A Metabolomic Approach to the Pathogenesis of Ventilator-induced Lung Injury

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ABSTRACT

Background: Global metabolic profiling using quantitative nuclear magnetic resonance spectroscopy (MRS) and mass spectrometry (MS) is useful for biomarker discovery. The objective of this study was to discover biomarkers of acute lung injury induced by mechanical ventilation (ventilator-induced lung injury [VILI]), by using MRS and MS.

Methods: Male Sprague–Dawley rats were subjected to two ventilatory strategies for 2.5 h: tidal volume 9 ml/kg, positive end-expiratory pressure 5 cm H_2O (control, n = 14); and tidal volume 25 ml/kg and positive end-expiratory pressure 0 cm H_2O (VILI, n = 10). Lung tissue, bronchoalveolar lavage fluid, and serum spectra were obtained by high-resolution magic angle spinning and ¹H-MRS. Serum spectra were acquired by high-performance liquid chromatography coupled to quadupole-time of flight MS. Principal component and partial least squares analyses were performed.

Results: Metabolic profiling discriminated characteristics between control and VILI animals. As compared with the controls, animals with VILI showed by MRS higher concentrations of lactate and lower concentration of glucose and glycine in lung tissue, accompanied by increased levels of glucose, lactate, acetate, 3-hydroxybutyrate, and creatine in bronchoalveolar lavage fluid. In serum, increased levels of phosphatidylcholine, oleamide, sphinganine, hexadecenal and lysine, and decreased levels of lyso-phosphatidylcholine and sphingosine were identified by MS.

Conclusions: This pilot study suggests that VILI is characterized by a particular metabolic profile that can be identified by MRS and MS. The metabolic profile, though preliminary and pending confirmation in larger data sets, suggests alterations in energy and membrane lipids. (**ANESTHESIOLOGY** 2014; 120:694-702)

ABOUT one third of patients admitted to the intensive care unit worldwide require mechanical ventilation, of whom more than two thirds have acute respiratory failure as the admitting diagnosis.[1] Although life saving in many patients, it is recognized that mechanical ventilation by itself may cause lung injury by repeated stretching of lung tissue during tidal mechanical breaths (ventilator-induced lung injury [VILI]), initiating or aggravating lung injury.[2–5] In addition, the use of large tidal volumes (V_T) in patients receiving mechanical ventilation may be associated with worse outcomes.[6–8] Unlike other clinical conditions, to date there is no specific biomarker that helps in the diagnosis or prognosis of acute lung injury (ALI), nor are there diagnostic tools to identify the appearance or progression of VILI.

The metabolomic approach is finding an increasing number of applications in critical illness,[9–11] and has been used for the diagnosis of sepsis in experimental models[12–14] and for the prediction of outcome in patients with trauma.[15,16] In

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What We Already Know about This Topic

- There are no biomarkers for ventilator-induced lung injury

What This Article Tells Us That Is New

- Metabolomic studies document changes in the lungs of experimental animals with ventilator-induced lung injury; these pilot data suggest that it is possible to identify ventilator-induced lung injury with metabolic investigations

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a pioneer study in a model of ALI induced by intratracheal administration of tumor necrosis factor-α and interleukin 1-β in mice, it was demonstrated that ¹H-nuclear magnetic resonance spectroscopy (MRS) of lung tissue is useful as a biomarker of inflammation-induced ALI.[17] Liquid chromatography–mass spectrometry (MS) has also been used to define the metabolic profile in hyperoxic-[18] and

γ-radiation–induced ALI.[19] More recently, ^{1}H-MRS has been used to identify the metabolome in patients with sepsis-induced ALI,[20] thus serving as a biomarker of this condition.

The discovery of biomarkers of VILI is useful for the identification of metabolic pathways involved in VILI and for a better understanding of the pathogenesis of this condition. The current pilot study was designed to identify metabolic biomarkers in an experimental model of VILI, a form of ALI not previously studied using MRS and MS through a data-driven metabolomics fingerprinting approach. Unlike preceding studies, which used samples from only one compartment (*e.g.*, lung tissue or serum), we studied different tissue compartments by analyzing lung tissue samples, bronchoalveolar lavage (BAL) fluid and serum. This allowed us to determine whether the inflammatory response is confined to the site of injury (the lung) or rather affects other organs. Our methods are likely to produce a solution that is very sample specific, and future ongoing studies will analyze how this solution replicates in an external sample. Our preliminary findings may enable sample size calculations for confirmatory studies and stimulate further research by applying metabolomics to improve the understanding of ALI.

