-0.526*	-0.655*	-0.502*	-0.522*
Lipid metabolites					
Phosphatidylcholines	-0.541†	-0.398†	-0.544*	-0.508*	-0.594*
Diglycerides	-0.454*	-0.341†	-0.445*	-0.494*	-0.536*
Triglycerides	-0.612*	-0.522*	-0.597*	-0.529*	-0.603*
Choline	0.747*	0.721*	0.731*	0.410†	0.527*
Hexadecenylcholine	0.567*	0.473*	0.591*	0.374†	N.S.

Definition of abbreviations: Eos, eosinophils; Lym, lymphocytes; Mac, macrophages; Neu, neutrophils; N.S. no significant correlations, $P > 0.05$; Total, total inflammatory cell numbers.

Moderate correlations are values of -0.5 to -0.3 (negative correlations) or 0.3 to 0.5 ; strong correlations are values of -1.0 to -0.5 (negative correlations) or 0.5 to 1.0 .

* Significant correlation, $P < 0.01$.

† Significant correlation, $P < 0.05$.

Lipid and sterol metabolism were also affected, with various lipid and sterol metabolites markedly lowered in the experimental asthmatic lungs (Figure 3C). Various PCs, DGs, and TGs were significantly reduced in the BALF, with a corresponding increase in downstream choline and hexadecenylcholine levels. Notably, substantial negative correlations have been found between PCs, DGs, and TGs with eosinophil, lymphocyte, and neutrophil levels, suggesting significant inverse associations of these lipid metabolites with inflammatory cells. PCs are associated with surfactant levels in the lungs, where decline of PCs can lead to reduced lung functions in asthma (22). TGs have been shown to protect the lungs by acting as antioxidants and conferring cytoprotective effects on epithelial, endothelial, and fibroblast cells against oxygen free radical injury (23). Choline has been reported to possess protective effects in asthma (24, 25), capable of suppressing inflammation and oxidative stress when given in substantial amounts. In a similar fashion, increases in choline levels have been observed in lungs exposed to silica dust (10) and in sepsis-induced lung injury (26). Increases in choline and hexadecenylcholine may possibly be an adaptive response in experimental asthmatic lungs to control the severity of inflammation, as a consequence of depleting other beneficial lipid metabolites. Supporting this hypothesis, strong positive correlations have been observed between choline and hexadecenylcholine levels with increasing macrophage and eosinophil levels.

In addition, considerable reductions in sterols, including cholic acid, cholesterol, and cortol, were also noted in asthmatic lungs of the mouse model (Figure 3C). In this study, these three sterol metabolites have been found to correlate inversely, especially to increasing eosinophil and neutrophil levels, and moderately to lymphocyte levels, implicating considerable relationships between sterols and these inflammatory cells. Cholesterol has multiple interactions with inflammation, and has been similarly reported to be reduced in serum of patients with asthma (27). Cortol is a metabolite of endogenous cortisol, which is produced from cholesterol and cholic acid (28). Cortisol is known to modulate immune responses in the lungs and exert broad anti-inflammatory activities to inhibit development of asthma (29,

30). Significant reduction in upstream metabolites, cholic acid and cholesterol, with corresponding decline in the downstream metabolite, cortol, suggests a possible dampening effect on cortisol metabolism, which may be an important feature in the development of asthma. So far, this is the first study that features comprehensive changes in lipid and sterol metabolism and specific correlations to inflammatory cells in experimental asthmatic lungs.

Dex (1 mg/kg), a potent corticosteroid, was shown in our recent study to exert protective effects in experimental asthma by modulating antioxidants and suppressing pro-oxidants (31). In this study, the same dose of Dex was similarly effective in suppressing airway inflammation and reversed many carbohydrate, lipid, and sterol changes in experimental asthmatic lungs. Dex restored levels of all three sugars, which possess protective effects in the lungs, suggesting that leverage of these sugars could be a beneficial effect induced by corticosteroids to attenuate lung inflammation. Another effect of Dex was to reverse the alterations in lipid metabolism, possibly by inducing increases in fatty acids, such as stearidonic, eicosapentaenoic, and petroselinic acid, as well as promoting other monoglycerides, DGs, and TGs. Finally, Dex was effective in reversing sterol metabolism, suggesting that reversal of sterol metabolism may also be a favorable effect induced by corticosteroids. Similar increases in fatty acids, PCs, and sterols have been observed in an experimental study of lungs treated with Dex (32), as this corticosteroid has been found to induce expression of the fatty acid synthase gene (33).

In this study, Dex was noted to be ineffective in halting the increase in lactate and malate levels, and even lead to further increases in creatinine levels. Dex also substantially induced many additional metabolite increases, which were unaffected in naive, sensitized, and asthmatic animals. It was beyond the scope of this study to investigate whether these metabolic changes are beneficial or adverse effects of corticosteroids, but our study suggests possible considerations of extensive pulmonary metabolic effects with the use of potent corticosteroids in asthma. A study involving rats under hypoxic conditions has also raised similar concerns of pulmonary hyperlipidemia with the use of Dex (32). Future studies may be specifically

TABLE 2. PEARSON CORRELATION COEFFICIENTS OF BRONCHOALVEOLAR LAVAGE FLUID METABOLITE LEVELS VERSUS VARIOUS INFLAMMATORY CELLS PERCENTAGES

	Mac%	Eos%	Lym%	Neu%
Energy metabolites				
Lactate	-0.448*	0.506*	N.S.	N.S.
Malate	-0.421†	0.465*	N.S.	N.S.
Carbohydrates				
Galactose	0.588*	-0.621*	N.S.	-0.493*
Mannose	0.569*	-0.602*	N.S.	-0.474*
Arabinose	N.S.	-0.362†	N.S.	N.S.
Sterols metabolites				
Cholesterol	0.624*	-0.575*	N.S.	-0.467*
Cortol	0.650*	-0.667*	N.S.	N.S.
Cholic acid	0.649*	-0.643*	N.S.	-0.426*
Lipid metabolites				
Phosphatidylcholines	0.650*	-0.615*	N.S.	-0.466*
Diglycerides	0.579*	-0.525*	N.S.	-0.419†
Triglycerides	0.630*	-0.620*	N.S.	-0.473*
Choline	-0.622*	0.683*	N.S.	0.477*
Hexadecenylcholine	-0.449*	0.489*	N.S.	N.S.

Definition of abbreviations: Eos%, percentage of eosinophils over total cells; Lym%, percentage of lymphocytes over total cells; Mac%, percentage of macrophages over total cells; Neu%, percentage of neutrophils over total cells; N.S. no significant correlations, $P > 0.05$; Total, total inflammatory cell numbers.

Moderate correlations are values of -0.5 to -0.3 (negative correlations) or 0.3 to 0.5 ; strong correlations are values of -1.0 to -0.5 (negative correlations) or 0.5 to 1.0 .

* Significant correlation, $P < 0.01$.

† Significant correlation, $P < 0.05$.

In short, these findings offer an overview of the metabolic profile of an experimental asthma model. The data obtained also provide a new understanding of metabolic pathway changes in experimental asthmatic lungs.

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