Materials and Methods

Experiments were carried out following the Principles of Laboratory Animal Care (EU 609/86 CEE, Spanish Real Decreto 1201/05), and the research protocol was approved by our Institutional Review Board. Methods were slightly modified from those used in previous work.[3,5] Male Sprague–Dawley rats (Harlan Iberica, Barcelona, Spain) weighing 342.3 ± 5.4 g (mean ± SD) were housed, acclimatized to a 12-h light/dark cycle, and maintained on Purina rat chow and water ad libitum before the experiments. On the day of the experiment, rats were anesthetized with intraperitoneal ketamine (90 mg/kg) and diazepam (5 mg/kg). The femoral vein was cannulated for the continuous intravenous infusion of ketamine (50 mg kg^{-1} h^{-1}) and midazolam (8 mg kg^{-1} h^{-1}). A surgical tracheotomy was performed and a 14-gauge cannula was secured in place and connected to a mechanical ventilator (Babylog 8000 Plus; Dräger, Lübeck, Germany) setting ventilatory parameters as in the control group (*vide infra*).

A 20-gauge catheter was inserted into the left carotid artery for monitoring arterial blood pressure (Model 66S; Hewlett Packard, Meyrin, Switzerland). Body temperature was maintained during the experiment by placing the animals over a thermopad (Challoner Marketing Ltd., Amersham, England).

Study Protocol

As in previous studies,[3,5] once monitoring had finished, and after an equilibration period of 15 min on mechanical ventilation, animals received one of two ventilatory strategies for 2.5 h: low V_T ventilation (V_T, 9 ml/kg; positive end-expiratory pressure, 5 cm H_2O, [CONTROL], n = 14) and high V_T ventilation (V_T, 25 ml/kg; zero positive end-expiratory pressure [VILI], n = 10). In both groups, respiratory rate was 70 breaths/min, inspiratory time 0.3 s, expiratory time 0.56 s, and F_{IO_2} 0.45.

Physiological and Biochemical Measurements

Mean arterial pressure, peak inspiratory pressure (PIP), and V_T were registered. Arterial blood ($t = 0$ min and $t = 150$ min) was obtained for measurement of blood gases and lactate concentration (Gem Premier 3000; IL Instrumentation Laboratory, Bedford, MA).

Animals were sacrificed by exsanguination at $t = 150$ min, and serum was obtained and frozen at –80°C for metabolomic studies. Thereafter, a BAL was performed by instilling once 10 ml of saline.

Finally, the thoracic cage was opened and lungs were excised and extracted. The right lung was frozen and stored at –80°C for metabolomic analysis. The left lung was removed and expanded by intratracheal instillation of 10% formaldehyde for histological analysis under light microscopy (hematoxylin and eosin). A modified lung injury score was used as previously reported.[3,21]

Metabolic Spectra Acquisition

Lung tissue, BAL fluid, and serum spectra were obtained for high-resolution magic angle spinning and ^{1}H-MRS (Bruker AMX500 nuclear magnetic resonance [NMR] spectrometer; Rivas-Vaciamadrid, Spain).

Serum spectra were also acquired by high-performance liquid chromatography coupled to quadupole-time of flight MS[22] (see Supplemental Digital Content 1, http://links.lww.com/ALN/B10, which includes a detailed description of methods on NMR and MS data acquisition).

NMR Data Treatment

Principal components analysis was applied in order to extract the most discriminative spectral subset from the total pool of metabolites. Partial least square (PLS) analysis[23] was applied on NMR data to investigate significant differences between groups. Potential biomarkers were selected from the PLS correlation plots by Hotteling T^2 tests. The NMR statistical analysis was performed with the Metabonomic package (rel.3.3.1[24]; see Supplemental Digital Content 1, http://links.lww.com/ALN/B10, for a detailed description of NMR data treatment).

MS Data Treatment

Primary data treatment (filtering and alignment) was accomplished with Mass Profiler Professional 2.0 (Agilent, Santa Clara, CA) software. Features were filtered by choosing the data that were present in 100% of samples in any group. Filtering was performed for comparisons of the VILI and the control groups. In total, 575 features (of 7,796) were selected for further data treatment. A Student t test ($P < 0.05$) was performed on the filtered data and eight significant masses were found.

Partial least square-discriminant analysis (PLS-DA) and orthogonal PLS-DA for each comparison calculated for filtered data sets were conducted using SIMCA-P+ 12.0.1 (Umetrics, Malm, Sweden). One hundred seventy-six masses from S-plot with a cutoff point of ±0.05 (after orthogonal PLS-DA) were checked through jack-knifing, and 44 significant masses from jack-knifing were added up to the list with the Student t test. Accurate masses of features representing significant differences were searched against the METLIN, KEGG, LIPIDMAPS, and HMDB databases (see Supplemental Digital Content 1, http://links.lww.com/ALN/B10, for a more detailed description of MS serum preparation, data treatment, quality control, and compound identification).

Mass spectrometry multivariate statistical calculations and plotting were done using SIMCA-P+ 12.0.1 (Umetrics). According to the method used, the number of analyses in relation to the number of events prevents definitive conclusions about the utility of these predictors in future samples. The interpretation of our results has to take into consideration that this level of discrimination does not necessarily reflect some underlying solution that can be used in future studies.

Other Statistical Analysis
Hemodynamic and biochemical variables as well as differences between the control and the VILI groups for individual metabolites were compared by an unpaired unequal variance Student t test ($P \leq 0.05$).

The correlation between each metabolite and several physiological variables at $t = 150$ min was assessed by Spearman rank correlation analysis. A P value of 0.05 or less was considered statistically significant. Data are shown as mean ± SD. We used the statistical package SPSS 17.0 (Chicago, IL).

Results

Effects of Ventilation
Rats ventilated with high V_T showed a significant increase in PIP over time, as well as a significant decrease in Pa_{O_2} and mean arterial pressure (table 1).

Histological Findings
All lungs analyzed from the control group were normal at macroscopic inspection and at light microscopy, whereas lungs from the high V_T group showed capillary congestion, interstitial edema, type-I alveolar cell necrosis, and hyaline membrane formation covering the denuded epithelial surface (lung injury score 0.0 ± 0.0 vs. 23.4 ± 10.1, respectively; $P < 0.001$; fig. 1).

MRS Results
Partial least square analysis was applied on NMR data to investigate significant differences between groups. As compared with the control group, animals with VILI showed higher concentrations of lactate and lower concentration of glucose and glycine in lung tissue; and higher concentration of glucose, lactate, acetate, 3-hydroxybutyrate, and creatine in BAL fluid (see Supplemental Digital Content 2, http://links.lww.com/ALN/B11, tables 1 and 2, which summarizes significant spectral regions of postulated metabolites).

Partial least square score plots of lung tissue and BAL fluid spectra (fig. 2) showed a perfect discrimination between the two groups.

MS Results
Partial least square-DA and orthogonal PLS-DA plots of serum samples showed a perfect discrimination between the control and the VILI groups (fig. 3).

Mass spectrometry analysis showed, among other changes, increased serum levels of phosphatidylcholine, oleamide, sphinganine, oxo-hexadecenal, and lysine, as well as decreased levels of lyso-phosphatidylcholine (lyso-phosphatidylcholine) and sphingosine (Supplemental Digital Content 2, http://links.lww.com/ALN/B11, table 2, which contains a lists of the identified compounds that are significantly different in serum samples from the control and the VILI groups by MS). Lyso-phosphatidylcholine, phosphatidylcholine, and fatty acid amides were confirmed with their characteristic fragments described in the literature.[25,26]

Table 1. Changes in Blood Gases, MAP, and Peak Inspiratory Pressure in Rats with VILI

		$t = 0$ min	$t = 150$ min	Delta	P Value
Pa$_{O_2}$ (mmHg)	Control	175 ± 10	183 ± 8	8 ± 12	0.04
	VILI	164 ± 10	133 ± 9	−31 ± 24	
MAP (mmHg)	Control	120 ± 16	93 ± 11	−28 ± 15	0.01
	VILI	127 ± 15	81 ± 10	−46 ± 15	
PIP (cm H$_2$O)	Control	16.4 ± 2.0	17.4 ± 1.3	1.5 ± 1.0	0.001
	VILI	26.4 ± 1.9	33.5 ± 3.3	8.6 ± 1.2	
HCO$_3^-$ (mM)	Control	16.4 ± 1.7	16.3 ± 1.9	−0.8 ± 1.1	0.5
	VILI	17.5 ± 1.5	16.6 ± 1.9	−0.4 ± 1.1	
Lactate (mM)	Control	2.6 ± 0.9	0.9 ± 0.3	−1.7 ± 0.9	0.07
	VILI	2.1 ± 0.9	1.1 ± 0.2	−1.1 ± 0.9	

Delta indicates the difference between $t = 0$ min and $t = 150$ min. P values refer to the comparison between delta in the control and the VILI groups for each variable. Values are mean ± SD.

MAP = mean arterial pressure; Pa$_{O_2}$ = partial pressure of oxygen in arterial blood; PIP = peak inspiratory pressure; VILI = ventilator-induced lung injury.
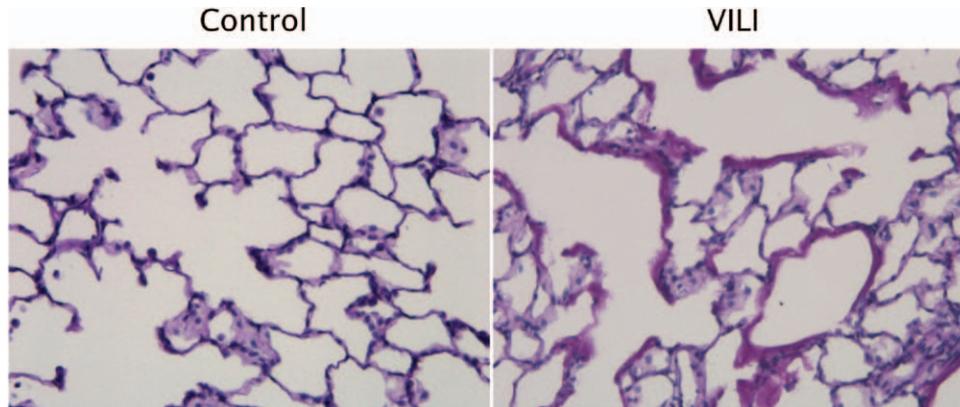
Fig. 1. Photomicrograph (light microscopy, hematoxylin and eosin) of representative lung slices (×20). Lungs from the control (low tidal volume) group were normal at macroscopic inspection and at light microscopy. Lungs from the ventilator-induced lung injury (VILI, high tidal volume) group showed capillary congestion, interstitial edema, alveolar epithelial necrosis, and thick hyaline membrane formation covering the denuded epithelial surface.
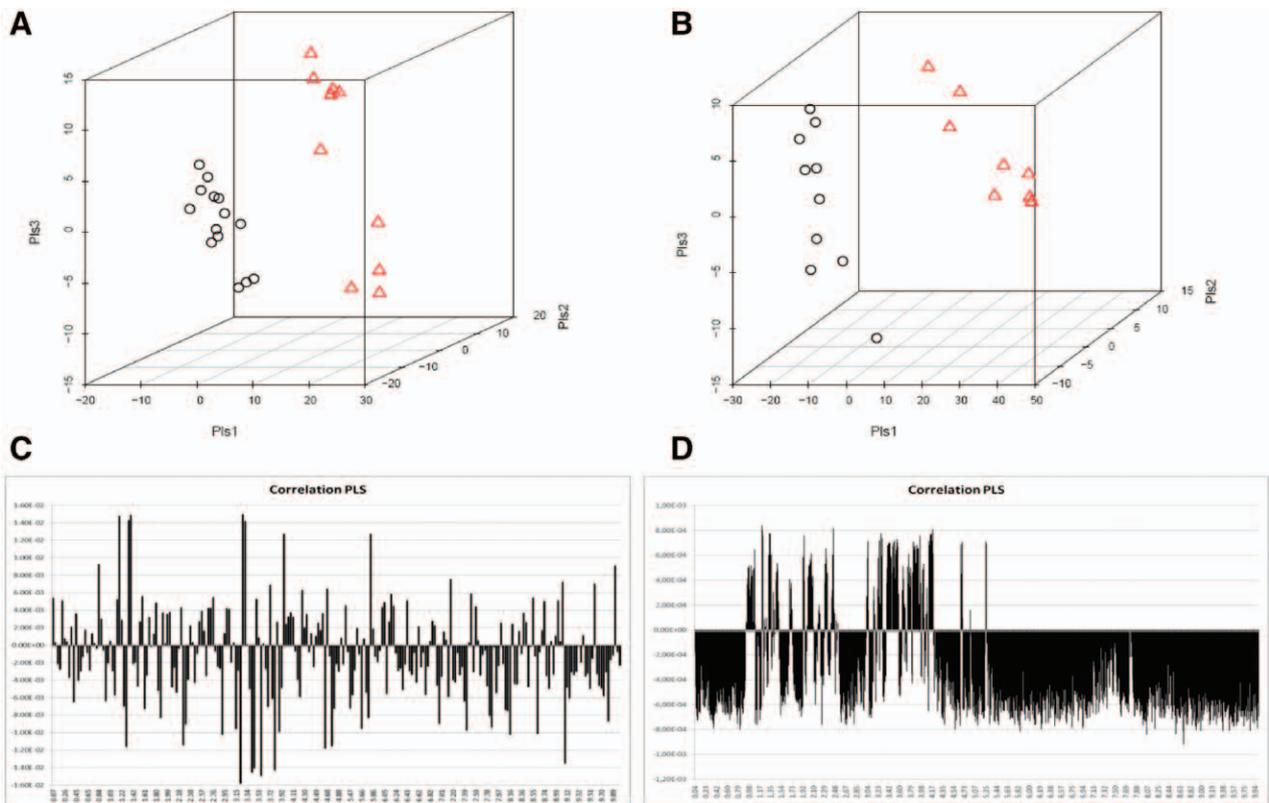

Fig. 2. Score and correlation plots of partial least squares (PLS) analyses performed on the ^{1}H-nuclear magnetic resonance spectra of lung tissue (*A* and *C*) and bronchoalveolar fluid (*B* and *D*) samples from the control (*circles*) and the ventilator-induced lung injury groups (*triangles*). The parameters for these models are (*A*) $R^2 = 0.998$, $Q^2 = 0.831$ and (*B*) $R^2 = 0.996$, $Q^2 = 0.812$. The most important bucket positions for the separation between ventilator-induced lung injury and control rats are selected with a significance level lower than 1.40e-02 and 7.00e-04, for lung tissue and bronchoalveolar fluid samples, respectively.

Correlation between Lung Injury and Metabolites

All metabolites identified by MRS whose concentration changed in VILI as compared with control animals, both in tissue and in BAL fluid samples (see Supplemental Digital Content 3, http://links.lww.com/ALN/B12, fig. 1, and Supplemental Digital Content 4, http://links.lww.com/

ALN/B13, fig. 2, containing representative ^{1}H-NMR spectra of lung tissue and BAL fluid samples), showed a significant correlation with PIP, Pao$_2$, and lung injury score at $t = 150$ min (table 3). None of the compounds identified by MS in serum showed a significant correlation with PIP, Pao$_2$, or lung injury score.
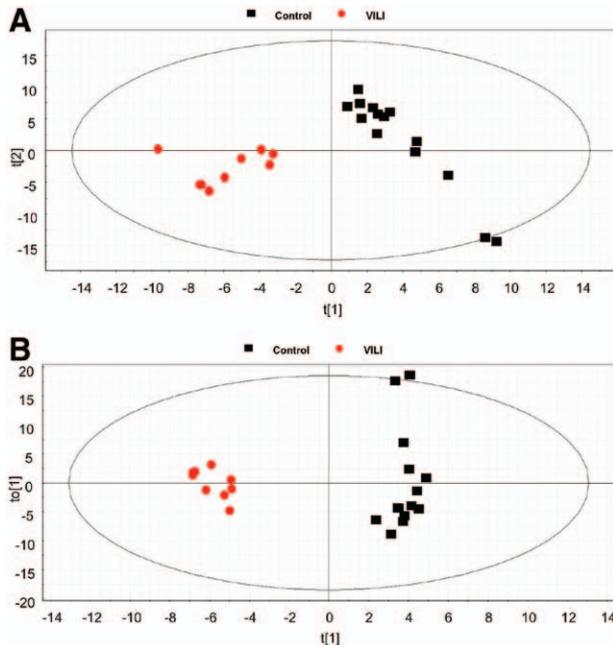
Fig. 3. Partial least squares analysis (*A*) and orthogonal partial least squares-discriminant analysis (*B*) plots of metabolite profiles in serum of control and ventilator-induced lung injury (VILI) (mass spectrometry). The parameters for these models are (*A*) $R^2 = 0.996$, $Q^2 = 0.781$ and (*B*) $R^2 = 0.979$, $Q^2 = 0.615$.

Table 2. Identified Compounds That Are Significantly Different in Serum Samples from the Control and the VILI Groups*

Sample	High	Low
Lung tissue†	Lactate	Glucose
		Glycine
BAL fluid†	Glucose	
	Lactate	
	Acetate	
	3-Hydroxybutyrate	
	Creatine	
Serum‡	Phosphatidylcholine	Lyso-phosphatidylcholine
	Oleamide	Sphingosine
	Sphinganine	Ethyl-dodecanoic acid
	Oxo-hexadecenal	Oxoisotretinoin
	Hydroxy-oxo-cholenoic acid	Octadecadienol
	Deoxycortisol	
	Lysine	

* See also table 1, Supplemental Digital Content 2, http://links.lww.com/ALN/B11. † ¹H-nuclear magnetic resonance spectroscopy. ‡ Mass spectrometry.
BAL = bronchoalveolar lavage; VILI = ventilator-induced lung injury.

Discussion

The main findings of this pilot study are: (1) ¹H-MRS and MS are useful to define the metabolome characteristic of VILI in this experimental model; (2) the specific metabolites changing in animals with VILI suggest alterations in energy pathways and membrane lipids; (3) the change in the level of the different metabolites correlate with physiological variables.

Cell Energy Metabolism

Our findings of decreased glucose and increased lactate levels in lung tissue from rats with VILI are consistent with increased glucose use and altered aerobic metabolism. Similarly, the increased creatine levels in BAL fluid indicate impairment of normal cell energy production, as creatine-phosphate can be hydrolyzed to obtain adenosine triphosphate under conditions of decreased adenosine triphosphate production, and be transported along with lactate into the alveolar space. In addition, acetate (which cannot be used under conditions of anerobic metabolism) and glucose (from glycogen degradation) can also be transported into the alveolar space and be detected in the BAL fluid determinations.

These results are in line with the findings reported by Serkova *et al.*,[17] who found decreased high-energy phosphates, energy balance, and energy charge, as well as an increased lactate/glucose ratio in lung tissue from a model of ALI in mice. Further evidence pointing to altered energy metabolism in ALI has been reported in a model of silica-induced lung inflammation[27] and in patients with sepsis-induced ALI.[20]

Membrane Lipids

In the current study we found a particular lipid profile revealed by MS in serum from rats with VILI. Levels of lyso-phosphatidylcholine and sphingosine were decreased, whereas levels of phosphatidylcholine, oleamide, sphinganine, and hexadecenal were increased (see Supplemental Digital Content 5, http://links.lww.com/ALN/B14, fig. 3, for a proposed relationship among the different lipids found to be altered in the current study). Changes in levels of membrane phospholipids have been reported in serum from patients with sepsis-induced ALI[20] and in lung tissue from mice subjected to silica-induced lung inflammation.[27]

Lysophospholipids. Lysophospholipids are generated by hydrolysis of the fatty acid ester bond of membrane phospholipids by the action of phospholipase A1 or A2, and are precursors of a different class of lipid mediators including platelet-activating factor and endocannabinoids.28 Decreased lysophospholipids could be explained in the context of activation of the remodeling pathway for phosphatidylcholine synthesis.

Phosphatidylcholine. Phosphatidylcholine is a major component of cell membranes.[28] The mechanism for increased phosphatidylcholine in our model cannot be determined, and could involve cell membrane injury and subsequent release of its components. However, membrane components released from injured membranes would not be expected to enter the circulation but would rather remain in the alveolar space were they are endocytosed and degraded by alveolar macrophages,

Table 3. Correlation between Physiological and Morphological Variables and the Change in the Concentration of Different Metabolites by ^{1}H-NMR Spectroscopy

Metabolite	PIP		Pa_{O_2}		LIS	
	R^2	P Value	R^2	P Value	R^2	P Value
Lung tissue						
Glucose	0.73	0.001	0.34	0.010	0.65	0.001
Lactate	0.56	0.010	0.32	0.010	0.39	0.010
Glycine	0.48	0.010	0.33	0.040	0.52	0.010
BAL fluid						
Glucose	0.92	0.001	0.63	0.001	0.77	0.001
Lactate	0.81	0.001	0.55	0.001	0.60	0.001
Acetate	0.65	0.001	0.24	0.004	0.28	0.004
Hydroxybutyrate	0.64	0.001	0.31	0.04	0.35	0.01
Creatine	0.89	0.001	0.72	0.001	0.79	0.001

BAL = bronchoalveolar lavage; LIS = lung injury score; NMR = nuclear magnetic resonance; Pa_{O_2} = partial pressure of oxygen in arterial blood; PIP = peak inspiratory pressure.

or used for surfactant synthesis by type II alveolar cells. Alternatively, lipid changes could set the scenario for the activation of the rate-limiting enzyme cytosine triphosphate:phosphocholine cytidylyltransferase, and increase *de novo* synthesis of phosphatidylcholine.

Oleamide. Oleamide is structurally related to anandamide,[29,30] another glycerolipid-derived regulatory molecule, and ligand for cannabinoid receptors. *Cannabinoids* have antiinflammatory and proapoptotic effects[31–33] and also play a role in the hypotension associated with various forms of shock.[31,34,35] Recent research has shown that the CB1 cannabinoid receptor is also coupled to the generation of the lipid second messenger *ceramide via* two different pathways. First, *via* sphingomyelin hydrolysis by *sphyngomyelinase*, yielding ceramide and phosphocholine (which is intermediate in the synthesis of phosphatidylcholine). The ceramide released has been linked to the regulation of metabolic functions, such as cannabinoid-mediated increased use of glucose and production of ketone bodies in primary astrocytes.[36] Second, the activation of palmitoyltransferase stimulating *de novo* synthesis of ceramide.[37–39] In the context of our findings, cannabinoid-mediated sphingomyelin hydrolysis yielding ceramide and phosphocholine could further provide substrate for phosphatidylcholine synthesis. In addition, cannabinoid-mediated activation of palmitoyltransferase could potentially explain the increased levels of *sphinganine* (one of the intermediate metabolites in *de novo* synthesis of ceramide).[40]

Sphingosine. Sphingosine can be released from ceramide, a process catalyzed by the enzyme ceramidase. Sphingosine can be phosphorylated *in vivo via* two kinases, sphingosine kinase type 1 and sphingosine kinase type 2, leading to the formation of sphingosine-1-phosphate, which is irreversibly transformed into hexadecenal and ethanolamine by sphingosine-1-phosphate lyase.[41,42] Thus, decreased levels of sphingosine in the current study could be explained by activation of ceramide synthase or by increased degradation to hexadecenal (the latter being supported by our findings

of increased levels of hexadecenal). Sphingosine and sphingosine-1-phosphate, which are metabolites of ceramide, have antiapoptotic effects[41,43,44] and play a variety of roles in diverse cellular activities such as cell growth, cell motility, and immunity[45,46] and in the regulation of vascular permeability.[47] *Hexadecenal* has been associated with apoptosis and cytoskeletal reorganization, leading to cell rounding, detachment, and eventual cell death by apoptosis in multiple cell types, including HEK293T, NIH3T3, and HeLa cells.[42]

Collagen/Elastin Metabolism
Levels of glycine were decreased in lung tissue in our model whereas lysine serum levels were increased. Glycine and lysine account for 30 and 3%, respectively, of amino acid residues in collagen, glycine being the most abundant amino acid in this protein. Previous studies have shown altered collagen metabolism in ALI. In paraquat-induced ALI, type III procollagen mRNA was higher in rats with ALI than in controls.[48] In silica-induced ALI in mice,[27] increased levels of glycine, lysine, glutamate, proline, and 4-hydroxyproline were found, suggesting the activation of the collagen pathway. In addition, increased type-I procollagen in alveolar lining fluid from patients with adult respiratory distress syndrome or ALI subjects has been reported.[49]

Potential Role of Metabolomic Changes in the Pathogenesis of ALI
In the current study the physiological relevance of the findings was further substantiated by the significant correlation between the concentration of different altered metabolites and variables indicative of lung dysfunction (impaired gas exchange) or injury (increased PIP and greater histological injury).

Our findings may help our understanding of the pathogenesis of VILI. Specifically, increased formation of cannabanoids could be related to sphyngomyelinase activation and increased formation of the lipid second messenger ceramide, as potentially important events in the pathogenesis

of VILI (Supplemental Digital Content 3, http://links.lww.com/ALN/B12, fig. 3). In addition, increased hexadecenal could play a role in cytoskeletal abnormalities in the context of cell mechanical trauma. The results of the current study supporting a role for the involvement of the ceramide–sphingosine pathway in the pathogenesis of VILI are also in line with the recognized function of *ceramide* as a signaling molecule in the inflammatory response.[45,50,51] Indeed, a protective effect of sphingosine 1-P lyase inhibition (resulting in increased levels of sphingosine 1-P) has been reported in lipopolysaccharide-induced ALI in mice.[52]

Regardless of the interest of our results, this is not a definitive study. The issue of type-I error inflation has to be considered because the methodology did not include adjustment of the analyses for the many inferences that were generated to create the solution. If subsequently validated in larger sample sets, our results may improve the understanding and management of ALI, providing suitable biomarkers that can be used in clinical practice. The strengths of our study lie in its originality and the rigorous way in which the metabolomics experiments were conducted. It further provides some pilot data with which a sample size calculation can be conducted for a definitive study.

Limitations

The current study has several limitations. First, we studied only one time in point after the insult. Second, there was no intervention to inhibit one of the pathways proposed to have a pathogenic role in VILI. Third, the relevance of serum analysis to identify metabolic biomarkers is under discussion as the insult was primarily localized to the lungs. It is possible that changes detected in serum could reflect lipoprotein-associated lipid metabolism in serum rather than alterations in lung metabolic and signaling pathways. Fourth, this is an animal model in which an exaggerated form of VILI was produced, using V_T not used in humans. However, the V_T used in the current study is well in the range of the V_T used in other investigations, ranging from 20 to 42 ml/kg (see study by de Prost[53] for excellent review). It is possible that the specific metabolomic changes herein reported cannot be reproduced with different ventilatory patterns. Specifically, of particular relevance would be the study, not approached in the current investigation, of metabolomic changes induced by VILI in preinjured lung models. Fifth, it is acknowledged that mechanical ventilation using V_T 9 ml/kg in the control group may already cause some degree of lung injury, and that a lower V_T would have been more appropriate in the control group.

In summary, we report for the first time metabolomic changes associated with VILI. Future studies should confirm the value of MRS and MS in the identification of biomarkers for the diagnosis of VILI, particularly in preinjured lungs, or other forms of ALI in larger sample sets.

Acknowledgments

Competing Interests

The authors declare no competing interests.

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